M.Sc. (Microbiology)
II - Semester
364 23

FOOD AND DAIRY
MICROBIOLOGY
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Food microbiology is the study of the microorganisms that inhibit, create, or contaminate food, including the study of microorganisms causing food spoilage, pathogens that may cause disease especially if the food is improperly cooked or stored, and those used to produce fermented foods, such as cheese, yogurt, bread, beer, and wine, and those with other useful roles, such as producing probiotics. The area of dairy microbiology is large and diverse. The bacteria present in dairy products may cause disease or spoilage. Some bacteria may be specifically added to milk for fermentation to produce products like yogurt and cheese.

Food science is a discipline concerned with all aspects of food that begins after harvesting, and ends with consumption by the consumer. It is considered as one of the agricultural sciences, and a field which is entirely distinct from the field of nutrition. The field of food microbiology is a very broad, encompassing the study of microorganisms which have both beneficial and deleterious effects on the quality and safety of raw and processed foods. It is important to understand the relationships among the various microorganisms making up the microflora of a food. In fact food microbiologists are concerned with the practical implications of the microflora of the food and the food microorganisms that can cause spoilage of food and disease in humans. The primary aim of microbiologists is to identify and quantitate food-borne microorganisms.

The production of safe food, basically free from pathogenic and spoilage organisms, is vitally important with the rapid changes in the pattern of food distribution now taking place in highly industrialized countries. Cities are so large that the inhabitants are becoming increasingly dependent on regular supplies of reasonably priced food, pre-packaged and available all the year round. Such foodstuffs, marketed over a wide area, must be microbiologically safe. Only companies with large capital resources and wide networking can afford the necessary amount of research and quality control to produce food to these high standards. Further, in order to keep the large food factories running economically, constant supply of vegetables and fruits are needed, of standard size and quality. This in turn has led to intensive farming practices with extremely high standards of hygiene and scientific principles of nutrition with rigid microbiological control at all stages.

This book, *Food and Dairy Microbiology*, is divided into four blocks that are further divided into fourteen units which will help to understand the basics of foods and related microorganisms, intrinsic factors, extrinsic factors, contamination and spoilage of cereal products, fruits, vegetables, meat products, sea foods, etc., food poisoning and food borne infections, general principles of food preservation, dairy microbiology, fermented milk products, microbial food fermentation, microbial products of food, fermented beverages, industrial production of enzymes (cellulases,
amylases, proteases, phytases, pectinases, lipases and glucose isomerases), food borne disease, food sanitation, food laws and quality control (HACCP, PFA, FPO, MFPO, BIS and AGMARK).

The book follows the self-instruction mode or the SIM format wherein each unit begins with an 'Introduction' to the topic followed by an outline of the 'Objectives'. The content is presented in a simple, organized and comprehensive form interspersed with 'Check Your Progress' questions and answers for better understanding of the topics covered. A list of 'Key Words' along with a 'Summary' and a set of 'Self Assessment Questions and Exercises' is provided at the end of each unit for effective recapitulation.
1.0 INTRODUCTION

Food microbiology is the study of the microorganisms that inhibit, create, or contaminate food, including the study of microorganisms causing food spoilage, pathogens that may cause disease especially if food is improperly cooked or stored, those used to produce fermented foods such as cheese, yogurt, bread, beer, and wine, and those with other useful roles such as producing probiotics.

Food may be contaminated from outside sources on the way from the field to the processing plant, or during transport, storage and distribution. There are thousands of different types of micro-organisms everywhere in air, water, soil and foods, and in the digestive tract of animals and human. Fortunately, the majority of microbes usefully functions in the environment and in some branches of food industry, such as production of wine, beer, dairy products, bakery products, etc. On the other hand unwanted food spoilage is generally caused by microbes and contamination of food with pathogens causes food safety problems. The microorganisms occurring on and in foods are divided into three groups: bacteria, yeast and moulds. Moulds are generally concerned in the food spoilage; their use in the food industry is limited. Yeasts are the most widely used microbes in the food industry due to their ability to ferment sugars to ethanol and carbon-dioxide. Some types of yeast, like baker’s yeasts are grown industrially, and some may be used as protein sources, mainly in animal feed. Bacteria are important in food microbiology may be divided into groups according to the fermentation product, for example lactic acid bacteria, propionic acid bacteria, acetic acid bacteria.
Bearing in mind the food constituent attacked, prolytic, saccharolytic and lipolytic bacteria may be distinguished.

Microorganisms are essential for the production of foods such as cheese, yogurt, bread, beer, wine and other fermented foods. Fermentation is one of the methods to preserve food and alter its quality. Yeast, especially Saccharomyces cerevisiae, is used to leaven bread, brew beer and make wine. Certain bacteria, including lactic acid bacteria, are used to make yogurt, cheese, hot sauce, pickles, fermented sausages and dishes. A common effect of these fermentations is that the food product is less hospitable to other microorganisms, including pathogens and spoilage-causing microorganisms, thus extending the food’s shelf-life. Some cheese varieties also require moulds to ripen and develop their characteristic flavours.

In this unit, you will study about food and related microorganisms in detail.

1.1 OBJECTIVES

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

- Discuss about food and related microorganism

1.2 GENERAL INTRODUCTION TO FOOD AND RELATED MICROORGANISM

In spite of their small size, microorganisms are of immense importance to man. They cause diseases, are a source of various foods and medicines, and dispose of our wastes. They are, in fact, responsible for the very air we breathe since free molecular oxygen was completely absent from the pre-biotic atmosphere, and this gas has accumulated only as a product of metabolism of primeval photosynthetic bacteria. Human beings have been making use of microorganisms, or their biochemical activities, since long before he even knew of their existence. The ancient Babylonians and Sumerians were brewing beer much as we do today and the Egyptians were baking leavened bread 2000 years earlier. Despite the antiquity of these microbiological practices, the first documentations of the structure of microorganisms did not occur until the advent of the first microscopes in the 17th century.

It was not until the 19th century that Louis Pasteur showed that microbes were not produced from mud or decaying organic matter. So Pasteur was considered to be the founder of industrial microbiology. Robert Koch was the forefather of medical microbiology. Koch, a German doctor could show that the causal agent of bovine anthrax was a bacterium, Bacillus anthracis. His remarkable work remains a landmark in microbiology about the nature of disease, and in proving the pathogenic activity of a disease-causing agent. We live in a time when microbiology has come of age. Microbial products are now being produced on large scale by industrial microbiologists. Some of the prominent examples are
We use microbes to make beer, wine, cheese, yogurt, sauerkraut, soy sauce, antibiotics, pesticides, gels, etc. Microbiological reactions are used in sewage treatment, in transforming the chemical structure of drugs, in cleaning clothes—bacterial enzymes are used in biological detergents—and even to extract metals such as copper and uranium from their mineral ores. New technologies such as gene cloning have been developed within the last few decades using which microbes may be used for the large-scale synthesis of valuable pharmaceutical products including human insulin, hormones, antiviral drugs and vaccines.

**Louis Pasteur’s Experiments**

By the 18th century some scientists had begun to realize that there was a causal relationship between the development of microorganisms in organic infusions and the chemical changes that took place in these infusions; microbes were the agents that brought about the chemical changes.

A great pioneer in these studies was a Frenchman, Louis Pasteur. He made a series of experiments. By conducting his studies using S shaped flasks, he demonstrated that air does contain microbes and, that they could be a cause of disease. Moreover, he demonstrated that fermentation process is brought about by the activity of microbes such as yeasts and bacteria. The popular belief was that fermentation of grape juice to wine was a natural chemical process, involving the breakdown of the protein, albumin. However, Pasteur could observe under his microscope some oval shaped yeast cells, and barely some rods commonly called bacteria. He believed that yeasts played a major role in fermentation.

In a series of classic experiments, Pasteur first showed that alcohol could be produced from grape–yeast mixtures. Using heat, he then destroyed the yeasts, whereupon alcohol failed to appear in the grape juice. However, when yeasts were returned to the flasks, fermentation took place, and wine was formed. Moreover, if care was taken to eliminate the bacteria, the wine would not become sour. Pasteur’s work thus seemed to indicate that microscopic yeasts and bacteria were tiny chemical factories that could bring about important chemical changes. Yeasts appeared to be vital to the fermentation process and bacteria made the wine sour.

Pasteur’s work appeared to demonstrate that microbes could be a cause of diseases, for if they could make the wine sick, perhaps they could also make the body ill as well. His experiments thus revealed the role of microbes in transforming the organic matter, and perhaps also in causing diseases. Pasteur believed that bacteria were present in the soil and air, and thus their spread could be controlled. If so, then perhaps diseases could also be controlled. Others believed that bacteria arose spontaneously from organic matter, as in the sick body, as long as the life force was present. Pasteur took the view of spontaneous generation seriously in order to develop his own theory of disease. He first showed that as air became purer, fewer microbes could be located. His swan-necked flasks offered the final...
defeat to the spontaneous generation theory. The flasks were left open to allow entry of any life force present in untreated air. The curvature of the neck prevented the microbes from reaching the broth. The flasks remained sterile. Thus, spontaneous generation was eliminated as a viable theory, and the idea that microbes existed in the environment was strengthened.

Robert Koch’s Experiments

The German doctor, Robert Koch is credited for his following main contributions to the development of microbiology. He stated that bacteria could be isolated and shown to cause disease. Based on his studies on anthrax, a disease infecting animals, he postulated his Germ Theory. He also postulated that a specific organism could be related to a specific disease and finally he developed the pure culture techniques. Koch had observed thread-like organisms in the blood of animals that had died of anthrax, a disease that was a serious threat to the farmers who had to kill their livestock periodically to prevent the disease from spreading. He injected laboratory animals with the blood of a dead sheep. He then performed an autopsy and noted that the tissue of the dead animal showed the presence of numerous thread-like organisms. Koch then isolated a few of these organisms and cultured (grew) them in the clear sterile vitreous humour of an ox’s eye, and watched their multiplication. He injected laboratory mice with a small sample containing spores and observed as anthrax symptoms developed in the mice. On autopsy, the blood was swarming with the thread like bacteria. Thus, he could establish that a specific microorganism would cause a specific disease. Ironically, Antony van Leeuwenhoek had first described the microorganisms 200 years earlier, but Koch was the scientist to prove that microorganisms cause diseases.

Koch accidentally observed that a slice of potato contained masses of bacteria in colonies, each colony separate and distinct from the others, and each composed of billions of rods. It occurred to him that bacteria could grow and multiply on solid surfaces, and he added gelatin to his broth to prepare a solid culture medium. When seeded onto the surface, bacteria formed visible colonies and each contained only one type of organism. Inoculations of laboratory animals could now be made with the assurance that only one type of microbe was involved. This was the breakthrough that had eluded Pasteur and that was to spark the further development of microbiology. At some period in history, Pasteur and Koch might have become friends and colleagues in their search for the agents of diseases. Pasteur became a symbol of French achievement and Koch, his German counterpart.

Other Noteworthy Experiments

Pasteur also discovered that bacteria were sensitive to temperature since chicken did not acquire anthrax at their normal body temperature of 42 °C, but did so when their temperature was lowered to 37°C. He also recovered anthrax spores from soil and demonstrated that cattle became infected by grazing in contaminated
fields. This explained the periodic recurrence of disease. Pasteur suggested that
dead animals be buried deeply in soil unfit for grazing, and where earthworms
would not bring the spores to the surface. A remarkable observation was made
that a group of test animals failed to acquire anthrax after being inoculated routinely.
Pasteur gave them a second inoculation with fresh and particularly virulent bacilli.
Still the animals lived. Pasteur then discovered that the first injection was with a
culture of bacilli that had been grown above normal temperature for several days.
He reasoned that the heated bacteria, instead of killing the animals had apparently
made them resistant to the second injection. This observation led to the formulation
of a basic principle of immunity that modified bacteria might be used to build
protection to infectious bacteria. This is one of the foundations on which immunization
with vaccines is based.

Pasteur’s work put France once more in the forefront of science. However,
it was Koch’s student, Georg Gaffky, who was the first to successfully grow the
typhoid bacillus. Friederich Loeffler, an assistant of Koch, isolated the organism
casing diphtheria. Another of his assistant, Kitasato, successfully grew the tetanus
bacillus, and also experimented with the plague bacillus. Elie Metchnikoff, a native
of Ukraine, who had come to Pasteur’s laboratory, discovered that white blood
cells engulf foreign organisms in the blood, and gave the name phagocytosis to the
process. Table 1.1 shows a list of prominent scientists and their contributions to
the development of microbiology.

Table 1.1 Prominent Scientists and Their Contributions

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<td>Louis Pasteur</td>
<td>Established the presence of microorganisms, explained the process of fermentation, developed the rabies vaccine.</td>
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<tr>
<td>Robert Koch</td>
<td>Found out the anthrax-causing bacillus.</td>
</tr>
<tr>
<td>Georg Gaffky</td>
<td>Grew the typhoid bacillus.</td>
</tr>
<tr>
<td>Friederich Loeffler</td>
<td>Isolated the organism causing diphtheria.</td>
</tr>
<tr>
<td>Kitasato</td>
<td>Grew the tetanus bacillus.</td>
</tr>
<tr>
<td>Metchnikoff</td>
<td>Explained phagocytosis, the process by which white blood cells engulf foreign organisms.</td>
</tr>
<tr>
<td>Christian Gram</td>
<td>Gram stain technique.</td>
</tr>
<tr>
<td>Winogradsky</td>
<td>Discovered chemosyntrophic bacteria.</td>
</tr>
<tr>
<td>Beijerinck</td>
<td>Nitrogen fixation.</td>
</tr>
<tr>
<td>Winogradsky and Beijerinck</td>
<td>Enrichment culture technique.</td>
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In the same year, Christian Gram, a Danish physician working in Berlin, noted that certain stained bacteria were losing their purple colour when treated with alcohol, whereas others retained it. He suggested that this procedure might be used to differentiate bacteria, and thus evolved the gram stain technique, the most important staining procedure in microbiology. Pasteur was almost at the climax of his career in 1885 when he successfully treated young Joseph Meister for rabies. He developed a vaccine for the rabies virus.

Koch, also reaching the height of his career, isolated the bacillus causing tuberculosis. He continued his studies regarding this disease. His work was interrupted when he visited Egypt and later India where cholera was raging. He isolated a comma-shaped microbe as the causative agent and confirmed the theory of John Snow that water was important for the transmission of the disease. In 1885, Koch was appointed Professor of Hygiene and Bacteriology in the University of Berlin. He continued his work on tuberculosis and in 1905 was honoured with the Nobel Prize. This age witnessed a series of discoveries unparalleled in medicine, most of which involved the identification of agents of disease. There was an increased awareness of the relationship of microbes to disease and methods of transmission. This led to sterile practices in hospitals, increased research in pest control and care in the preparation and consumption of food.

Winogradsky discovered chemosynthetic bacteria, growing in completely inorganic environments, obtaining energy by the oxidation of reduced inorganic compounds and using CO₂ as its carbon source. Winogradsky and Beijerinck by their independent studies explained the role played by microbes in fixing atmospheric nitrogen. This is done by both, free-living bacteria and those in symbiotic associations. Winogradsky and Beijerinck also developed a new and important technique for the isolation of a particular type of microorganism from a mixed population through the modification of such factors as the carbon source, energy supply, temperature, and pH value of the growth medium. The technique is known as enrichment culture. In the last part of the 19th century, microbiology became an established discipline with a distinct set of concepts and techniques. During the same period, a science of general biology also emerged, with the published work of Charles Darwin. However, microbiology and general biology had little coherence. For half a century after the death of Pasteur in 1895, microbiology and general biology developed almost completely independent of one another.

Early 20th Century

The second great advancement in biology in the early 20th century was the creation of a new discipline, ‘genetics’ which most scientists at that time thought will have no significant impact on microbiology. Continuous research in the following years revealed that bacteria during the course of their evolution change their genetic material to survive the ever-changing environment. It was the work by Avery, McLeod and McCathy on the process of transformation in bacteria disclosed that
genetic matter in living organisms is constituted by DNA (Deoxyribonucleic Acid). Microbiology, thus, set the stage for the second major revolution in biology—the advent of molecular biology—to which it made many contributions of fundamental importance.

Microorganisms in Food

The ecological role of microorganisms and their importance in all the geochemical cycles (C, N, P, S, and water cycles) in nature is well documented. Since the human food supply consists basically of plants and animals or products derived from them, it is understandable that our food supply can contain microorganisms. In most cases, microorganisms use our food supply 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-flavours by means of breakdown of a product or synthesis of new compounds they can 'spoil' food. This is a normal consequence of the action of microorganisms, since one of their functions in nature is to convert reduced forms of carbon, nitrogen, and sulphur in dead plants and animals to the oxidized forms required by plants, which in turn are consumed by animals. So by carrying out their reactions they frequently render our food supply unfit for consumption. To prevent this, we minimize the contact between microorganisms and our food and also eliminate them from our food, or at least adjust conditions of storage to prevent their growth. From the numerous studies conducted using microorganisms, we now know that when the microorganisms involved are pathogenic, their association with our food supply becomes critical from a public health perspective. This is because many of our foods support the growth of pathogenic microorganisms or at least serve as a vector for them. Interactions between microorganisms and our food are sometimes beneficial, as exemplified by the many cultured products consumed and enjoyed. In order to understand the governing factors in these interactions and to find out the reason for such interaction it is necessary that we develop a strong knowledge base related to food microbiology. As some foods support the growth of microorganisms more readily than others and some foods are very stable in regard to microbial deterioration, this brings our quest to know food as the substrate, and so the characteristics of a food are an important consideration. The type of microorganisms present and the environmental conditions also are important. However, the food or substrate dictates what can or cannot grow. By understanding the characteristics of the food or substrate, one can make predictions about the microbial flora that may develop. Knowledge of the factors that favour or inhibit the growth of microorganisms is essential to understand the principles of food spoilage and preservation. From numerous past studies we know that the chief compositional factors of a food that influence microbial activity are hydrogen ion concentration, moisture, oxidation–reduction potential, nutrients, and the presence of inhibitory substances or barriers.
Microbial Types in Foods

Following are the types of microorganisms present in food:

**Raw Foods:** In general, the numbers and types of microorganisms present in a finished food product are influenced by the general environment from which the food was originally obtained, the microbiological quality of the food in its raw or unprocessed state, the sanitary conditions under which the product is handled and processed, the adequacy of subsequent packaging, handling, and storage conditions in maintaining the flora at a low level.

In producing good-quality market foods, it is important to keep microorganisms at a low level for reasons of public health and product shelf life. Excessively high numbers of microorganisms in fresh foods present cause for alarm. It should be kept in mind that the inner parts of healthy plant and animal tissues are generally sterile and that it is possible to produce many foods free of microorganisms. The number of microorganisms in a fresh food product, then, may be taken to reflect the overall conditions of raw product quality, processing, handling, storage, and so on.

**Ground Meat Products:** Commercial ground meats generally consist of trimmings from various cuts. Ground meat provides a greater surface area, which itself accounts in part for the increased flora. In some commercial establishments, the meat grinders, cutting knives, and storage utensils are rarely cleaned as often and as thoroughly as is necessary to prevent the successive buildup of microbial numbers. One heavily contaminated piece of meat is sufficient to contaminate others, as well as the entire lot, as they pass through the grinder. Both *bacilli* and *clostridia* are found in meats of all types.

The addition of soy protein (soybean flour, soy flakes, textures soy protein) at levels of 10 to 30 per cent to ground meat patties is fairly widespread in the fast-
food industry. Why bacteria grow faster in the meat-soy blends than in non-soya controls is not clear. The soy itself does not alter the initial flora. It is possible that soy protein increases the surface area of soy-meat mixtures so that aerobic bacteria of the type that predominate on meats at refrigerator temperatures are favoured. When meat animals are slaughtered for human consumption, meat from the carcasses is removed by meat cutters. In a study of sausage made from hot-boned pork, significantly higher counts of mesophiles and lipolytics were found in the product made from hot-boned pork than in the cold-boned product. The meats discussed in this section are livers, kidneys, hearts, and tongues of bovine, porcine, and ovine origins. They differ from the skeletal muscle parts of the respective animals in having both higher pH and glycogen levels, especially in the case of liver. It is estimated that at least 80 per cent of fresh beef in the US leaves the packaging plant in vacuum packages, and because of the longer shelf life of this product, its microbiological quality is of great interest. Vacuum packaging is achieved by placing meats into plastic bags or pouches followed by the removal of air using a vacuum packaging machine and the closure of bag or pouch, often by a heat sealer. From the microbiological standpoint, the most dramatic effect of this process is the change in the gaseous environment of the product. While not all $O_2$ is removed before sealing, some of what remains is consumed by the aerobic flora and the meat itself, resulting in an increased level of $CO_2$ which is inhibitory to the flora. The relative quantities of $O_2$ and $CO_2$ in the headspace of stored vacuum packages are controlled largely by the degree to which the plastic package impedes the flow of these gases.

**Poultry Products:** Whole poultry tends to have a lower microbial count than cut-up poultry. Poultry meat represents an important food source of salmonellae to humans. Of the various cooked poultry products, precooked turkey rolls have been found to have considerably lower microbial numbers of all types. The microbial flora of fresh poultry consists largely of pseudomonads and other closely related gram-negative bacteria, as well as coryneforms, yeasts, and other organisms. The incidence of microorganisms in seafood such as shrimp, oysters, and clams depends greatly on the quality of water from which these animals are harvested. Assuming good-quality water, most of the organisms are picked up during processing. In the case of breaded raw shrimp, the breading process is expected to add organisms if not properly done or if the ingredients are of poor microbiological quality. Under proper handling and storage conditions, the predominant flora is gram positive. While yeasts, molds, and gram-negative bacteria may be found along with lactic acid bacteria, most or all of these types are more heat sensitive than gram positives and are more likely to be destroyed during pasteurization. Milk continues to serve as a vehicle for certain disease. Outbreaks generally involve the consumption of raw milk, certified raw milk, homemade ice cream, containing fresh eggs, and dried and pasteurized milks contaminated after heat processing (Refer Figure 1.2).
Fig. 1.2 Raw and Processed Poultry Products

Actinomyces and Related Organisms: Actinomyces constitute a diverse and large group of gram positive filamentous and/or branching bacilli. Since they are filamentous and may take few days to several weeks to grow, actinomyces are mistaken for fungi.

Some species of Actinomyces do not form an outer covering (endospores) during harsh environmental condition as done by most types of bacteria. Actinomyces are responsible for causing several diseases human beings. They also play a vital role in forming and controlling the nutrient content of the soil. They produce a number of enzymes that are directly responsible for the soil nutrient cycle. These enzymes help in degrading organic plant material such as lignin and chitin, thus enriching the soil. Thus, actinomyces have a major role in the production of compost. Many of the found Actinomyces varieties or species are the basic or opportunistic disease-causing microbes or pathogens of humans beings and most of the other mammals. In animals they attack the oral cavity of the infected organisms. In rarest of the rare instances these bacteria has tendency to cause actinomycosis which is a disease notified or characterized by the formation of abscesses in the region of the mouth, in and around the lungs part and in the gastrointestinal tract of the vectors. Some vital species are also responsible for the odour or the smell of top of the soil which is sometimes or often particularly noticeable or ascertained during or even after the rain occurs.
Rickettsiae are Gram-Negative Bacteria which are mostly coccoid shaped. Sometimes, rod-shaped variants of the bacteria are also found. Unlike the other types of bacteria, *rickettsiae* require a living host for growth and survival. They are called obligate intracellular parasites as they utilize the metabolic energy of the host. *Rickettsiae* usually infect all mammals. They tend to live and multiply in and around the vicinity of the gastrointestinal tract. Arthropods such as ticks, lice and fleas act a carrier. Because *rickettsia* cannot live in artificial nutrient content or in the laboratory environment, they are grown either inside the tissues or inside the embryo during cultures by using typically chicken embryos. Earlier, they were regarded as microorganisms positioned somewhere in between the viruses and true bacteria. The majority of rickettsiae are susceptible to antibiotics, especially the tetracycline group. They have also been found to be associated with a variety of different types of plant diseases (Refer Figure 1.4).

**Fig. 1.4 A Carrier of Rickettsia**

**Fungi in Food:** The hyphae in most of the usually occurring varieties of fungi contain chitin, which is nitrogenous substance. The composition of the fungi depends on its stage of growth and the environmental conditions prevailing in the growth medium. The upper tips of the fungi which are called are filled with a gelatinous substance called cytoplasm; the rest is made up of central vacuole. The hyphae usually spread out and grow over their food material and form a visible mesh.

Fungi play an important role in food industry. They help in processing foods because being heterotrophs, (organisms that get their nutrition from other organisms) they feed by absorption, that is, they secrete powerful enzymes into their environment that break down complex organic molecules into smaller molecules, which they then absorb. Thus, by permitting the growth of the right fungi, it is possible to change the constituents of food and thereby its flavour, taste and texture. The following are few examples (Refer Figures 1.5).
• Many types of cheese are ripened through the action of fungi. The characteristic flavour and texture of blue cheese is attributed to the fungus Penicillium roquefortii. Blue cheese is prepared by inoculating curd with spores of the fungus. The germinating spores are allowed to grow for some time in the curd until the cheese develops the required flavour.

• Chocolate industry makes use of the fungi Candida krusei and a species of Geotrichum. These fungi help in fermenting the cacao beans, thereby reducing their bitterness and impart the characteristic chocolate flavour.

• Because of their high-protein content, fungi are used in the preparation of meat substituent foods.

• Yeast is another fungus important in food industry. It is extensively used in bakeries and confectionery industry.

• In many parts of the world, mushrooms form an important part of diet. Mushrooms, due to their high-protein content, are consumed as an alternative source of protein by vegetarians. Because of their high nitrogen-content, mushrooms spoil easily when they come in contact with atmospheric oxygen, producing obnoxious odour.

![Fig. 1.5 Some Types of Edible Fungi](image)

**Check Your Progress**

1. Who is considered as the forefather of medical microbiology?
2. Where are microbiological reactions used?
3. How is gene cloning developed?
4. What did Pasteur discovered?
5. What are Rickettsiae? What is their shape and what are the organisms that are effected by Rickettsiae?
1.3 RELEVANCE OF MICROBIOLOGY

Food microbiology covers various aspects of microorganisms that are important for food safety and food spoilage. The major areas covered by this discipline are as follows:

- Mechanisms of molecules concerned with the presence, persistence and transmission of food-borne pathogens in the entire food chain.
- The ability of microorganisms to colonize and infection on consumers.
- Systems of food models.
- Studies on population investigating the diversity of food-borne pathogens.
- Diagnostic methods.
- Mycotoxins.

Food microbiology is a sub-discipline of microbiology concerned with the in-depth study of fungi, viruses and bacteria that either grow in or are transmitted by foods. Though bacteria are frequently related with food poisoning and spoilage, there are some species that help in preserving food through fermentation. Food microbiology is a vast field that includes not only microbiology, but also biochemistry, engineering, statistics and mathematical modeling.

The fields of food microbiology and food safety are considered as dynamic fields of microbiology and food science, respectively. These fields have gained more importance in the last 30 years, mainly due to recurrence of food-borne diseases, and changes in food habits and practices of consumers, health status, techniques of food preservation, responses of microorganisms to these techniques, food production chain systems, etc.

Most of the food diseases are attributed to the following listed groups of pathogenic bacteria:

- *Salmonella* and *Shigella*
- *Clostridium perfringens*
- *Bacillus cereus*
- *Staphylococcus aureus*
- *Clostridium Botulinum*
- *Yersinia enterocolitica*
- *Escherichia coli*
- *Listeria monocytogenes*
- *Campylobacter jejuni*
Food and Related Microorganism

Food microbiology is concerned with studying the microbiological, chemical and physical hazards caused by these bacteria in foods and beverages, and thereby systematizing the process of food production, so as to ensure public health. It is a very interesting, challenging as well as exciting field that is concerned with the study of food-borne microorganisms.

**Bacteria, Yeasts and Molds:** Bacteria, yeasts and molds are small forms of living beings that cannot be seen by the naked eye. Just like human beings, they eat, grow, reproduce and die. They are found everywhere, on mountains, in oceans, on the bodies of animals, human beings and even in the air. They can be harmful as well as useful. They help in our bodily processes by breaking down complex food into simpler substances.

Bacteria, yeasts and molds all of them have one thing in common, that is, they all have only one cell. We can compare their body with a factory, which has only one machine, but is capable of carrying out major functions like converting complex foods into simpler substances. The single cell is capable of carrying out all the functions that are necessary for its survival.

The cell is divided into the nucleus, cytoplasm and the cell wall. The nucleus is the control centre of the cell and its main functions are to direct division of the cell and formation of new cells. The function of the cytoplasm is to convert food material into energy. The cell membrane or the wall holds everything together and controls the movement of all the material to and fro from the cell.

Let us now discuss bacteria, yeasts and molds one by one.

**Bacteria:** The largest group of microorganisms is made up of bacteria. There is a misconception that all bacteria harm us, however, the reality is that only a very small number of them are actually pathogenic in nature.

Regardless of minor differences, most bacteria are classified on the basis of five basic cell shapes. They are also classified on the basis of their arrangement, as follows:

- **Diplococci:** Cocci that are grouped in pairs; known to cause pneumonia.
- **Streptococci:** Cocci that are arranged in chains; known to cause ‘strep throat’.
- **Staphylococci:** Cocci that are arranged in bunches; known to cause ‘staph infections’.

The structure of a bacterium’s cell is shown in Figure 1.6.
Bacteria vary in sizes, but on an average, their size is known to be about 1/25,000 of an inch. Bacteria are also classified on the basis of their requirements of oxygen. Those that grow only in the presence of oxygen are known as aerobes, those that grow only in the absence of oxygen are known as anaerobes, and those which can grow either with or without oxygen are known as facultative anaerobes. Anaerobes mainly grow in places with no air, such as in deep water or soil, or in canned or vacuum packed foods that have not been carefully processed or handled.

Microorganisms, like any other living being, need food to grow and reproduce. Their food should be such that it easily passes through the cell membrane, such that it can be processed and energy is released. Since some foods are quite complex to pass through the cell membrane, they must be broken down into simpler substances, and this task is accomplished with the help of some enzymes produced within the bacterial cells.

**Yeasts**: As shown in Figure 1.7, yeasts are small single-celled plants that belong to the family of fungi. The basic difference between fungi and other plants is that they lack chlorophyll. Yeasts mainly survive on starches and sugars. With the help of these starches and sugars, they produce carbon dioxide and alcohol.

Yeasts can be harmful as well as useful to human beings. They are used to raise bread dough and fermented wines, beer, brandy, whisky. They also play a significant role in the production of vinegar.

Yeasts are quite commonly found on plants, leaves, flowers, salt water and soil. Sometimes they are also found on the surface of skin and in the intestinal tracts of warm blooded animals. Here, they live either symbiotically or as parasites.
Moulds: As shown in Figure 1.8, molds belong to the family of fungi. They are found widely and are distributed everywhere in the nature. They grow in conditions where air and moisture are available. All of us have seen the growth of mold on damp clothes, old shoes, stale bread. This growth, which is visible by the naked eye, is actually a colony of millions of mold cells growing together. However, they may vary in their appearance. While some of them may appear fluffy, others may look glossy, moist or slimy.

Primary Sources of Food Related Microorganisms

Soil, Water and Air: The species of bacteria that are found in soil and water include Acinetobacter, Alcaligenes, Bacillus, Citrobacter, Clostridium, Corynebacterium, Enterobacter, Micrococcus, Pseudomonas. The species of molds commonly found in soil and air include Aspergillus, Rhizopus, Penicillium, Trichothecium, Botrytis and Fusarium. A large number of yeasts are also found in soil and air. Their number is more in soil and air as compared to water. The genera of bacteria Bacillus and Micrococcus, the yeast genus of Torulopsis, and many genera of mold are also found in air and dust.

Plants and Their Related Products: The genera of plant-borne bacteria include Acetobacter, Flavobacterium, Lactobacillus, Leuconostoc, Listeria,
Food and Related Microorganism

Pediococcus and Streptococcus. The spoilage of vegetables and fruits is caused by molds like Botrytis cinerea, Geotrichum candidum, R. stolonifer, Phytophora sp, etc, and the yeasts that are associated with the species of plants include Torula, Rhodotorula, Saccharomyces, etc.

Animals and Human Beings: A variety of microorganisms are found in the intestinal tract of human beings and animals. The main bacteria that are found include Bacteroides, Escherichia, Lactobacillus, Proteus, Salmonella, Shigella, Staphylococcus, Streptococcus, Clostridium, Citrobacter, Enterobacter and Pseudomonas. The yeast Candida is also found quite commonly in the intestinal tract of the human beings. Some genera of bacteria are specifically associated with the nasal cavity, and hands and mouth of man. Thus, a food handler can easily contaminate food in a food processing plant through Micrococcus and Staphylococcus. Hides, feeds and fecal matter of animals can also contaminate food.

Food Handling Equipment: If equipment and utensils used for handling food are not properly sterilized before use, they can also lead to contamination.

Check Your Progress
6. What does food microbiology covers?
7. What is the biggest similarity between bacteria, yeasts and moulds?
8. Where does anaerobes mainly grow?
9. What is the basic difference between fungi and other plants?
10. Where are moulds found and mostly grown?

1.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Robert Koch was the forefather of medical microbiology.
2. Microbiological reactions are used in sewage treatment, in transforming the chemical structure of drugs, in cleaning clothes—bacterial enzymes are used in biological detergents—and even to extract metals such as copper and uranium from their mineral ores.
3. New technologies such as gene cloning have been developed within the last few decades using which microbes may be used for the large-scale synthesis of valuable pharmaceutical products including human insulin, hormones, antiviral drugs and vaccines.
4. Pasteur discovered that bacteria were sensitive to temperature since chicken did not acquire anthrax at their normal body temperature of 42 °C, but did so when their temperature was lowered to 37°C.
5. *Rickettsiae* are Gram-Negative Bacteria which are mostly coccoid shaped. Sometimes, rod-shaped variants of the bacteria are also found. Unlike the other types of bacteria, *rickettsiae* require a living host for growth and survival. *Rickettsiae* usually infect all mammals. They tend to live and multiply in and around the vicinity of the gastrointestinal tract. Arthropods such as ticks, lice and fleas act as a carrier.

6. Food microbiology covers various aspects of microorganisms that are important for food safety and food spoilage.

7. Bacteria, yeasts and moulds all of them have one thing in common, that is, they all have only one cell.

8. Anaerobes mainly grow in places with no air, such as in deep water or soil, or in canned or vacuum packed foods that have not been carefully processed or handled.

9. The basic difference between fungi and other plants is that they lack chlorophyll.

10. Moulds are found widely and are distributed everywhere in the nature. They grow in conditions where air and moisture are available.

### 1.5 SUMMARY

- In spite of their small size, microorganisms are of immense importance to man. They cause diseases, are a source of various foods and medicines, and dispose of our wastes.
- Human beings have been making use of microorganisms, or their biochemical activities, since long before he even knew of their existence.
- The ancient Babylonians and Sumerians were brewing beer much as we do today and the Egyptians were baking leavened bread 2000 years earlier.
- Despite the antiquity of these microbiological practices, the first documentations of the structure of microorganisms did not occur until the advent of the first microscopes in the 17th century.
- It was not until the 19th century that Louis Pasteur showed that microbes were not produced from mud or decaying organic matter. So Pasteur was considered to be the founder of industrial microbiology.
- Robert Koch was the forefather of medical microbiology. Koch, a German doctor could show that the causal agent of bovine anthrax was a bacterium, *Bacillus anthracis*. His remarkable work remains a landmark in microbiology about the nature of disease, and in proving the pathogenic activity of a disease-causing agent.
- Microbiological reactions are used in sewage treatment, in transforming the chemical structure of drugs, in cleaning clothes—bacterial enzymes are used...
in biological detergents—and even to extract metals such as copper and uranium from their mineral ores.

- New technologies such as gene cloning have been developed within the last few decades using which microbes may be used for the large-scale synthesis of valuable pharmaceutical products including human insulin, hormones, antiviral drugs and vaccines.

- By the 18th century some scientists had begun to realize that there was a causal relationship between the development of microorganisms in organic infusions and the chemical changes that took place in these infusions; microbes were the agents that brought about the chemical changes.

- The German doctor, Robert Koch is credited for his following main contributions to the development of microbiology. He stated that bacteria could be isolated and shown to cause disease. Based on his studies on anthrax, a disease infecting animals, he postulated his Germ Theory.

- Pasteur also discovered that bacteria were sensitive to temperature since chicken did not acquire anthrax at their normal body temperature of 42 °C, but did so when their temperature was lowered to 37°C.

- Pasteur gave them a second inoculation with fresh and particularly virulent bacilli. Still the animals lived. Pasteur then discovered that the first injection was with a culture of bacilli that had been grown above normal temperature for several days.

- The second great advancement in biology in the early 20th century was the creation of a new discipline, ‘genetics’ which most scientists at that time thought will have no significant impact on microbiology.

- In general, the numbers and types of microorganisms present in a finished food product are influenced by the general environment from which the food was originally obtained, the microbiological quality of the food in its raw or unprocessed state, the sanitary conditions under which the product is handled and processed, the adequacy of subsequent packaging, handling, and storage conditions in maintaining the flora at a low level.

- The addition of soy protein (soybean flour, soy flakes, textures soy protein) at levels of 10 to 30 per cent to ground meat patties is fairly widespread in the fast-food industry. Why bacteria grow faster in the meat-soy blends than in non-soya controls is not clear.

- Whole poultry tends to have a lower microbial count than cut-up poultry. Poultry meat represents an important food source of salmonellae to humans.

- The microbial flora of fresh poultry consists largely of pseudomonads and other closely related Gram-Negative Bacteria, as well as coryneforms, yeasts, and other organisms.
• The incidence of microorganisms in seafood such as shrimp, oysters, and clams depends greatly on the quality of water from which these animals are harvested.

• While yeasts, moulds, and gram-negative bacteria may be found along with lactic acid bacteria, most or all of these types are more heat sensitive than gram positives and are more likely to be destroyed during pasteurization.

• Actinomycetes constitute a diverse and large group of gram positive filamentous and/or branching bacilli. Since they are filamentous and may take few days to several weeks to grow, actinomycetes are mistaken for fungi.

• Rickettsiae are Gram-Negative Bacteria which are mostly coccoid shaped. Sometimes, rod-shaped variants of the bacteria are also found. Unlike the other types of bacteria, rickettsiae require a living host for growth and survival.

• Rickettsiae usually infect all mammals. They tend to live and multiply in and around the vicinity of the gastrointestinal tract. Arthropods such as ticks, lice and fleas act a carrier.

• The hyphae in most of the usually occurring varieties of fungi contain chitin, which is nitrogenous substance.

• Fungi play an important role in food industry. They help in processing foods because being heterotrophs, (organisms that get their nutrition from other organisms) they feed by absorption, that is, they secrete powerful enzymes into their environment that break down complex organic molecules into smaller molecules, which they then absorb.

• Food microbiology covers various aspects of microorganisms that are important for food safety and food spoilage.

• Food microbiology is a sub-discipline of microbiology concerned with the in-depth study of fungi, viruses and bacteria that either grow in or are transmitted by foods.

• Food microbiology is concerned with studying the microbiological, chemical and physical hazards caused by these bacteria in foods and beverages, and thereby systematizing the process of food production, so as to ensure public health.

• Bacteria, yeasts and moulds are small forms of living beings that cannot be seen by the naked eye. Just like human beings, they eat, grow, reproduce and die.

• Bacteria, yeasts and moulds all of them have one thing in common, that is, they all have only one cell.

• Anaerobes mainly grow in places with no air, such as in deep water or soil, or in canned or vacuum packed foods that have not been carefully processed or handled.
The basic difference between fungi and other plants is that they lack chlorophyll. Yeasts mainly survive on starches and sugars.

Yeasts can be harmful as well as useful to human beings. They are used to raise bread dough and fermented wines, beer, brandy, whisky. They also play a significant role in the production of vinegar.

Yeasts are quite commonly found on plants, leaves, flowers, salt water and soil. Sometimes they are also found on the surface of skin and in the intestinal tracts of warm blooded animals.

Moulds are found widely and are distributed everywhere in the nature. They grow in conditions where air and moisture are available.

1.6 KEY WORDS

- **Aerobic bacteria**: Bacteria requiring oxygen for growth.
- **Anaerobic bacteria**: Bacteria which can survive in the absence of oxygen.
- **Mould**: Mould is a type of fungi.
- **Yeasts**: Those fungi which are generally not filamentous but unicellular and ovoid or spheroid and which reproduce by budding or fission.

1.7 SELF ASSESSMENT QUESTIONS AND EXERCISES

**Short Answer Questions**

1. What is the role of microorganism in food?
2. How is fungi helpful in food industry?
3. What are the various classifications of bacteria?
4. List the general characteristics of moulds.
5. What are the various classifications of bacteria?

**Long Answer Questions**

1. Trace the history of evolution of microbiology. Highlight the contributions of Pasteur and Koch in the development of microbiology.
2. Explain the characteristics of yeasts and yeast-like fungi. Add a note on their industrial uses.
3. Write a note on relevance of microbiology.
4. Define ‘bacteria’. How are they classified? Give details of the bacteria important in food bacteriology.
5. What are moulds? State some industrial uses of moulds.
6. Write a note on primary sources of food related microorganisms.

1.8 FURTHER READINGS

UNIT 2  INTRINSIC FACTORS

2.0  INTRODUCTION

Intrinsic Factor (IF), also known as Gastric Intrinsic Factor (GIF), is a glycoprotein produced by the parietal cells of the stomach. It is necessary for the absorption of vitamin B12 later on in the ileum of the small intestine. In humans, the gastric intrinsic factor protein is encoded by the GIF gene. Haptocorrin is another glycoprotein secreted by the salivary glands which binds to vitamin B12. Vitamin B12 is acid sensitive and in binding to Transcobalamin I it can safely pass through the acidic stomach to the duodenum. In the less acidic environment of the small intestine, pancreatic enzymes digest the glycoprotein carrier and vitamin B12 can then bind to intrinsic factor. This new complex is then absorbed by the epithelial cells of the ileum. Inside the cells, B12 dissociates once again and binds to another protein, Transcobalamin II; the new complex can then exit the epithelial cells to be carried to the liver.

The intrinsic factor is secreted by the stomach, and so is present in the gastric juice as well as in the gastric mucous membrane. The optimum pH for its action is approximately 7. Its concentration does not correlate with the amount of HCl or pepsin in the gastric juice, for example intrinsic factor may be present even when pepsin is largely absent. The site of formation of the intrinsic factor varies in different species. In pigs it is obtained from the pylorus and beginning of the duodenum; in human beings it is present in the fundus and body of the stomach. The limited amount of normal human gastric intrinsic factor limits normal efficient absorption of B12 to about 2 µg per meal, a nominally adequate intake of B12.

In this unit, you will study about various intrinsic factors like buffering capacity, redox-potential, antimicrobial barriers and constituents, nutrient content and pH in detail.
2.1 OBJECTIVES

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

- Understand what intrinsic factors are
- Discuss about various intrinsic factors

2.2 INTRINSIC FACTORS

Intrinsic Factor (IF), also known as Gastric Intrinsic Factor (GIF), is a glycoprotein produced by the parietal cells of the stomach. It is necessary for the absorption of vitamin B12 later on in the ileum of the small intestine. In humans, the gastric intrinsic factor protein is encoded by the GIF gene.

The intrinsic or food-related factors include pH, moisture content, water activity or availability, oxidation-reduction potential, physical structure of the food, available nutrients, and the possible presence of natural antimicrobial agents. We will discuss following intrinsic parameters in detail:

- Buffering Capacity
- Redox-Potential
- Antimicrobial Barriers and Constituents
- Nutrient content
- pH

Buffering Capacity

Buffer capacity is a measure of the efficiency of a buffer in resisting changes in pH. Conventionally, the buffer capacity ($\beta$) is expressed as the amount of strong acid or base, in gram-equivalents, that must be added to 1 liter of the solution to change its pH by one unit.

The pH of a food is critical because food with low pH favors the growth of yeasts and molds. In neutral or alkaline pH foods, such as meats, bacteria are more dominant in spoilage and putrefaction. Most microorganisms grow best at pH values around 7.0 (6.6–7.5), whereas few grow below 4.0 (Refer Table 2.1). Adverse pH affects the functioning of enzymes and the transport of nutrients into the cell.

<table>
<thead>
<tr>
<th>Food Items</th>
<th>pH</th>
<th>Microorganism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruits</td>
<td>≤ 3.5</td>
<td>Mold and Yeast Spoilage</td>
</tr>
<tr>
<td>Meats and sea foods</td>
<td>≥ 5.6 and above</td>
<td>Bacteria as well as Mold and Yeast Spoilage</td>
</tr>
<tr>
<td>Vegetables</td>
<td>Lower pH Values than Fruits</td>
<td>Subjected More to Bacterial than Fungal Spoilage</td>
</tr>
</tbody>
</table>
Some foods are better able to resist changes in pH than others. Those that tend to resist changes in pH are said to be buffered. In general, meats are more highly buffered than vegetables. Buffering capacity of meats is contributed mainly by various proteins present in it. Vegetables are generally low in proteins and, consequently, lack the buffering capacity to resist changes in their pH during the growth of microorganisms.

**Redox-Potential**

The oxidation-reduction potential of a food also influences spoilage. Microorganisms display varying degrees of sensitivity to the Oxidation–Reduction Potential (O/R, Eh) of their growth medium. The O/R potential of a substrate may be defined generally as the ease with which the substrate loses (oxidized) or gains electrons (reduced). The more highly oxidized a substance, the more positive will be its electrical potential and more it favours growth of aerobic microorganism. For example, aerobic bacteria such as some members of the genus *Bacillus*, most molds and yeasts (Refer Table 2.2). The more highly reduced a substance, the more negative will be its electrical potential, and more it favours growth of anaerobic microorganisms. For example, when meat products, especially broths are cooked, they often have lower Oxidation-Reduction Potentials, i.e., they present a reducing environment for growth. These products with their readily available amino acids, peptides, and growth factors are ideal media for the growth of anaerobes, including *Clostridium*. Some aerobic bacteria actually grow better under slightly reduced conditions, and these organisms are referred to as microaerophiles such as lactobacilli and campylobacters. Some bacteria have the capacity to grow under either aerobic or anaerobic conditions. Such types are referred to as facultative anaerobes. Some molds and yeasts encountered in and on foods are aerobic, although a few tend to be facultative anaerobes.

<table>
<thead>
<tr>
<th>Table 2.2 Eh Requirements of Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Food Items</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Plant Juices</td>
</tr>
<tr>
<td>Meats Broths</td>
</tr>
<tr>
<td>Cheeses</td>
</tr>
</tbody>
</table>

The O/R Potential of a Food is determined by the Following:

- Aerobes can lower the Eh of their environment while anaerobes cannot. As aerobes grow, O$_2$ in the medium is depleted, resulting in the lowering of Eh. The result is that the medium becomes poorer in oxidizing and richer in reducing substances.
- The Eh of a medium can be reduced by microorganisms by their production of certain metabolic byproducts such as H$_2$S, which has the capacity to...
Intrinsic Factors

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lower Eh to ~300 mV. Because \( \text{H}_2\text{S} \) reacts readily with \( \text{O}_2 \), it will accumulate only in anaerobic environments.

- Among the substances in foods that help to maintain reducing conditions are –SH groups in meats and ascorbic acid and reducing sugars in fruits and vegetables.

**Antimicrobial Barriers and Constituents**

Many foods contain natural antimicrobial substances, which make them stable against attack by certain microorganisms. Some plant species are known to contain essential oils that possess antimicrobial activity. Coumarins found in fruits and vegetables exhibit antimicrobial activity. Cow’s milk and eggs also contain antimicrobial substances. Eggs are rich in the enzyme lysozyme that can lyse the cell walls of contaminating Gram-Positive Bacteria. Herbs and spices often possess significant antimicrobial substances; generally fungi are more sensitive than most bacteria. Sage and rosemary are two of the most antimicrobial spices. Aldehydic and phenolic compounds that inhibit microbial growth are found in cinnamon, mustard, and oregano. Table 2.3 summarised antimicrobial constituents of some of the food.

**Table 2.3** Antimicrobial Constituents of Some of the Food

<table>
<thead>
<tr>
<th>Food Items</th>
<th>Antimicrobial Substances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cloves</td>
<td>Eugenol</td>
</tr>
<tr>
<td>Garlic</td>
<td>Allyl isothiocyanate</td>
</tr>
<tr>
<td>Thyme</td>
<td>Rosmarinic Acid</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>Cinnamic Aldehyde and Eugenol</td>
</tr>
<tr>
<td>Mustard</td>
<td>Allicin</td>
</tr>
<tr>
<td>Sage</td>
<td>Eugenol and Thymol</td>
</tr>
<tr>
<td>Oregano</td>
<td>Carvacrol (Isothymol) and Thymol</td>
</tr>
<tr>
<td>Cow’s Milk</td>
<td>Lactoferrin, Conglutinin, and the Lactoperoxidase System</td>
</tr>
<tr>
<td>Raw Milk</td>
<td>Rotavirus Inhibitor</td>
</tr>
<tr>
<td>Eggs</td>
<td>Enzyme Lysozyme that can Lyse the Cell Walls of Contaminating Gram-Positive Bacteria</td>
</tr>
<tr>
<td>Fruits, Vegetables, Tea, Molasses, and other Plant Sources</td>
<td>The Hydroxycinnamic Acid Derivatives (P-coumaric, Ferulic, Caffeic, and Chlorogenic Acids)</td>
</tr>
<tr>
<td>Raw Egg White</td>
<td>Ovotransferrin that Inhibits <em>Salmonella enteritidis</em></td>
</tr>
</tbody>
</table>

However, spices also can sometimes contain pathogenic and spoilage organisms. Enteric bacteria *B. cereus*, *Clostridium perfringens*, and *Salmonella* species have been detected in spices. Microorganisms can be eliminated or reduced by ethylene oxide sterilization. This treatment can result in Salmonella-free spices and herbs and a 90% reduction in the levels of general spoilage organisms. Green and black teas also have well-documented antimicrobial properties because of their polyphenol contents which are active against bacteria, viruses, and fungi and may have anticancer properties.
Nutrient Content

The types and proportions of nutrients present in the food are all important in determining what organisms are most likely to grow. Consideration must be given to: (i) Foods for energy (ii) Goods for growth (iii) Accessory food substances, or vitamins, which may be necessary for energy or growth.

- **Carbohydrates**: Foodstuffs contain two energy-rich components, namely carbohydrates and fats. Microorganisms depend on these for their energy requirements. They break down the sugar present in the food into simpler sugars for their consumption. Very few microorganisms are able to utilize complex carbohydrates such as starch and cellulose as sources of energy by first degrading these compounds into simple sugars. Fats are also used by microorganisms as source of energy, but these compounds are attacked by a relatively small number of microbes in foods. Carbohydrates, especially the sugars, are most commonly used, but other carbon compounds may also serve as carbon source, for example esters, alcohols, peptides, amino acids, and organic acids and their salts. Complex carbohydrates, for example cellulose, can be utilized by comparatively few organisms, and starch can be hydrolysed by only a limited number of organisms. 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. Maltose is not attacked by some yeasts. Bacteria often are identified and classified on the basis of their ability or inability to utilize various sugars and alcohols. Most organisms, if they utilize sugars at all can use glucose.

- **Fats**: A limited number of types 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 hydrolysed with the aid of lipase to glycerol and fatty acids, which then can serve as an energy source for the hydrolyzing organism or other organisms. In general, aerobic microorganisms are more commonly involved in the decomposition of fats than are anaerobic ones, and the lipolytic organisms usually are also proteolytic. Direct oxidation of fats containing unsaturated fatty acids usually is chemical.

- **Proteins**: Hydrolysis products of proteins, i.e., peptides and amino acids, serve as energy source for many proteolytic organisms when a better energy source is lacking and as foods for energy for other organisms that are not proteolytic. Meats, for example, may be low in carbohydrate and therefore decomposed by proteolytic species, for example Pseudomonas species, with successive growth of weakly proteolytic or non-proteolytic species that can utilize the products of protein hydrolysis. Organisms differ in their ability to use individual amino acids for energy. Microorganisms differ in their ability to use various nitrogenous compounds as the source of nitrogen for growth. Many organisms are unable to hydrolyse proteins and, hence,
Intrinsic Factors

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cannot get nitrogen from them without the help from a proteolytic organism. One protein may be a better source of nitrogenous food than another because of different products formed during hydrolysis, especially peptides and amino acids. Peptides, amino acids, urea, ammonia, and other simpler nitrogenous compounds may be available to some organisms but not to others or may be usable under some environmental conditions but not under others. Some of the lactic acid bacteria grow best with polypeptides as nitrogen foods, cannot attack casein, and do not grow well with only a limited number of types of amino acids present. The presence of fermentable carbohydrates in a substrate usually results in acid fermentation and suppression of proteolytic bacteria and, hence, in what is called a ‘sparing’ action on the nitrogen compounds. Also, the production of obnoxious nitrogenous products is prevented or inhibited. Many types of moulds are proteolytic, but comparatively few genera and species of bacteria and very few yeasts are actively proteolytic.

In general, proteolytic bacteria grow best at pH values near neutral and are inhibited by acidity, although there are exceptions, such as proteolysis by the acid-proteolytic bacteria that hydrolyse protein while producing acid. The basic source of nitrogen for the microorganisms is the protein. A large number of various microorganisms breaks down and thrive on the amino acids present in the protein rich foods stuffs. Some microbes, for example, are able to utilize nucleotides and free amino acids, while others are able to utilize peptides and proteins.

- Minerals: Carbon for growth may come partly from carbon dioxide, but more often it comes from organic compounds. The minerals required by microorganisms are nearly always present at the low levels required, but occasionally an essential mineral may be tied up so that it is unavailable, lacking, or present in insufficient amounts. An example is milk, which contains insufficient iron for pigmentation of the spores of Penicillium roquefort. Bacteria causing septicemia usually have the ability to bind some of the iron in blood. Only strains which can compete for transferring iron are able to grow well in human blood.

- Accessory Food Substances or Vitamins: Some microorganisms are unable to manufacture some or all of the vitamins needed and must have them furnished. Most natural plant and animal food-stuffs contain an array of these vitamins, but some may be low in amount or lacking. Thus, meats are high in B vitamins and fruits are low, but fruits are high in ascorbic acid. Egg white contains biotin but also contains avidin, which ties up biotin, making it unavailable to microorganisms and eliminating as possible those spoilage organisms which must have biotin supplied. The processing of foods often reduces the vitamin content. Thiamine, pantothenic acid, the folic acid group, and ascorbic acid (in air) are heat labile, and drying causes the loss of 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 the accessory growth factors.

**Hydrogen Ion Concentration (pH)**

Hydrogen ion concentration is defined as the number of molecules of hydrogen ions (H⁺) present in a litre of the solution. If the pH is 7, then it means that the solution is neutral. At pH values above 7, the solution would be basic while at values less than 7 it behaves as acid.

- **Effect of pH on Microbes:** Every microorganism has a minimal, a maximal, and an optimal pH for growth. Microbial cells are significantly affected by the pH of food because they apparently have no mechanism for adjusting their internal pH. In general, yeasts and moulds are more acid tolerant than bacteria. The inherent pH of foods varies, although most foods are neutral or acidic. Foods with low pH values (acidic) below 4.5 usually are not readily spoiled by bacteria and are more susceptible to spoilage by yeasts and moulds. A food with an inherently low pH would therefore tend to be more stable microbiologically than a neutral food. The excellent keeping quality of the following foods is related to their restrictive pH: fruits, soft drinks, fermented milks, sauerkraut and pickles. Some foods have a low pH because of inherent acidity; others, for example the fermented products, have a low pH because of developed acidity from the accumulation of lactic acid. It has been well established that most microorganisms grow best at pH values around 7.0, while few grow below 4.0.

- **Effect of pH on Bacteria:** Bacteria tend to be more fastidious in their relationships to pH than moulds and yeasts, with the pathogenic bacteria being the most fastidious. The range of pH not only depends on the substrate on which microorganisms thrive, but also its acidic property; for example, acids could be of different varieties such as hydrochloric acid, lactic acid, phosphoric or tartaric acid. The action of microorganisms in relation to all these acids would be different in different circumstances and changes accordingly. So there could not be any fixed mechanism to denote the exact pH for the optimal growth of microorganisms. Certain bacteria produce some substances during their mode of action such as lactic acid by lactobacillus which reduces the pH from 7 to 5. Most bacteria favour a pH near neutrality, although some, such as the acid formers, favour moderate acidity, and others, for example the actively proteolytic bacteria, can grow in media with a high (alkaline) pH as found in the white of a stored egg.

- **Effect of pH on Moulds:** Moulds are a type of fungi which can grow over a wide range of pH values than can most yeasts and bacteria. Many types of moulds grow at acidity too strong for yeasts and bacteria.

- **Effect of pH on Yeast and Fungi:** In general, yeast and fungi are more acid tolerant than bacteria. It has been well established that most microorganisms grow best at pH values around 7.0, whereas few grow
below pH 4.0. Most types of meats have a final pH of about 5.6 and above; this makes these products susceptible to bacteria as well as to mould and yeast spoilage. Most fermentative yeasts are favoured by a pH of about 4.0 to 4.5, as in fruit juices, and film yeasts grow well on acid foods such as sauerkraut and pickles. On the other hand, most yeasts do not grow well in alkaline substrates and must be adapted to such media.

- **Role of Buffers in Food**: The buffers in a food, i.e., the compounds that resist changes in pH are important not only for their buffering capacity, but also for their ability to be 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. Vegetable juices have low buffering power, permitting an appreciable decrease in pH with the production of only small amounts of acid by the lactic acid bacteria during the early part of sauerkraut and pickle fermentations. This enables the lactic acid bacteria to suppress the activity of undesirable pectin-hydrolysing and proteolytic organisms. Milk, on the other hand, is fairly high in protein (a good buffer) and therefore permits considerable growth and acid production by lactic acid bacteria in the manufacture of fermented milks before growth of the starter culture (the culture which actually performs the fermentation) is finally suppressed.

- **pH and Food Preservation**: The pH of a product can be readily determined with a pH meter, but this value alone may not be sufficient for predicting microbial responses. 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. The inhibitory properties of many of the organic acids, including acetic, benzoic, citric, lactic, propionic, and sorbic acids make them widely used as acidulants or preservatives in foods. Also, changes in acidity are not always evident from pH measurements. Not only are the rates of growth of microorganisms affected by pH, so are the rates of survival during storage, healing, drying, and other forms of processing. Also, the initial pH may be suitable, but because of competitive flora or growth of the organism itself, the pH may become unfavourable. Conversely, the initial pH may be restrictive, but the growth of a limited number of micro-organisms may alter the pH to a range that is more favourable for the growth of many other microorganisms.

### Check Your Progress

1. Define intrinsic factors.
2. What are the various intrinsic factors?
3. How is Oxidation–Reduction Potential of a food determined?
4. What are nutrient content?
5. Write in brief about fats.
2.3 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Intrinsic Factor (IF), also known as Gastric Intrinsic Factor (GIF), is a glycoprotein produced by the parietal cells of the stomach. It is necessary for the absorption of vitamin B12 later on in the ileum of the small intestine. In humans, the gastric intrinsic factor protein is encoded by the GIF gene.

2. We will discuss following intrinsic parameters in detail:
   - Buffering Capacity
   - Redox-Potential
   - Antimicrobial Barriers and Constituents
   - Nutrient content
   - pH

3. The Oxidation–Reduction Potential (O/R Potential) of a food is determined by the following:
   - Aerobes can lower the Eh of their environment while anaerobes cannot. As aerobes grow, O₂ in the medium is depleted, resulting in the lowering of Eh. The result is that the medium becomes poorer in oxidizing and richer in reducing substances.
   - The Eh of a medium can be reduced by microorganisms by their production of certain metabolic byproducts such as H₂S, which has the capacity to lower Eh to ~300 mV. Because H₂S reacts readily with O₂, it will accumulate only in anaerobic environments.
   - Among the substances in foods that help to maintain reducing conditions are –SH groups in meats and ascorbic acid and reducing sugars in fruits and vegetables.

4. The types and proportions of nutrients present in the food are all important in determining what organisms are most likely to grow. Consideration must be given to (i) foods for energy, (ii) foods for growth, and (iii) accessory food substances, or vitamins, which may be necessary for energy or growth.

5. A limited number of types 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 hydrolysed with the aid of lipase to glycerol and fatty acids, which then can serve as an energy source for the hydrolyzing organism or other organisms. In general, aerobic micro-organisms are more commonly involved in the decomposition of fats than are anaerobic ones, and the lipolytic organisms usually are also proteolytic. Direct oxidation of fats containing unsaturated fatty acids usually is chemical.
2.4 SUMMARY

- **Intrinsic Factor (IF),** also known as Gastric Intrinsic Factor (GIF), is a glycoprotein produced by the parietal cells of the stomach.
- It is necessary for the absorption of vitamin B12 later on in the ileum of the small intestine. In humans, the gastric intrinsic factor protein is encoded by the GIF gene.
- The intrinsic or food-related factors include pH, moisture content, water activity or availability, oxidation-reduction potential, physical structure of the food, available nutrients, and the possible presence of natural antimicrobial agents.
- Buffer capacity is a measure of the efficiency of a buffer in resisting changes in pH. Conventionally, the buffer capacity ($\beta$) is expressed as the amount of strong acid or base, in gram-equivalents, that must be added to 1 liter of the solution to change its pH by one unit.
- The pH of a food is critical because food with low pH favors the growth of yeasts and molds. In neutral or alkaline pH foods, such as meats, bacteria are more dominant in spoilage and putrefaction.
- Most microorganisms grow best at pH values around 7.0 (6.6–7.5), whereas few grow below 4.0. Adverse pH affects the functioning of enzymes and the transport of nutrients into the cell.
- The oxidation-reduction potential of a food also influences spoilage. Microorganisms display varying degrees of sensitivity to the Oxidation–Reduction Potential (O/R, Eh) of their growth medium.
- The O/R potential of a substrate may be defined generally as the ease with which the substrate loses (oxidized) or gains electrons (reduced). The more highly oxidized a substance, the more positive will be its electrical potential and more it favours growth of aerobic microorganism.
- Many foods contain natural antimicrobial substances, which make them stable against attack by certain microorganisms. Some plant species are known to contain essential oils that possess antimicrobial activity.
- Coumarins found in fruits and vegetables exhibit antimicrobial activity. Cow’s milk and eggs also contain antimicrobial substances. Eggs are rich in the enzyme lysozyme that can lyse the cell walls of contaminating gram-positive bacteria.
- Herbs and spices often possess significant antimicrobial substances; generally fungi are more sensitive than most bacteria.
- Sage and rosemary are two of the most antimicrobial spices. Aldehydic and phenolic compounds that inhibit microbial growth are found in cinnamon, mustard, and oregano.
The types and proportions of nutrients present in the food are all important in determining what organisms are most likely to grow. Consideration must be given to (i) foods for energy, (ii) foods for growth, and (iii) accessory food substances, or vitamins, which may be necessary for energy or growth.

A limited number of types of microorganisms can obtain their energy from fats but do so only if a more readily usable energy food, such as sugar, is absent.

Hydrolysis products of proteins, i.e., peptides and amino acids, serve as energy source for many proteolytic organisms when a better energy source is lacking and as foods for energy for other organisms that are not proteolytic.

Carbon for growth may come partly from carbon dioxide, but more often it comes from organic compounds.

The minerals required by microorganisms are nearly always present at the low levels required, but occasionally an essential mineral may be tied up so that it is unavailable, lacking, or present in insufficient amounts.

Some microorganisms are unable to manufacture some or all of the vitamins needed and must have them furnished.

Most natural plant and animal food-stuffs contain an array of these vitamins, but some may be low in amount or lacking.

Every microorganism has a minimal, a maximal, and an optimal pH for growth. Microbial cells are significantly affected by the pH of food because they apparently have no mechanism for adjusting their internal pH.

Bacteria tend to be more fastidious in their relationships to pH than moulds and yeasts, with the pathogenic bacteria being the most fastidious.

Moulds are a type of fungi which can grow over a wide range of pH values than can most yeasts and bacteria.

The pH of a product can be readily determined with a pH meter, but this value alone may not be sufficient for predicting microbial responses.

### 2.5 KEY WORDS

- **Intrinsic factor**: Intrinsic Factor (IF), also known as Gastric Intrinsic Factor (GIF), is a glycoprotein produced by the parietal cells of the stomach.

- **Buffer capacity**: Buffer capacity is a measure of the efficiency of a buffer in resisting changes in pH.

- **Oxidation–Reduction Potential**: The Oxidation–Reduction Potential (O/R, Eh) potential of a substrate may be defined generally as the ease with which the substrate loses (oxidized) or gains electrons (reduced).
2.6 SELF ASSESSMENT QUESTIONS AND EXERCISES

### Short Answer Questions
1. Define intrinsic factors?
2. Briefly describe the various intrinsic parameters.
3. What is redox potential?
4. What are carbohydrates?
5. What is the effect of pH on microbes?

### Long Answer Questions
1. Write a note on intrinsic factors. Also, explain the various parameters of intrinsic factors.
2. Explain with the help of table Eh requirements of microorganisms.
3. What are the antimicrobial barriers and constituents?
4. Write a note on various nutrient contents.
5. Discuss about the Hydrogen Ion Concentration (pH) and effect of pH on various microorganisms.

2.7 FURTHER READINGS


UNIT 3 EXTRINSIC FACTORS

3.0 INTRODUCTION

Extrinsic factors are the factors which are outside the body. Risk factor external to the body, that causes injury. The factors that effect the growth of microorganisms are temperature of storage, relative humidity of environment and gaseous atmosphere. Microbes are capable of growing under a wide range of temperatures, starting from −34°C to 90°C. The organisms that grow at less than 20°C and have temperature in the range of 20°C to 30°C, are known as psychrophiles or psychrotrophs, the ones that grow in the temperature range of 20°C and 45°C and have optimum temperature between 30°C and 40°C are known as mesophiles, and lastly, the organisms that grow at temperatures more than 45°C and have optimum temperature within 55°C to 65°C, are known as thermophiles.

In order to select temperature for storage, the type and quality of the food should also be taken into account. For example, bananas fare well when stored at a temperature in the range of 13°C to 17°C, whereas other vegetables do better when stored at about 10°C temperature. Apart from selecting proper temperature, it is also important to maintain relative humidity of the storage temperature, such that foods are prevented against any kind of spoilage.

The relative humidity of the storage chamber must be such that there is no excessive drying of the food or absorption of moisture by the food. Surface spoilage of foods can be inhibited by using a controlled atmosphere that surrounds the food. Increasing the amount of carbon dioxide in the atmosphere by about 10 per cent can help in retarding the fungal rotting of fruits. Further increase in the amount can prevent rotting of animal products like meat and fish. Several spoilage microorganisms can be avoided, if ozone gas is added. Ethylene has also been found to be effective in increasing the resistance of fruits against fungal attack.

In this unit, you will study about various extrinsic factors like temperature of storage, relative humidity of environment and gaseous atmosphere in detail.
3.1 OBJECTIVES

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

- Understand about extrinsic factors
- Discuss about various extrinsic factors

3.2 EXTRINSIC FACTORS

Extrinsic factors are the factors which are outside the body. Risk factor external to the body, that causes injury. The extrinsic parameters of foods are not substrate dependent. They are those properties of the storage environment that affect both the foods and their microorganisms. Extrinsic or environmental factors include temperature, relative humidity, gases (CO₂, O₂) present, and the types and numbers of microorganisms present in the food. Temperature and relative humidity are important extrinsic factors determining whether a food will spoil.

- Temperature of Storage
- Relative Humidity of Environment
- Gaseous Atmosphere

Temperature of Storage

Microorganisms Grow Over a Wide Range of Temperatures

Therefore, it is important to consider the temperature growth ranges for organisms which will help in selecting the proper temperature for the storage of different types of foods. Based on their temperature requirements, micro-organisms are placed into three groups as shown in Table 3.1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Grow Well</th>
<th>Optimum</th>
<th>Microorganisms Found Most Commonly in Foods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychrophiles</td>
<td>At or below 7°C</td>
<td>20°C–30°C</td>
<td>Pseudomonas and Enterococcus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>These organisms grow well at refrigerator temperatures and cause spoilage of meats, fish, poultry, eggs, and other foods normally held at this temperature.</td>
</tr>
<tr>
<td>Mesophiles</td>
<td>20°C–45°C</td>
<td>30°C–40°C</td>
<td>May be found on foods held at refrigerator temperatures. They apparently do not grow at this temperature but do grow at temperatures within the mesophilic range if other conditions are suitable. It should be pointed out that some organisms can grow over a range from 0°C to &gt;40°C. One such organism is Enterococcus faecalis.</td>
</tr>
<tr>
<td>Thermophiles</td>
<td>At and above 45°C</td>
<td>55°C–65°C</td>
<td>Some sp. of Bacillus, Pseudomonas, Clostridium, Geobacillus, Acidothiobacillus, and Thermoaerobactera. They are of great interest to the food microbiologist and food technologist in the canning industry.</td>
</tr>
</tbody>
</table>
Molds are able to grow over wide ranges of temperature as do bacteria. Many molds are able to grow at refrigerator temperatures. Notably some strains of *Aspergillus*, *Cladosporium*, and *Thamnidium*, may be found growing on eggs, sides of beef, and fruits.

Yeast grow over the psychrotrophic and mesophilic temperature ranges but generally not within the thermophilic range.

The quality of the food product must also be taken into account in selecting a storage temperature. Although it would seem desirable to store all foods at refrigerator temperatures or below, this is not always best for the maintenance of desirable quality in some foods. For example, bananas keep better if stored at 13-17 degree C than at 5-7 degree C. A large number of vegetables are favored by temperatures of about 10 degree C, including potatoes, celery, cabbage, and many others. In every case, the success of storage temperature depends to a great extent upon the Relative Humidity (RH) of the storage environment and the presence or absence of gases such as CO\(_2\) and O\(_3\). At higher relative humidities microbial growth is initiated more rapidly, even at lower temperatures (especially when refrigerators are not maintained in a defrosted state). When drier foods are placed in moist environments, moisture absorption can occur on the food surface, eventually allowing microbial growth.

Relative Humidity (RH) of Environment

In general, the higher the temperature of the environment, the lower is its RH and vice versa. Water activity or \(a_w\) is the partial vapor pressure of water in a substance divided by the standard state partial vapor pressure of water. In the field of food science, the standard state is most often defined as the partial vapor pressure of pure water at the same temperature. Using this particular definition, pure distilled water has a water activity of exactly one. As temperature increases, \(a_w\) typically increases, except in some products with crystalline salt or sugar.

The RH of the storage environment is important both from the standpoint of water activity (\(a_w\) the amount of moisture or water available in food for microorganisms to grow) within foods and the growth of microorganisms at the surfaces.

- When foods with low \(a_w\) values are placed in environments of high RH, the foods pick up moisture. Therefore, when dried foods are placed in moist environments, moisture absorption can occur on the food surface, eventually allowing microbial growth. Improperly wrapped meats such as whole chickens and beef cuts tend to suffer much surface spoilage in the refrigerator, due to the generally high RH of the refrigerator.
- Foods with a high \(a_w\) lose moisture to the atmosphere when placed in an environment of low RH and thereby become undesirable.
Extrinsic Factors

In selecting the proper environmental conditions of RH, consideration must be
given to both the possibility of surface growth and the desirable quality to be
maintained in the foods in question. By altering the gaseous atmosphere, it is possible
to retard surface spoilage without lowering the RH.

Presence and Concentration of Gases in the Environment

The atmosphere in which food is stored also is important:

- **O₂**: This is especially true with shrink-packed foods because many plastic
films allow oxygen diffusion, which results in increased growth of surface-
associated microorganisms.

- **CO₂**: Excess CO₂ can decrease the solution pH, inhibiting microbial growth.
Storing meat in a high CO₂ atmosphere inhibits gram-negative bacteria,
resulting in a population dominated by the lactobacilli.

- **Ozone (O₃)**: O₃ is the other atmospheric gas that has antimicrobial
properties. It has been shown to be effective against a variety of
microorganisms, but because it is a strong oxidizing agent, it should not be
used on high-lipid-content foods since it would cause an increase in rancidity.

The observation that food storage atmosphere is important has led to the
development of Modified Atmosphere Packaging (MAP). For example, modern
shrink-wrap materials and vacuum technology make it possible to package foods
with controlled atmospheres. These materials are largely impermeable to oxygen,
which prolongs shelf-life by a factor of two to five times compared to the same
product packaged in air. With a carbon dioxide content of 60% or greater in the
atmosphere surrounding a food, spoilage fungi will not grow, even if low levels of
oxygen are present. Recently, it has been found that high-oxygen MAP also may
be effective. This is due to the formation of the superoxide (O₂-)
anion inside cells
under these conditions, which then transformed to highly toxic peroxide and oxygen,
resulting in antimicrobial effects. Some products currently packaged using MAP
technology include delicatessen meats and cheeses, pizza, grated cheese, some
bakery items, and dried products such as coffee.

Check Your Progress

1. Define extrinsic factors.
2. What are the various extrinsic factors that effect microbial growth?
3. What should be taken in consideration while selecting a storage temperature?
4. What happens to microbial growth in higher relative humidities?
5. What happens when drier foods are placed in moist environments?
3.3 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Extrinsic factors are the factors which are outside the body. Risk factor external to the body, that causes injury.

2. Temperature and relative humidity are important extrinsic factors determining whether a food will spoil.
   - Temperature of Storage
   - Relative Humidity of Environment
   - Gaseous Atmosphere

3. The quality of the food product must also be taken into account in selecting a storage temperature.

4. At higher relative humidities microbial growth is initiated more rapidly, even at lower temperatures (especially when refrigerators are not maintained in a defrosted state).

5. When drier foods are placed in moist environments, moisture absorption can occur on the food surface, eventually allowing microbial growth.

3.4 SUMMARY

- Extrinsic factors are the factors which are outside the body. Risk factor external to the body, that causes injury.

- The extrinsic parameters of foods are not substrate dependent. They are those properties of the storage environment that affect both the foods and their microorganisms.

- Extrinsic or environmental factors include temperature, relative humidity, gases (CO₂, O₂) present, and the types and numbers of microorganisms present in the food.

- It is important to consider the temperature growth ranges for organisms which will help in selecting the proper temperature for the storage of different types of foods.

- Molds are able to grow over wide ranges of temperature as do bacteria. Many molds are able to grow at refrigerator temperatures. Notably some strains of Aspergillus, Cladosporium, and Thamnidium, may be found growing on eggs, sides of beef, and fruits.

- Yeasts grow over the psychrotrophic and mesophilic temperature ranges but generally not within the thermophilic range.
Extrinsic Factors

- The quality of the food product must also be taken into account in selecting a storage temperature. Although it would seem desirable to store all foods at refrigerator temperatures or below, this is not always best for the maintenance of desirable quality in some foods.
- In general, the higher the temperature of the environment, the lower is its RH and vice versa.
- Water activity or $a_w$ is the partial vapour pressure of water in a substance divided by the standard state partial vapour pressure of water.
- In general, the higher the temperature of the environment, the lower is its RH and vice versa. Water activity or $a_w$ is the partial vapor pressure of water in a substance divided by the standard state partial vapor pressure of water.
- In the field of food science, the standard state is most often defined as the partial vapor pressure of pure water at the same temperature.
- As temperature increases, $a_w$ typically increases, except in some products with crystalline salt or sugar.
- The RH of the storage environment is important both from the standpoint of water activity ($a_w$, the amount of moisture or water available in food for microorganisms to grow) within foods and the growth of microorganisms at the surfaces.
- $O_2$ is especially true with shrink-packed foods because many plastic films allow oxygen diffusion, which results in increased growth of surface-associated microorganisms.
- Excess CO$_2$ can decrease the solution pH, inhibiting microbial growth. Storing meat in a high CO$_2$ atmosphere inhibits gram-negative bacteria, resulting in a population dominated by the lactobacilli.
- $O_3$ is the other atmospheric gas that has antimicrobial properties. It has been shown to be effective against a variety of microorganisms, but because it is a strong oxidizing agent, it should not be used on high-lipid-content foods since it would cause an increase in rancidity.

3.5 KEY WORDS

- **Extrinsic factors**: Extrinsic factors are the factors which are outside the body. Risk factor external to the body, that causes injury.
- **Water activity**: Water activity or $a_w$ is the partial vapor pressure of water in a substance divided by the standard state partial vapor pressure of water.
3.6 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions
1. What are extrinsic factors?
2. What are the various extrinsic factors that effect microbial growth?
3. How does temperature effect storage?

Long Answer Questions
1. Write a note on extrinsic factors and the factors effecting microbial growth.
2. Discuss about relative humidity on environment.
3. Write a note on presence and concentration of gases in the environment.

3.7 FURTHER READINGS


4.0 INTRODUCTION

Food contamination is the introduction or occurrence of a contaminant in food. A contaminant is any biological or chemical agent, foreign matter, or other substance unintentionally added to food that may compromise food safety or suitability. Among these contaminants are biological, chemical or physical agents in, or condition of, food with the potential to cause an adverse health effect. The contamination of food by chemicals is a worldwide public health concern and is a leading cause of trade problems internationally. Contamination may occur through environmental pollution, as in the case of toxic heavy metals, Poly Chlorinated Biphenyl (PCBs) and dioxins, or through the intentional use of chemicals, such as pesticides, animal drugs and other agrochemicals. Food additives and contaminants resulting from food manufacturing and processing can also adversely affect health. When foods are contaminated with unsafe levels of pathogens, chemical contaminants, or metals, they can pose substantial health risk to consumers and place severe economic burden on individual communities or nation. Cross-contamination of food is a common factor in the cause of food borne diseases. Cross-contamination is the contamination of a food product from another contaminated source. Foods can become contaminated by microorganisms (bacteria and viruses) from many different sources during the food preparation and storage process.
Spoilage of food may be due to chemical or biological causes; the latter include action of inherent enzymes, growth of micro-organisms, invasion by insects, and contamination with trichinae and worms. About one-fourth of the world’s food supply is lost through the action of micro-organisms alone. The ‘spoilage’ concept includes concepts about edibility, means the food is unfit to eat or fit to eat. Spoilage is decomposition. Many foods may not be decomposed, but harbor certain kinds of bacteria, or their toxins, in number or amounts which make the food poisonous and thus unfit for human consumption. Harvested foods decompose from the moment they are harvested due to attacks from enzymes, oxidation and microorganisms. These include bacteria, mould, yeast, moisture, temperature and chemical reaction.

In this unit, you will study about contamination and spoilage of cereals, cereal products, fruits, vegetables, meats, meat products, fish, sea foods, eggs, poultry and canned foods in detail.

4.1 OBJECTIVES

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

- Understand what contamination and spoilage is
- Discuss the contamination and spoilage of cereals, cereal products, fruits, vegetables, meats, meat products, fish, sea foods, eggs, poultry and canned foods

4.2 CONTAMINATION AND SPOILAGE

Food contamination refers to the presence of harmful chemicals and microorganisms in food, which can cause consumer illness. This article addresses the chemical contamination of foods, as opposed to microbiological contamination, which can be found under foodborne illness.

The impact of chemical contaminants on consumer health and well-being is often apparent only after many years of processing and prolonged exposure at low levels. Unlike food-borne pathogens, chemical contaminants present in foods are often unaffected by thermal processing. Chemical contaminants can be classified according to the source of contamination and the mechanism by which they enter the food product.

Food spoilage is the process where a food product becomes unsuitable to ingest by the consumer. The cause of such a process is due to many outside factors as a side-effect of the type of product it is, as well as how the product is packaged and stored. Due to food spoilage, one-third of the world’s food produced for the consumption of humans is lost every year. Bacteria and various fungi are the cause of spoilage and can create serious consequences for the consumers, but there are preventative measures that can be taken.
Foods are not only of nutritional value to those who consume them but often are ideal culture media for microbial growth. Microorganisms can be used to transform raw foods into gastronomic delights, including chocolate, cheeses, pickles, sausages, and soy sauce. Wines, beers, and other alcoholic products also are produced through microbial activity. On the other hand, microorganisms can degrade food quality and lead to spoilage and hence can serve as vehicles for disease transmission. Food spoilage is a major concern throughout the world. This can occur at any point in the course of food production process: growth, harvesting, transport, storage, or final preparation.

Food Spoilage

When foods become harmful and unsuitable for consumption due to damage of original nutritional value, texture and flavour, it is known as food spoilage.

Common Types of Spoilage

The most common types of spoilage include the following:

- **Microbial Spoilage**: Yeasts, molds and bacteria cause this type of spoilage. Growth of yeasts causes fermentation which results in yeast metabolism. True yeast and false yeast are the two classifications of yeasts. True yeast metabolizes sugar and as a result, alcohol and carbon dioxide are produced. This is known as fermentation. False yeast grows as a dry film on a food surface. Also, it occurs in foods that have an environment of high sugar or high acid.

  Molds grow in filaments thereby forming a tough mass which is visible as 'mould growth'. Spores are formed by the molds which after drying float through the air to find optimal conditions to start the growth cycle again. Eating moldy food may cause nausea or vomiting. Yeasts as well as moulds can thrive in high acid foods like fruits, tomatoes, jams, jellies and pickles and are easily destroyed by using heat.

- **Fungal Spoilage**: Figure 4.1(a) to (d) show some common types of fungal spoilage.

Fig. 4.1 (a) Rot in Grapes caused by Botrytis Cinerea
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**NOTES**

- **Bacterial Spoilage**: Bacteria are microorganisms that are found in round, rod or spiral shapes. Different kinds of bacteria cause different kinds of spoilage. They are divided into two groups, namely spore-forming and non-spore-forming.

- ** Spoilage Due to Enzymes**: If uncooked foods are not used while fresh, enzymes cause undesirable changes in color, texture and flavor. Enzymes can be destroyed easily by heat processing.

- ** Spoilage Due to Oxidation**: Atmospheric oxygen on reacting with some components of food may cause rancidity or color changes.

- ** Spoilage Due to Low Temperature**: Sometimes, the internal structure of the food gets damaged due to very low temperature. The examples are shown in Figure 4.2 (a) and (b).

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**Fig. 4.1 (b)** Rot in Strawberry due to *Botrytis Cinerea*

**Fig. 4.1 (c)** Black Mummy Rot of Grapes caused by *Guignardia Bidwellii*

**Fig. 4.1 (d)** Watery Soft Rot due to *Sclerotinia Sclerotiorum*
Infestations by Insects and Rodents: These also lead to heavy loss of food stocks.

The various types of spoilage can also be discussed in reference to the following food products:

**Fruits and Vegetables:** Diseases in fruits and vegetables are generally caused because of microbial spoilage. Bacterial soft rot, commonly occurring in fruits is due to the *Erwinia carotovora* and *P. marginalis*. These microbes break down the pectin thereby giving rise to soft, mushy consistency with a bad odour and a water-soaked appearance. Vegetables, for example, carrot, celery, beet, garlic, onion, lettuce, potato, cabbage, tomato, watermelon are affected by this disease.

Watery soft rot of vegetables or sour rot is caused by *Geotrichum candidum*. It occurs in vegetables like bean, carrot, lettuce, cabbage, onion, garlic, radish, etc. Similarly *Rhizopus* soft rot is caused by *R. stolonifer* in vegetables like cabbage, cucumber, pumpkin, and carrot, and radish, watermelon thereby making them soft and mushy. Other important microbes causing diseases in the vegetables are bacterial blight of celery, black rot of cabbage and cauliflower, ring rot of potatoes, bacterial speck of tomatoes, etc.

**Cereals:** Owing to the low water activity in cereals like wheat, rice, rye, corn they remain comparatively protected from the attack of microbes, if they are stored properly. The flour made from these cereals also remains protected due to the action of bleaching agents used during milling operation. If favourable conditions are there, bacteria and molds might grow on these cereals, thereby spoiling them.

**Bakery Products:** In case of commercially baked breads, the content of moisture is insufficient for the growth of microbes except molds. The common bread mold is *R. stolonifer* causing ropiness in bread, particularly if it is stored in high humidity or wrapped while still warm. Owing to high sugar content in most of the cakes, the
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Possibility of spoilage is quite low in them, although molds can still cause spoilage in them. The process of baking is such that it makes spoilage by microbes difficult. The icings, toppings, nuts, cherries sprinkled on the cake can however get spoiled easily.

**Dairy Products:** Microbial spoilage occurs quite readily in dairy products like butter, milk, cream, cheese. Milk is an excellent medium for a variety of microbial spoilage. Fresh as well as refrigerated raw milk contains several bacteria belonging to the genera of *Streptococcus, Leuconostoc, Lactobacillus, Pseudomonas, Bacillus, Microbacterium, Propionibacterium, Micrococcus*, etc.

**Meat, Poultry and Sea Foods:** Micro flora coming from the animal’s lymph nodes, intestinal tract, hide, processing equipments cause fresh meat to spoil. In meats microbial spoilage occurs due to bacteria from internal sources such as *Mucor, Rhizopus and Thamnidium*. They produce whiskers on beef, *Penicillium* produces green patches, *Cladosporium* causes black spots.

**Eggs:** *Pseudomonas* species, *proteus* species, penicillium, aspergillus, flavobacterium and salmonella are common spoilage organisms in eggs.

**Fitness or Unfitness of Food for Consumption**

According to Thom and Hunter, ‘A product is considered fit for food, if a discriminating consumer, aware of its history of production and seeing the material himself is ready to eat and conversely the same product is spoiled when such an examiner refuses it as food.’ On the basis of this definition, you can say that the fitness of food is dependent on the person who is judging it, as what one person judges to be fit may be unfit for another person. For example, Britishers generally like their meat ‘high’ that is, having a strong flavour developed by hanging or aging, whereas Americans consider this meat as spoilt. Similarly, people who are starving might eat food they would not consume under normal conditions.

Generally, the following criteria are used for measuring the fitness of food:

- **Desired Stage of Maturity:** Fruits should be consumed at a specific stage of maturity when they are just ripe. Similarly, sweet corn should be young enough to be tender and milky in character. Poultry should also be consumed young.

- **Pollution Free:** If fertilized with sewage, vegetables should not be consumed raw. Oysters that have been obtained from contaminated water should not be consumed. Food contaminated by flies or rodents should be spurned.

- **Microbial Attack or Enzymes Action:** Sometimes there is very little difference between harmless growth and spoilage by microorganisms. The same type of food change may be considered sometimes desirable and sometimes undesirable. For example, some people may consider sour milk as spoilt, but may easily consume cultured butter milk. Meat purification means spoilage, while putrefactive changes in Limburger cheese is considered normal in the ripening process.
Causes of Spoilage

Natural deterioration of organic matter is known as food spoilage. Nature’s every component is broken down in simpler parts so that it can again become a part of soil.

The main or biggest symptom of food spoilage is bad odour. Thus, it is believed that the foods that have a bad odour must be thrown out. If foods having bad odour are kept in the refrigerator for some time, they transfer their odour to other foods, making them disagreeable to eat. Smoked salmon’s smell permeates into cake and nobody prefers to eat a cake smelling like a salmon. Nowadays, products are available in the market that help in reducing the intensity of refrigerator odour and foul taste transfer among the foods. Refrigerator purifiers and small air filters are also available in the market.

The various factors responsible for the spoilage of food are as follows:

Air and Oxygen: We have all studied that air comprises of about 80 per cent nitrogen, 20 per cent oxygen and some other gases. If the amount of oxygen in the air is too high, then antioxidants are produced by our bodies in order to refute or counter the undesirable reactions. When an organism loses all support from its various physiological systems, its chemical structure starts reacting with the oxygen in the air. For example, aerobic bacteria or molds are able to colonize flesh areas which are exposed to the air. They form colonies on the food and start metabolizing the organism’s flesh and divide at the rate of one division per twenty minutes per cell of bacteria.

Oxidizing enzymes react with oxygen and cause the process of food spoilage, for example, vegetable enzymes like catase and peroxidase cause browning of some foods, such as, apples and potatoes. Enzymes are basically substances which increase the speed of chemical and enzymatic reactions with oxygen in the organic matter, thereby hastening degeneration of the matter. Cooking at high temperature is done to refute these chemical reactions. The food does not become inedible by the reactions of the enzymes, but if they get combined with the microbial infestations, then food can get spoilt and become risky for consumption.

Water: It is found in abundance in nature, and all organisms are made up of at least 70 per cent water. When an organism is alive, the water found in it is known as bound water; since it is chemically connected to body’s other substances. A certain level of chemical concentration is maintained in all living beings and is said to be similar to the concentration of sea water from which all living beings originate. Fluids of a particular concentration flow around the cells and each cell is carefully controlled and regulated by the central control system of the organism. This process is known as homeostasis in higher organisms.

After a plant or animal has been cut into smaller parts, the bound water in it gets exposed to the environment. The organism’s flesh on being exposed to water
soaks up the water and dilutes the concentration levels within the flesh with that of the concentration levels surrounding it. This sometimes causes explosion of the organism’s cells as it gets too full of water. This process of tissue deterioration is the major cause of food spoilage. The moisture intake also serves as a perfect vector for microbial infestations and results in food hygiene breakdown. Apart from this, the excess free water in and around the cells provides bacteria the perfect medium to operate. Water within an organism can be controlled by:

- Dehydration
- Freezing
- Addition of Food Preservatives

**Light:** When food spoilage occurs due to light, it is known as photo degeneration.

We know that all foods are exposed to the light at one time or the other. Light, which can be artificial or natural, is a form of energy. It is made of different wave lengths. At the outer end of the light spectrum lies infrared and ultraviolet light that varies in intensity in different parts of the world. These light radiations are considered to be harmful and can cause dead and live tissue to react in harmful manner. Foods change in nature when they are exposed to the sources of light. In solid food like meat, since the density of the material is high, deep penetration of light is blocked, and thus changes occur only on the surface of the substances. In case of liquids, the penetration of light is much deeper and thus the effects of photo generation are more substantial.

**Microbial Growth:** Microbes play an important role in the balance of nature. We find that some bacteria enjoy a symbiotic relationship with living organisms and in some cases they develop parasitical relationship with them. The organisms that are healthy are able to keep bacteria that are parasitical in nature at bay through their immune system and homeostasis. However, it has also been observed that the parasitical bacteria are always on a lookout for signs of weakness and are generally successful in bringing about rapid deterioration in sick organisms. They also ensure that they are recycled back into the environment with the help of tissue deterioration and degeneration processes. There is one more group of bacteria that can cause food spoilage at low temperatures; these are known as psicrophilic bacteria. In extremely cold climates, weaker animals die and remain frozen until they start thawing when the spring commences. Certain categories of bacteria develop in cold carcass and thereby start deterioration. The spoilage of food by these bacteria can be controlled by implementing and establishing a good health regime.

**Temperature:** This is probably the single most important environmental variable which is under our control. In order to prevent the food from spoilage, it is imperative to control the temperature. Temperature regulates several changes in the nature of...
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It starts by slowing down chemical reactions within the food. Then, it prevents the growth of bacteria and sometimes even destroys it through the process of cooking. The regulation of temperature also controls the deterioration of vitamins and prevents dehydration and ripening of food.

Temperature is professionally controlled, these days. It has been observed that over freezing can result in cracking of the surfaces thereby puncturing the cells and causing the flesh to become soft and pulpy. Pigment is lost due to this and food loses much of its nutritive value, this process is known as freezer burning. Vegetables and fruits in the cold storage are maintained at temperature of about 10°C. Meats are chilled at about 4°C and frozen food at –18°C for a period less than six months.

Characteristics of Spoilt Food

The following are the common characteristics of spoilt food:

- **Slime:** In this case, as bacteria touch one another, the food becomes slimy.
- **Sour:** In this case, food goes sour when microorganisms produce acids.
- **Discolored:** This occurs due to microbial growth.
- **Gas:** Bacteria sometimes produce gas as a byproduct when there is a microbial growth.
- **Odour:** Due to the breakdown of bacterial cells, there is a rotten smell.

Foods can be classification on the basis of ease of spoilage as follows:

- **Stable/Non-Perishable Foods:** These do not get spoilt unless handled carelessly, for example, sugar, flour.
- **Semi-Perishable Foods:** These if properly handled and stored, remain unspoilt for a long period, for example, potatoes, apples.
- **Perishable Foods:** These refer to foods that spoil readily unless special preservative methods are used, for example, meats, fish, poultry, eggs and milk.

Factors Affecting Kinds and Numbers of Microorganisms in Food

The kind of microbial spoilage of foods depends on the kinds and numbers of these agents present in the environment. A variety of bacteria, yeasts, molds and enzymes are present in most of the raw foods. Owing to specific environmental conditions, only a small proportion of them are able to grow rapidly and cause food spoilage. Usually a single or sometimes two or three types of organisms are present in the food. If the first type of organisms starts with the spoilage, then other organisms move to produce secondary spoilage.

The kinds and numbers of microorganisms that will be present in the food depend on the kind and extent of contamination, earlier opportunities for growth of a particular organism, and earlier treatments that the food has received.
The number of microorganisms in the food is increased due to contamination and it may even introduce new kinds of microbes in the food. For example, in case of butter, the wash water may contain surface taint bacteria; plant equipment may also add spoilage microbes to the food during the process of processing; washing machines may add microbes to the eggs and dirty boats may add them to the fish. The increased microorganism’s bio-burden, which causes spoilage, makes the efforts of preservation more difficult. It makes the process of spoilage more rapid.

Pretreatments may destroy or sometimes remove some microbes, add organisms, or change the proportions of those present in the food, already. For example by washing the vegetables thoroughly, surface taint bacteria may be removed. In case washing is done by some germicidal solution, the numbers of infectants may further decrease.

Treatments with the help of rays, ozone, sulphur dioxide or germicidal vapours may also reduce numbers. High temperature also reduces the numbers of microbes spoiling food. Thus, storage under various conditions also helps in reducing the numbers on microbial infectants spoiling the food.

**Food Deterioration**

Preservation of food entails keeping the food in such a state that it does not get spoilt for a long time, thereby making it palatable for an extended period of time. In cases where food has not been preserved, you must have seen its spoilage in several ways. For example, if bread is kept out of the refrigerator for a few days, a spongy or mouldy growth is seen on it, which may be white, green or black in colour (Refer Figure 4.3). The bread becomes unfit for consumption and has to be discarded. In case, there is a bad smell, fermentation (in the form of gas or bubbles) or mouldy appearance on the food, it means that the item has been spoilt and is not fit for consumption. Soft brown spots on fruits and vegetables also indicate food spoilage.

![Spoilt Bread](Fig. 4.3 Spoilt Bread)
The reason to study food spoilage is that it will help us to remove the conditions that make them rot and preserve the food in an appropriate manner. The terms that are generally used while discussing food deterioration are as follows:

- **Deterioration of Food**: It is generally defined as a series of continuous degenerative or degradative changes that occur in a food item which may affect the wholesomeness of the product, thereby resulting in a reduction of its quality and serviceability. Deterioration is a continuous process and begins the moment an animal is slaughtered or a plant is harvested. It continues till the product is no longer recognizable as a food product or is literally reduced to a fine powder. It is important to note that some of the techniques that are used in preservation of food products are freezing, blanching, drying, usage of additives and canning.

- **Detection of Deterioration**: There are various ways of detecting the onset of deterioration of food, some of which have been discussed as follows:
  - **Sensory evaluation**: In most of the cases, generally physical senses are used to detect deterioration in foods. Senses like smelling, seeing and tasting are used to find out about food deterioration.
  - **Check of common characteristics**: Senses are also used to find out the following characteristics of a food item.
    - **Colour**: It is the attribute of appearance of food items that helps in the identification of their condition. An abnormal colour is an indication of its deteriorated condition.
    - **Odour**: This characteristic of food items makes it perceptible to the sense of smell. The smells can be spicy, fruity, burnt, sharp, metallic, sweet, rancid.
    - **Texture**: This refers to the physical properties of food that can be tender, tough, crispy, soft. An abnormal texture indicates that there is a deterioration of food. Abnormal textures can be water-soaked, slimy, soft, hard, brittle or spongy. In case of liquids, the consistency can become abnormal.
    - **Taste**: It is a term used for four sensations like sweet, sour, salty and sweet. An off-flavor can be offending to the taste as well as smell indicating the deteriorated condition. An off-flavor may also be a pleasant odour which may not be characteristic of the product. Terms such as fishy, musty and sour can be used for explaining off-tastes.

**Causes of Food Deterioration**

The major causes of food deterioration are microbes, enzymatic action, chemical reactions, physical changes, time, insects and rodents. It is important to note that these causes are not isolated in nature. Causes like the action of bacteria, insects,
light are also agents that deteriorate crops in the fields or warehouses. They increase the multiplication and activities of bacteria on the food. Thus, many forms of deterioration may be going on simultaneously in the food so as to deteriorate it. For the purpose of food preservation, elimination or minimization of the causes is necessary.

**Microbes:** Bacteria, yeasts and moulds are the primary microbes that are involved in the deterioration of foods. Thousands of genera and species of microbes are found in nature and are associated with food in one way or the other. However, it is essential to note that not all microbes are involved in the spoilage of food and some of them are also used in the preservation of food products. Examples of such microbes are lactic acid bacteria that help in preserving foods such as cheese, sauerkraut, etc. Some microbes are also used for producing alcohol (like wine) or in the making of beer. These exceptions are the cases where microbes are especially cultivated by the selective inoculation process or by controlled conditions to favour the growth (of a kind of bacteria) over those that are less desirable. The microbes attack almost all constituents of food. Some of the bacteria help in the fermentation of sugars and some hydrolyze starches and cellulose. Various others hydrolyze fats, thereby, producing rancidity. There are also some that help in the digestion of proteins and produce odours and are putrid. Some form acids and make the food sour, while others produce gas making the food foamy. They also form pigments, produce toxins and give rise to food-borne illnesses.

- **Bacteria:** These are unicellular microbes found in many forms out of which three are predominant. These are the spherical-shaped cocci, rod-shaped bacilli and spiral-shaped spirilla. Some of the bacteria produce spores and are resistant to heat, chemicals and other conditions that are considered adverse. The spores of bacteria are far more resistant to most conditions of processing than food enzymes that are natural. The bacteria associated with food are small and are able to penetrate the smallest possible openings and many can pass through the natural pores of the shell of an egg.

- **Moulds:** They are larger than bacteria and yeast and are structurally more complex. They grow in the form of a network of fibers that are hair-like and are known as mycelia possessing fructifying bodies that produce mould spores known as conidia. The black formation on the bread and the blue-coloured veins seen on the cheese occur due to the presence of conidia. Mycelia are a micron in thickness and like bacteria they too can penetrate the smallest possible openings.

- **Yeast:** They are larger than bacteria and measure about 20 microns in length and 10 microns in breadth. However, yeasts are smaller than moulds. Yeasts are mostly cream, tan or grey in colour and are spherical or ellipsoidal in shape. These are associated with all types of food products like vegetables, meat, cheese, poultry, fruits, etc. In cases where bacterial inhibitors are added, yeasts can dominate but otherwise bacteria are generally dominant.
Some of the yeasts are also found in foods such as molasses, fruit, sugar, honey, etc. Yeasts that are tolerant to salt grow as a film on brine food and on salted food and ham.

**Food Enzymes:** The action of food enzymes is the next major cause of food deterioration. These are basically organic catalysts and are produced by animal and plant cells or bacteria. You have read earlier that microbes possess enzymes thereby producing fermentation, rancidity and putrefaction of foods. Similarly, plants and animals that are uninfected have their own enzyme whose activity largely survives harvest and slaughter. There have been examples of cereals whose grains have been recovered after sixty years of storage and still possess properties like respiration, germination and growth which are all enzyme-controlled functions. These enzymes continue to catalyze chemical reactions within the food unless they are inactivated by heat, chemicals or any other means (Refer Figure 4.4). Some such reactions are highly desirable unless they are not allowed to go too far, like ripening of tomatoes after they have been picked and natural tenderizing of beef upon aging. However, it is important to regulate ripening and tenderizing as after an optimum point it results in food deterioration and on weakening the tissues become more prone to microbial infections.

**Fig. 4.4 Food Enzymes**

**Chemical Reactions:** Chemical reactions are another significant cause of food deterioration. These reactions are very complex in food. They exclude the enzymatic actions that are responsible for such deteriorative changes. These changes can be oxidation, changes in colour, reactions between a food container and its content and protein coagulation.

**Temperature:** The rate of deterioration is significantly affected by the temperature also. According to the rule of Van’t Hoff, the rate at which deterioration can take
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place can be ascertained. The rule says that for every 10°C increase in the temperature, the rate of the chemical reaction doubles. The chemical reactions taking place in the food also bring about changes in the food for every 10°C increase. It reduces the shelf-life of the food by half and the rate of chemical reactions is doubled.

Physical Changes: Changes in appearance will not cause a food to be spoiled but may cause deteriorative changes in the food items making them unsuitable for human consumption. Such physical changes are the following:

- Freezing causes undesirable changes in many food items like destruction of emulsions and texture. Products that are emulsified such as salad dressing and mustard (containing fat or oil and water mixture) are unable to combine without special processing or additives. If such products are frozen, the emulsion will be destroyed and the fat and water will separate into different layers. The fruits and vegetables that are first frozen and then allowed to thaw will have a disrupted texture resulting in cracked skins thereby making it possible for the microbes to attack them. Thus, we find that canned fruits and vegetables becomes soft and mushy due to uncontrolled freezing.

- Cold damage to food need not be caused by extreme freezing. Like other living systems, many fruits and vegetables have a requirement of optimum temperature after harvest. Common refrigeration temperatures of about 5°C hold the fruits and vegetables and weaken or deteriorate them. It results in development of off-colour, surface pitting and various other forms of decay. Some fruits such as bananas, squash, lemons, tomatoes, etc. should always be held at a temperature no lower than 10°C for maximum possible retention of quality.

- In the moderate temperature range, much of the food is handled between 10°C and 38°C. Within this range, for every 10°C rise in the temperature, the rate of chemical reactions double including the rate of many enzymatic as well as non-enzymatic reactions. Excess heat can denature proteins, break emulsions, dry out foods by removing moisture and destroy vitamins. In the case of green vegetables, excessive heat leads cell walls and membranes to lose their integrity and release acids and enzymes. This results in the development of a soft texture as well as development of off-colours and flavours. In the case of a muscle tissue, the excessive heat denatures proteins which then clump together and enzymes are inactivated. The result is toughening of texture, loss of water holding capacity, cooked or caramel flavors and development of off-colours.

- Dehydration is a kind of physical change that causes deterioration of food and is simply defined as loss of water from the food product. Foods that are fresh, chilled or frozen are more prone to dehydration. We are all aware that food items contain a substantial amount of water, for example, products of meat contain about 70 to 75 per cent water, fresh fruits and vegetables contain 80 to 95 per cent water and so on. As water vapours continually...
move from an area of high concentration to an area of low concentration, improper storage conditions and packaging result in dehydration. The symptoms of dehydration are dryness or shriveling on the food’s surface. The development of off-colours or having a darkening effect is also sometimes observed. This process is also known as freezer burn and as wilting in the case of fruits and vegetables.

- Excessive moisture leads to gross changes resulting in deterioration of food. The foods that are dehydrated and freeze-dried are more prone to this type of food deterioration. These food items are very hygroscopic (which readily take up and retain moisture). If such foods are not packaged properly the food item becomes lumpy or caked as excessive moisture is present in them. This condition further deteriorates food and may result in bacterial growth and oxidation. Surface moisture can also result from slight changes in relative humidity causing lumping and caking and defects like motting, crystallization and stickiness. The surface of the food can become a pool for the multiplication of bacteria or growth of mould due to the slightest possible condensation. In a package that is moisture proof, fruits and vegetables can give off moisture from respiration and perspiration. This moisture in its turn is trapped within the package and supports the growth of microbes.

- When a particular food item suffers mechanical damage its tissues that have been damaged become prone to other forms of deterioration. Mechanically-damaged foods are easy prey for the attack of microbes and the damaged area serves as a port of entry for them. Mechanical abrasion destroys the cell walls of food and results in the release of enzymes of the food. Once these enzymes are liberated the enzymes initiate the process of deterioration also known as autolysis. It results in softening of the texture and development of off flavors.

- Light is also a form of physical change that causes deterioration of food and results in fading of colour in many products. Riboflavin, Vitamin A and Vitamin C are destroyed by light. Milk that is stored in bottles develops a flavour when exposed to the sun as it causes light-induced fat oxidation and changes in the protein. However, not all wavelengths which make up natural or artificial light are absorbed by the components of the food equally and not all are destructive. Some foods that are sensitive can be protected from light by impervious packaging or by incorporating compounds into glass and films that may be transparent that screen out specific wavelengths. Another problem related to light is the greening of potatoes, also known as sun burn. It is the result of the exposure of potatoes to sunlight during growth (or after digging) and to artificial light during display, for sale. After they are exposed for more than two days, there is a development of green colour as the skin and flesh of potatoes are affected. Due to the exposure of light, an alkaloid called chlorophyll and solanine are produced. These green tubers acquire a
bitter and pungent taste and if eaten in a large quantity, they may prove to be poisonous. Although chlorophyll is tasteless and harmless, solanine is a bitter and harmful component.

- Time (the aging process) is also a major cause of food deterioration. There is a time after slaughter, harvest or manufacture of food when the quality of food is at its peak but this is a transitory period. The growth of microorganisms, destruction due to insects, action of food enzymes, non-enzymatic interaction of food constituents, loss of flavour, effects of heat, cold, moisture, oxygen, and light all grow with time. The longer the duration, the greater is the impact of destruction. Although certain cheeses, sausages, wines and other fermented foods improve with aging, the quality of the majority of foods decreases with time and the major objective of handling food and practices of preservation is to capture and maintain freshness.

- All foods have a definite storage life which is determined by the type of food, processing and packaging method, storage environment to a great extent (Refer Figure 4.5). The longer the food is stored, the greater is the opportunity for changes related to deterioration occur.

- Insects mainly destroy cereal grains, fruits and vegetables. The problem of insects relates not only to the amount it can eat but they damage the food and open it for the attack of microbes. The attacks of insects, rodents and rats can be controlled by using chemicals such as methyl bromide, ethylene oxide and propylene oxide. The use of these chemicals is prohibited in food items that are high in moisture content because the possibility of formation of toxic substances increases. After processing, sometimes the eggs of the insects persist and are laid in the food, like in the case of flour. In the case of rodents, they not only eat foods in quantities but also contaminate them. Their urine and droppings may contain several kinds of disease-causing
bacteria. They can be directly or indirectly involved in the transmission of diseases like salmonellosis, leptospirosis, and murine typhus. Insects and rodents may be effectively controlled by the following means:

- Keeping insects and rodents out of the storage facilities by pest-proofing the building
- Following good housekeeping practices
- Using appropriate control measures to exterminate pests

### Check Your Progress

1. What is food contamination?
2. What is food spoilage?
3. What are the criteria used for measuring the fitness of food?
4. Give the characteristics of spoilt food.

### 4.3 CONTAMINATION AND SPOILAGE OF VARIOUS THINGS

Following are the various types of contaminations that can be seen on the various things:

#### Spoilage of Fruits and Vegetable Juices

It has been estimated that one-fourth of all fruit produce harvested is spoiled before consumption. Spoilage of fresh fruits and vegetables usually occurs during storage and transport and while waiting to be processed (Refer Figure 4.6). Unlike many other foods, fruits and vegetables after picking and before processing are ‘alive’ for an extended time. The resulting respiration of these products and the normal ripening process complicate an independent discussion of the microbiological spoilage of fruits and vegetables.

![Fig. 4.6 Fruits Showing Microbial Growth](image_url)
Contamination of Fruits and Vegetables

During Transportation: As soon as fruits and vegetables are gathered into boxes, lugs, baskets, or trucks during harvesting, they are subject to contamination with spoilage organisms from each other and from the containers unless these have been adequately sanitized. During transportation to market or the processing plant, mechanical damage may increase the susceptibility to decay and growth of microorganisms and refrigeration during transportation will slow such growth. Washing the fruit or vegetable may involve a preliminary soaking or may be achieved by agitation in water, or preferably, by a spray treatment. Soaking and washing by agitation tend to distribute spoilage organisms from damaged to whole foods. Reused water is likely to add organisms, and the washing process may moisten surfaces enough to permit growth of organisms during a holding period. Washing with detergent or germicidal solutions will reduce the numbers of microorganisms on the foods.

Contamination in Handling: Sorting spoiled fruits or vegetables or trimming spoiled parts removes microorganisms, but additional handling may result in mechanical damage and therefore, greater susceptibility to decay. When these products are sold in the retail market without processing, they are not ordinarily subjected to much further contamination, except for storage in the market in contaminated bins or other containers, possible contact with decaying products, handling by salespeople and customers, and perhaps spraying with water or packing with chipped ice. This spraying not only gives a fresh appearance to the vegetables and delays decomposition, but also adds organisms, for example, psychrotrophs, from water or ice and gives a moist surface to encourage their growth on longer storage.

Contamination in Processing: In the processing plant, fruits and vegetables are subjected to further contamination and chances for growth of microorganisms, or numbers and kinds of organisms may be reduced by some procedures. Adequate washing at the plant causes a reduction in numbers of microorganisms on the food, as do peeling by steam, hot water, or lye and blanching (heating to inactivate enzymes, etc.). Sweating of products during handling increases numbers, processes such as trimming, mechanical abrasion or peeling, cutting, pitting or coring, and various methods of disintegration may add contaminants from the equipment involved. In fact, every piece of equipment coming in contact with the food can be a significant source of microorganisms unless it has been cleaned and sanitized adequately. Modern metal equipment with smooth surfaces and without cracks, dead ends, etc., is made to facilitate such treatments. Examples of possible sources of contamination of foods with microorganisms are trays, bins, tanks, pipes, flames, tables, conveyor belts and aprons, fillers, blanchers, presses, screens, and filters. Wooden surfaces are difficult to clean and sanitize and therefore are especially likely to be sources of contamination, as are cloth surfaces, for example, on conveyor belts. Neglected parts of any food-handling system can build up numbers of microorganisms contaminating the food. Hot-water blanching, although it reduces
Contamination by Microbes: Build-up of populations of microorganisms on equipment as a result of microbial growth in the exudates and residues from fruits and vegetables may greatly influence the amount of contamination of the foods and the growth of the contaminants. Not only is there the possibility of the addition of large numbers of organisms from this source, there is also the likelihood that these organisms are in their logarithmic phase of growth and therefore able to continue rapid growth. This effect is especially evident on vegetables following blanching. The heat treatment reduces the bacterial content considerably, damages many of the surviving cells, and consequently lengthens their lag period. On the other hand, the actively growing contaminants from the equipment can attain large numbers if enough time is allowed before freezing, drying, or canning; such growth is usually the cause of very high bacterial counts.

Contamination by Spoiled Items: Inclusion of decayed parts of fruits increases the numbers of microorganisms in fruit juices. Numbers in orange juice, for example, and numbers of coliforms are increased greatly by the inclusion of fruits with soft rots. Heating grapes before extraction reduces numbers of organisms in the expressed juice, but pressing introduces contamination.

Contamination by Equipment: The kinds of microorganisms from equipment will depend on the product being processed, for that product will constitute the culture medium for the organisms. Thus, pea residues would encourage bacteria that grow well in a pea medium and in tomatoes those organisms which can develop in tomato juice. As the equipment is used throughout the day, the organisms can continue to build up. At the end of the run, however, when the equipment is cleaned and sanitized, the total numbers of microorganisms thereon are greatly reduced, and if the operation is efficient, only the resistant forms survive. Therefore, spores of bacteria are likely to survive, and if conditions for growth are present while the equipment is idle, these spore formers may increase in numbers, especially in poorly cleaned parts. The thermophilic spore formers so troublesome to canners of vegetables build up in this manner and add to the difficulty of giving the foods an adequate heat process. The numbers of such organisms on poorly cleaned and sanitized equipment may be high at the start of a day’s run and decrease as the day progresses, but the reverse usually is true. A layoff during the run permits a renewed increase in numbers. It is obvious that the numbers of microorganisms that enter foods from equipment depend on the opportunities given to these organisms for growth and that these opportunities are the result of inadequacy of cleaning and sanitizing, combined with favourable conditions of moisture and temperature for an appreciable period of time.
Contamination and Spoilage of Cereals

Harvested grains are infected by microorganisms from soil and insects. Grains are infected by bacteria which belong to *Pseudomonadaceae* and *Lactobacillaceae*. Washing the grains remove some of the microorganisms. The milling processes further reduce the number of organisms. Bacteria in wheat flour contain spores of *Bacillus*. A freshly baked loaf of bread is practically free of viable microorganisms, but is subject to contamination by mold spores from air during cooling and before wrapping. During slicing, contamination takes place from microorganisms in the air. The contamination of grains and cereal products with moulds is a major concern because of the presence of mycotoxins. To reduce contamination by moulds and to avoid conditions which allow their growth is a major challenge for microbiologists undertaking research activity in the field.

Cereals are important foods which provide bulk of our dietary requirements. They are also source of carbohydrates which are metabolized by body for energy generation. Besides, cereals also provide minerals, proteins and vitamins.

Type of Cereal Products

- **Whole Cereals:** Only the husk of the grain is removed, for example rice, wheat, gram, lentils, etc.
- **Milled Grain Products:** Made by removing the bran and usually the germ of the seed and then crushing the kernel into various sized pieces, for example wheat flour, maida, semolina (rawa), etc.
- **Processed Cereals:** Weaning food, breakfast cereals, breads, etc.
- **Ready Mixes:** Cake and bakery products, pasta, macaroni.

Various spoilage factors influencing the quality of cereals are:

- **Physical:** Physical losses are caused by spillages, which occur due to use of faulty packaging materials.
- **Physiological:** Physiological losses include respiration and heating in grains, temperature, humidity and oxygen.
- **Biological:** Biological losses occur due to micro-organisms, insects, rodents, natural microflora of harvested grains.

Cereal Grains and Meals

Due to low moisture content grains and flours usually have long shelf life if these are properly harvested or stored under proper conditions where microbial growth is not supported. Moulds can rapidly grow on grains and corn when these products are stored in at high temperature and humidity. For example, ear rot of corn (Refer Figure 4.7) can result in major economic losses. The spoilage mainly occurs due
to moisture absorption during storage leading to fungal growth. Wet mash of the
grains or a mash of the meals will undergo an acid fermentation by lactic acid
bacteria and coliform normally present on plant surfaces. This is followed by
alcoholic fermentation by yeasts as soon as the acidity has increased enough to
favor them. Finally moulds will grow on the top surfaces, where air is available.
Before bulk packaging and storage, the whole grains are fumigated to reduce
microbial load and increase storage period.

Fig. 4.7 Fungal Spoilage of Corn

Major factors involved in spoilage of stored grains by moulds are:
- Type and number of microorganisms.
- Moisture content of more than 12-13%.
- Storage temperature.
- Physical damage.

Microorganisms Spoiling Cereal Grains

Common bacterial genera spoiling grains are *Pseudomonas, Micrococi, Lactobacillus* and *Bacillus*.

Common moulds spoiling grains:
- *Aspergillus, Penicillium, Rhizopus, Mucor, Fusarium*.
- Many of these moulds can produce mycotoxins. For example: Stem rot
  and head blight of wheat (Refer Figure 4.8) and barley caused by *Fusarium
culmorum* and *Fusarium graminearum*. 
Contamination and Spoilage

Contamination of Grains by the Ascomycete: *Claviceps purpurea* causes ergotism, a toxic condition. Hallucinogenic alkaloids produced by this fungus can lead to altered behavior, abortion, and death if infected grains are eaten.

**Spoilage of Flours**

The process of flour making such as washing and the milling reduce the microbial content and hence microbial load. Moisture content of less than 15% does not allow growth of moulds. Most moulds and bacteria in flours can grow only above 17% moisture, thus moistening of flours is essential for spoilage by microbes. Because of the variations in microbial content of different lots of flour, different type of spoilage result. If acid-forming bacteria are present, an acid fermentation begins, followed by alcoholic fermentation by yeasts and then acetic acid by *Acetobacter species*.

In the absence of lactis and coliforms, micrococci have been found to acidify the paste, and in their absence, species of *Bacillus* produce lactic acid, gas, alcohol, acetone, small amount of esters and other aromatic compounds, which develop characteristic odor.

**Spoilage of Bread**

Bread is a major product prepared using flours. Dough is prepared from flours which undergo fermentation for which desirable microorganisms must grow. If this fermentation exceeds the required limits, it causes souring. Excessive growth of proteolytic bacteria reduces the gas holding capacity which is otherwise required for dough rising (Refer Figure 4.9).
Bacterial Spoilage of Bread

Following are the bacterial spoilage of bread:

**Ropiness in Bread**

- The chief causative organism is mucoid variant of *Bacillus subtilis* or *B. licheniformis*, with their spores surviving baking temperatures (not more than 100°C). These spores can germinate once they get favourable conditions.
- The ropy condition is the result of capsulation of the bacillus, together with hydrolysis of the flour proteins (gluten) by proteinases of the organism and of starch by amylase to give sugars that encourage rope formation.
- In one stage the slimy material can be drawn out into long threads when the bread is broken and pulled apart.

**Chalky Bread**: This spoilage is characterized by development of white chalk-like spots, which are caused by growth of yeast-like fungi *Endomycopsis fibuligera* and *Trichosporon variable*.

**Red or Bloody Red Bread**: Due to the growth of bacteria *Serratia marcescens* which produces brilliant red color on starchy foods giving blood-like appearance.

Mould Spoilage of Bread

Following are the mould spoilage of bread:

**Green Spored Mould**: The green growth most likely is *Penicillium expansum* or *P. stoloniferum*, *A. niger* with its greenish or purplish brown to black conidal heads and yellow pigment diffusing into the bread.

**Black Mould**: The black growth is characteristic of *Rhizopus stolonifer* with its white cottony mycelium and black dots of sporangia.

**Red Bread Mould**

- *M. (Neurospora) sitophila*, whose pink conidia give a pink or reddish color to its growth.
- A red color in the crumb of dark bread has been caused by *Geotrichum aurantiacum*. 
Mold Spoilage is favored by

- Heavy contamination after baking, due to air heavily laden with mold spores, a long cooling time, considerable air circulation, or a contaminated slicing machine.
- Slicing in that more air is introduced in to the leaf.
- Wrapping, especially when bread is still warm.
- Storage in a warm humid place.

**Contamination and Spoilage of Fish**

Fish is a very perishable food due to its high nutritional content. Fish have a high content of non-protein nitrogen, and autolytic changes caused by their enzymes increase the supply of nitrogenous foods (amino acids and amines) and glucose for bacterial growth, which produce trimethylamine, ammonia, lower fatty acids, aldehydes, hydrogen sulfides, mercaptans, indoles which are indicative of putrefaction.

**Storage and Processing Conditions also affect Microbial Growth**

- Chilling temperature: *Pseudomonas, Acinetobacter, Moraxella* and *Flavobacterium*.
- Higher temperature: *Micrococcus, Bacillus*.
- Packing under carbon dioxide and addition of low concentrations of sodium chloride favor growth of lactic acid bacteria and *Photobacterium phosphoreum*.
- Heavily wet-salted fish support growth of yeasts.
Contamination and Spoilage

- Salt fish are spoiled halotolerant or by halophilic bacteria of the genera *Serratia*, *Micrococcus*, *Bacillus*, *Alcaligenes*, *Pseudomonas*.
- Smoked fish are mainly spoiled by moulds.
- Addition of organic acid select for lactic acid bacteria and yeasts.
- A musty or muddy odor and taste of fish has been attributed to the growth of *Streptomyces* species in the mud at the bottom of the body of water and the absorption of the flavor by the fish.
- Pasteurization kills vegetative bacteria but spores of *Clostridium* and *Bacillus* survive and may grow, particularly in unsalted fish.

Discoloration of the Fish Flesh may Occur during Spoilage

- Yellow to greenish yellow colors caused by *Pseudomonas* fluorescence, yellow micrococci.
- Red or pink colors from growth of *Sarcina*, *Micrococcus*, *Bacillus* sp. or by moulds or yeasts.
- Chocolate brown color by *Asporogenous* yeast.

Contamination and Spoilage of Seafoods

The term seafood covers fish, shellfish, crabs, shrimps, oysters, clams, mollusks from all waters–fresh, marine, warm, or cold. Most shrimp, crabs, crawfish, lobsters, and other crustaceans are consumed fully cooked, and hence most bacteria, viruses, and parasites are either inactivated or reduced to a level at which infection may not occur. However, foods that are transported and consumed in an uncooked state are an increasingly important source of food-borne infection. The problem becomes more serious because of rapid movement of people and products around the world. International trade in uncooked foods, aided by rapid air transport, provides many opportunities for disease transmission.

Many molluscan shellfish (primarily oysters, clams, and mussels) are customarily consumed raw or with minimal heating and have caused foodborne illness outbreaks and mortality. Raw sewage can contaminate shellfish-growing areas; in addition, waterborne pathogens such as *Vibrio* are more prevalent in the water column during the warm months (for example, in Chesapeake Bay on the mid-Atlantic coast of the United States). Viruses also can be a problem. Oysters are filter feeders that process several liters of water per day, leading to the potential concentration of at least 100 types of enteric viruses. Heavy rainfall in shellfish areas can cause runoff of pathogens from adjacent septic systems and contaminate coastal waters. Often it is necessary to ban shellfish harvesting until the animals void pathogens from their digestive systems. Alternatively, shellfish from contaminated areas can be moved to clean waters to allow them to clean their digestive systems.
### Table 4.1 Organism Spoiling Seafoods

<table>
<thead>
<tr>
<th>Type of Seafood</th>
<th>Spoiling Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shell Fish</td>
<td>Spoiled by Acinetobacter, Moraxella and Vibrio.</td>
</tr>
<tr>
<td>Chilled Shrimps</td>
<td>Acinetobacter, Moraxella, and Vibrio.</td>
</tr>
<tr>
<td>Crabmeat</td>
<td>At chilling temperature- deteriorated by <em>Pseudomonas, Acinetobacter, Moraxella.</em></td>
</tr>
<tr>
<td></td>
<td>At higher temperature- Proteus.</td>
</tr>
<tr>
<td>Raw Lobsters</td>
<td><em>Pseudomonas, Alcaligenes, Flavobacterium, Bacillus.</em></td>
</tr>
<tr>
<td>Oysters, and Clams</td>
<td>Fecal coliforms like <em>Escherichia, fecal streptococci, S. aureus,</em></td>
</tr>
<tr>
<td></td>
<td><em>Salmonella enterica, serovar enteritidis and typhimurium.</em></td>
</tr>
<tr>
<td>Crabs and Oysters</td>
<td>May contain species of <em>Vibrio,</em> including <em>V. parahaemolyticus</em></td>
</tr>
<tr>
<td>Smoked Salmon and Shrimps</td>
<td>Pathogenic <em>L. monocytogenes</em></td>
</tr>
</tbody>
</table>
| Oysters             | Oysters remain in good condition as long as they are kept alive in the shell at chilling temperature, but they decompose when they are dead. Oysters are high in protein and sugars.  
  - At temperature near freezing- *Pseudomonas, Acinetobacter, Moraxella.*  
  - At higher temperature- souring may be the result of the fermentation of the sugars by coliform bacteria, streptococci, lactobacilli, and yeast to produce acid and sour odour.  
  - Spoilage by an asporogenous yeast cause pink oyster.                     |

### Contamination and Spoilage of Meat Products

Meat is as an ideal medium for many organisms because:

- **Moisture Content**: Surface of the meat may be little moist to allow mold growth, still moisture to encourage yeasts, and very moist to favor bacterial growth.
- **A Higher pH**: favourable pH for most microbial growth.
- **Nutritional Content**: rich in nitrogenous foods, supplied with minerals and accessory growth factors. Also has some fermentable carbohydrates (glycogen).

Spoilage organisms can grow on fresh, cured, dried or cooked meats

#### Spoilage in Fresh Meat

Muscles of healthy animals do not contain any bacteria or fungi but as soon as animals are slaughtered, meat is exposed to contaminants. The number of spoilage organisms on meat just after slaughter is a critical factor in determining shelf life. Chopping and grinding of meats can increase the microbial load as more surface area is exposed and more water and nutrients are available.
Contamination and Spoilage

NOTES

- Pseudomonas sp. is the predominant spoilage bacteria in aerobically stored raw meat and poultry.
- Shewanella putrefaciens can produce sulfides and ammonia, which not only smell bad but also cause color changes in meat.
- Brochothrix thermosphacta is often a significant spoilage organism on fresh meat stored aerobically at refrigeration temperatures.
- Enterobacteriaceae, particularly species of Serratia, Enterobacter, and Hafnia, are major causes of spoilage in vacuum-packed, high pH fresh meats. These organisms are facultative anaerobes that produce organic acids, hydrogen sulfide and greening of meats.
- Lactic acid bacteria grow on meat and poultry packaged under vacuum and modified atmospheres, producing organic acids from glucose by fermentation. This gives rise to off-odors which may be accompanied by gas and slime formation and greening of meat.
- Psychrophilic, anaerobic Clostridium spp. are associated with spoilage of vacuum-packaged meats. ‘Blown pack’ meat spoilage is characterized by excessive gas formation with off odors due to formation of butyric acid, butanol and sulfurous compounds.
- Yeasts and moulds grow relatively slowly on fresh meat and do not compete well with bacteria. Therefore, they are a minor component of spoilage flora.

Processed Meat

Curing is employed for flavor and color development. Salt serves to prevent microbial growth during and after curing. Nitrite or nitrate serves to stabilize red meat color, contribute to cured meat flavor, retard rancidity, and prevent the germination of clostridial spores. The isomers sodium ascorbate and erythorbate are used to stabilize color, to speed curing, and to make the cure more uniform. Sugar is involved for color stabilization, flavoring, and substrate for lactic fermentation, also moderating the harsh flavor of sodium chloride. Phosphates are used in most pumped meats (bacon, ham, roast beef, pastrami, etc.) to increase water binding. All curing ingredients may be expected to contain microorganisms, and care should be taken to ensure that undesirable ones are not introduced to products during ingredient application.

Smoking

Primary purposes of smoking meat are:
- Development of aroma and flavor.
- Preservation.
- Creation of new products.
- Development of color.
- Formation of a protective skin on emulsion-type sausages.
- Protection from oxidation.
The antimicrobial properties of smoking result from the activities of some of the smoke ingredients and the heat that is associated with wood smoking.

## Spoilage in Processed Meat

Spoilage organisms can grow on processed and cooked cured meats, so they are best stored chilled, under a vacuum or modified atmosphere.

- **Pseudomonas** spp. are not usually important causes of spoilage in processed meats because of their sensitivity to curing salts and heat pasteurization and their inability to grow well in meats packed with a vacuum or high carbon dioxide atmosphere. However, when packages have been opened and there has been insufficient curing, these bacteria may spoil refrigerated processed meats.

- Some cold- and salt tolerant *Enterobacteriaceae* have been found to cause spoilage in some specific processed meats, such as ham or bacon.

- Lactic bacteria produce sour off-flavors, gas, slime, and greening, and this spoilage may be more severe than in fresh meat because of the presence of added carbohydrates. Competitive ability of different LAB strains is related to pH and water activity of the meat, cooking and storage temperatures and oxygen and carbon dioxide levels.

- Sporeformers (*Clostridium* and *Bacillus*) are usually not a spoilage problem in processed meats because of the presence of nitrite and other curing salts. However, faulty cooking/cooling procedures, including long cooling periods and temperature abuse, has allowed growth of these organisms in some cases. Spores of these organisms may be introduced with spices or other ingredients.

- Yeasts cause some spoilage in processed meats but are generally only important when sulfite is used as a preservative or when meats have been irradiated or are stored aerobically in the cold.

### Table 4.2 General Types of Spoilage of Meats

<table>
<thead>
<tr>
<th>Condition</th>
<th>Products Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface Slime</td>
<td>Caused by species of <em>Pseudomonas</em>, <em>Acinetobacter</em>, <em>Moraxella</em>, <em>Alcaligenes</em>, <em>Streptococcus</em>, <em>Leuconostoc</em>, <em>Bacillus</em> and <em>Micrococcus</em>. Temperature and the availability of moisture influence the kind of microorganisms causing surface slime. At chilling temperatures, high moisture will favor the <em>Pseudomonas</em>- <em>Alcaligenes</em> group; with less moisture, micrococci and yeast will be encouraged; and with still less moisture, moulds may grow. At room temperatures, micrococci and other mesophilic grow well.</td>
</tr>
<tr>
<td>Changes in Fat</td>
<td>The unsaturated fat in meat gets oxidized by lipolytic species of <em>Pseudomonas</em> and <em>Achromobacter</em> or by yeasts which produce off odours (rancidity) due to hydrolysis of fats and production of aldehydes and acids.</td>
</tr>
<tr>
<td>Off Odors and Off Tastes</td>
<td>Volatile acid like formic, acetic, butyric, and propionic acid produce sour odor. Cold storage flavor or stale flavor. <em>Actinomyces</em> produce musty or earthy flavor. Yeast also cause sliminess discoloration and off odor and taste defects.</td>
</tr>
</tbody>
</table>
Contamination and Spoilage

NOTES

Change in Colour of Meat Pigments
The red colour of meat called its “bloom” may be changed to shades of green, brown or gray as a result of the production of oxidizing compounds like peroxides or by H2S production by bacteria. Lactobacillus and Leuconostoc sp. cause the greening of the sausage.

Surface Colour Change
The red pigment producing bacteria, Aerobacter aerogenes, caused red spots on meat. Blue color surface is caused by Pseudomonas syringae yellow discoloration is caused by Micrococcus or Flavobacterium. Chromobacterium lividum give greensh-blue to brownish-black spots on stored beef. The purple “stamping ink” discoloration of surface fat is caused by yellow pigmented cocci and rods. When the fat become rancid and peroxides appear the yellow color changes to greenish shade and later becomes purplish to blue.

Yeast Spoilage: Under aerobic condition yeasts may grow on the surface of meats, causing sliminess, lipolysis, off-odors and tastes, and discoloration - white, cream, pink or brown - due to pigments in the yeast.

Aerobic Growth of Moulds may cause stickiness, whiskers (when meat is stored near freezing temperature, a limited amount of mycelia growth may take place without sporulation. Such a fuzzy white growth can be caused by a number of moulds including Thermidium chaetoalvei, Mucor, Rhizopus), black-spot (Cladosporium herbarum), white-spot (Sporotrichum carnis), green patches (species of Penicillium), off odor and off taste.

B. Spoilage Under Anaerobic Condition
Souring
Sour odor and taste caused by production of formic, acetic, butyric, propionic and higher fatty acids or other fatty acids such as lactic or succinic. Souring can occur by a) action of the meat’s own enzymes during aging or ripening b) anaerobic production of fatty acids or lactic acids by bacterial action c) proteolysis without putrefaction caused by facultative or anaerobic bacteria and sometimes called ‘stinking sour fermentation’.

Putrefaction
True putrefaction is the anaerobic decomposition of proteins with the production of foul smelling compounds like hydrogen sulphide, mercaptans, indol, scatol, ammonia and amines. It is usually caused by species of Clostridium, but facultative bacteria also may cause or assist in putrefaction such as Pseudomonas and cloacilenes (P. putrefaciens). Proteus are also putrefactive.

Taint
Inexact word applied to any off-taste or off-odor. The term “bone taint” of the meat refers to either souring or putrefaction near to the bones, especially in hams. Usually it means putrefaction.

Contamination and Spoilage of Eggs

Spoilage of Egg
The hen’s egg is an excellent example of a product:

Factors that Protect Egg from Spoilage
There are several extrinsic and intrinsic mechanisms through which the egg protects itself from the microbial invasion:

- The outer waxy shell membrane; the shell; and the inner shell membrane, each is effective to some degree in retarding the entry of microorganisms.
- Internally, lysozyme is present in egg white, which is quite effective against Gram-Positive Bacteria.
- Egg white also contains avidin, which forms a complex with biotin, thereby making this vitamin unavailable to microorganisms.
- In addition, hen egg albumen contains ovotransferrin, which chelates metal ions, particularly Fe, and ovoflavoprotein, which binds riboflavin. At its normal pH of 9–10, egg albumen is cidal to Gram-positive bacteria and yeasts at both 30 degree C and 39.5 degree C.
Factors Promoting Spoilage

- The nutrient content of the yolk material.
- pH in fresh eggs (about 6.8) make it an excellent source of growth for most microorganisms.

**Table 4.3 Spoilage of Eggs by Bacteria and Moulds**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Spoilage of Eggs by Bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>Fresh eggs are generally sterile particularly the inner contents. However, breaks or cracks in egg shell taking place due to transportation or mechanical damage may allow microorganisms to enter in to the egg yolk and cause spoilage on storage.</td>
<td></td>
</tr>
<tr>
<td><strong>2. Different Types of Rots</strong></td>
<td></td>
</tr>
<tr>
<td>Green Rot caused by <em>Pseudomonas fluorescens</em>, a bacterium that grows at 0 degree C. The white of egg is bright green color. This stage is noted with difficulty in candling but visible when the egg is broken. The content of egg fluoresce strongly under ultraviolet light.</td>
<td>Colorless Rot caused by <em>Pseudomonas, Acinetobacter, Alcaligenes, certain coliform bacteria</em>. These dots are detected readily by candling, for the yolk usually is involved, except in very early stages, disintegrates or at least shows a white incrustation. The odor varies from a scarcely detectable one to fruity to highly offensive.</td>
</tr>
<tr>
<td>Black Rots caused by many species of <em>Proteus, some species of Pseudomonas and Aeromonas</em>. Eggs are almost opaque to the candling lamp, because the yolk become blackened and then break down to give the whole egg contents a muddy brown color and also there is strong putrid odor due to the formation of H2S.</td>
<td>Pink Rots are caused by strains of <em>Pseudomonas</em> and may at times be a later stage of some of the green rots. They resemble the colorless rots, except for a pinkish precipitate on the yolk and a pink color in the white.</td>
</tr>
<tr>
<td>Red Rots are caused by species of <em>Serratia</em>, are mild in odor and are not offensive.</td>
<td></td>
</tr>
<tr>
<td><strong>B. The Spoilage of Eggs by Fungi</strong></td>
<td></td>
</tr>
<tr>
<td>In storage atmosphere of high humidity a variety of moulds may cause superficial fungal spoilage, first in the form of a fuzz or whiskers covering the shell and later as more luxuriant growth.</td>
<td></td>
</tr>
<tr>
<td>Egg are stored usually near-freezing temperature, which is high enough for slow mycelial growth of some moulds but too low for sporulation, while other moulds may produce asexual spores. Moulds causing spoilage of eggs include species of <em>Penicillium, Cladosporium, Sporotrichum, Mucor, Thamnidium, Botrytis</em>, and <em>Alternaria</em>.</td>
<td></td>
</tr>
</tbody>
</table>

**The Spoilage of Eggs by Fungi goes Through Stages of Mold Growth**

- Very early mold growth is termed pin-spot molding because of small, compact colonies of mold appearing on the shell and usually just inside it. The color of these pin spots varies with the kind of mold spoiling the egg: *Penicillium* species cause yellow or blue or green spots inside the shell. *Cladosporium* species give dark-green or back spots, and species of *Sporotrichum* produce pink spots (Refer Figure 4.10).
The final stage of spoilage by moulds is fungal rotting, after the mycelium of the mold has grown through the pores or cracks in the egg. Jellying of the white may result and colored rots may be produced, for example fungal red rot by *Sporotrichium* and a black color by *Cladosporium*.

The hyphae of the mold may weaken the yolk membrane enough to cause it to rupture after which the growth of the mold is stimulated greatly by the food released from the yolk.

Approximately 31% of meat consumed worldwide is poultry. The two principal types of poultry meat consumed are chicken and turkey. The shelf life of poultry depends on a combination of factors, including the number and types of microorganisms initially present, storage temperature, atmosphere and packaging conditions. The chilling process before packaging, whether by air or water, influences the type and number of contaminants on the carcass in the package. It is important to freeze the poultry fast in order to keep it in good condition for several months. Freezing further reduces the number of microorganisms in the poultry meat provided the temperature is maintained quite low (~18 degree C or below).

**Spoilage**

Most bacterial growth takes place on the surfaces, i.e., the skin, the lining of the body cavity, and any cut surfaces, and the decomposition products diffuse slowly into the meat. Isolates from poultry and poultry products include members of numerous genera (Refer Table 4.4)
**Table 4.4 Microbes Involved in Spoilage of Poultry**

<table>
<thead>
<tr>
<th>Product</th>
<th>Microbes Isolated from Poultry</th>
<th>Microorganisms Isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incoming Birds</td>
<td>Bacteria: Acinetobacter, Moraxella, Pseudomonas, Flavobacteria,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Staphylococcus, Micrococcus</td>
<td></td>
</tr>
<tr>
<td>After Processing</td>
<td>Bacteria: Acinetobacter, Moraxella, Pseudomonas, Flavobacteria,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Staphylococcus, Micrococcus, Cytophage, Entero bacter, Alcaligenes,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salmonella, Campylobacter</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yeast: Trichosporon, Torulopsis, Candida, Rhodotorula,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moulds: Penicillium, aspergillus, Alternaria</td>
<td></td>
</tr>
</tbody>
</table>

**Microbes Involved in Spoilage of Refrigerated Poultry**

<table>
<thead>
<tr>
<th>Product</th>
<th>Microbes Isolated from Poultry</th>
<th>Microorganisms Isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Eviscerated Carcasses Held at 10 degree C Or Below</td>
<td>Bacteria: Spoiled mostly by bacteria, such as Pseudomonas fluorescens, P. putida, Acinetobacter, Moraxella.</td>
<td></td>
</tr>
<tr>
<td>Above, 10 degree C</td>
<td>Yeast: Spoiled less extent to Torulopsis and Rhodotorula, micrococii usually predominate, there also is growth of Alcaligenes and Flavobacterium.</td>
<td></td>
</tr>
<tr>
<td>Dark Meat, pH 5.8-6.7</td>
<td>Bacteria: Acinetobacter, Alteromonas, Pseudomonas.</td>
<td></td>
</tr>
<tr>
<td>White Meat, pH 5.7-5.9</td>
<td>Yeast: Pseudomonas and others.</td>
<td></td>
</tr>
<tr>
<td>Chicken Wrapped in Oxygen-impermeable Films</td>
<td>Microaerophilic bacteria, lactic acid bacteria, and others.</td>
<td></td>
</tr>
<tr>
<td>Vacuum-Packaged Chicken</td>
<td>Yeast: Entero bacter and others.</td>
<td></td>
</tr>
<tr>
<td>Iced, Cut-Up Poultry</td>
<td>Yeast: Often develops a slime accompanied by an odor described as tainted, acid, sour, and dishraggy. This effect is caused chiefly by species of Pseudomonas, Alcaligenes also may be concerned.</td>
<td></td>
</tr>
</tbody>
</table>

**Contamination and Spoilage of Canned Food**

Canning is a method of preserving food in which the food contents are processed and sealed in an airtight container made up of tin (tin can), wrought iron canisters. The idea is to make food available and edible long after the processing time. This is an important method of packaging food for long term storage. Normally food is stored in metallic containers along with heat treatment. The heat treatment varies depending upon type of food. There is always a chance that microorganisms may survive if the heat treatment is not proper thereby leading to spoilage of food. Although the canning process is usually designed to provide a sterile product, spoilage in these products does occur as a result of:

- Preprocess Spoilage
- Inadequate Processing
- Post-Processing Contamination

To prevent the food from being spoiled before and during containment, a number of methods are used: pasteurisation, boiling (and other applications of high temperature over a period of time), refrigeration, freezing, drying, vacuum treatment,
antimicrobial agents that are natural to the recipe of the foods being preserved, a sufficient dose of ionizing radiation, submersion in a strong saline solution, acid, base, osmotically extreme (for example very sugary) or other microbially-challenging environments.

**Causes of Spoilage in Canned Foods**

Usually the incidences of food spoilage in cans are low. The spoilage of can could be due to biological or chemical reasons or combination of both. The biological spoilage is primarily due to microbial growth while chemical spoilage is due to hydrogen produced due to reaction of acid in food and iron on can. The degree of swelling can also be increased by high summer temperature and high altitudes. Certain other factors, such as overfilling, buckling, denting or closing the can while cool may also be responsible for spoilage of foods in cans.

**Chemical Spoilage**

The chemical spoilage in most cases is due to production of hydrogen gas produced in can because of action of acid of food on iron of can. This spoilage is termed as Hydrogen swell. It occurs due to following factors:

- Increased storage temperature.
- Increased acidity of food.
- Improper exhaust.
- Presence of soluble sulfur and phosphorous compounds.
- Improper timing and lacquering of can at internal surfaces.

**Biological Spoilage**

The cause of biological spoilage is microbial activity. In heat treated cans, the growth of microorganisms occur due to:

- **Leakage of Can:** It occurs because of manufacturing defects, punctures or rough handling. Bacteria are introduced into can by either in holes or improper seams. In this type, the microorganisms are not usually heat resistant and wide array of organisms had been found to cause spoilage as it is post processing contamination. Microbes may also get entry into can due to cold water, used to cool cans after heat treatment. Leakage may also be responsible for release of vacuum, which can favor the growth of microorganisms.

- **Survival of Organisms after the Administration of Heat Treatment:** It includes sub-optimal heat treatment, faulty retort operations, excessive microbial load and contamination in product, change in consistency of the product.
Microbial Spoilage of Canned Food

Spoilage by Thermophilic Spore Forming Bacteria: Spoilage by these types of bacteria is most prevalent in under processed heat treated canned foods. Their spores survive the heat treatment and undergo vegetative cell formation and subsequent growth in canned conditions. The spores of thermophilic bacteria do not germinate to cause spoilage in heat-treated canned products stored at 30°C or below. If can temperature increases to 40°C or high, the spores germinate, multiply, and spoil the canned food.

Flat Sour Spoilage

- This is caused by souring bacteria. Name is derived from the fact that the ends of can remain flat during souring, or the development of lactic acid in the food by the flat sour bacteria.
- Gas is not produced (therefore flat), only acid is produced.
- Because the can retains a normal outward appearance, the detection of spoilage from outside is not possible thereby culturing of contents become necessary to detect the type of organisms.
- Occurs more frequently in low acid foods, such as peas and corns and is caused by Bacillus stearothermophilus (spores of thermophiles are more heat resistant and may serve the heat process to cause flat sour spoilage). Flat sour spoilage of acid foods, such as tomatoes or tomato juice is caused by facultative thermophilic Bacillus coagulans.
- Can do not bulge (flat) but pH falls and food tastes sour, off odors may be apparent.

TA Spoilage

- The bacterium causing this type of spoilage has been nick named TA, which is short for thermophilic anaerobe not producing H₂S or for the species of Clostridium thermosaccharolyticum.
- It produces acid and gas in foods.
- The gas, a mixture of CO₂ and H₂, swells the can
- Spoiled food produces sour or cheesy smell
- Blackening of the food may also be observed.

Sulfide or Sulfur Stinker Spoilage

- Caused by Desulfotomaculum nigeriensis.
- The spores of this bacterium have considerably less heat resistance than those of flat sour and TA bacteria, hence their appearance in canned foods is indicative of gross underprocessing.
Contamination and Spoilage

NOTES

- This type of spoilage occurs in low acid foods and H$_2$S, formed in canned peas or corn, is evident by odor when the can is opened. In corn, a bluish gray liquid is evident in which blackened germs and grey kernels of corn float. Peas usually give the H$_2$S odour but without any TA bacteria, but manure can also be an original source.

Spoilage by Mesophilic Spore formers: Spoilage by mesophilic microorganisms results from underprocessing and is caused by spore forming bacteria of the genera Bacillus and Clostridium.

Spoilage by Mesophilic Clostridium Species

- Species of Clostridium may be sugar fermenting, for example *C. butyricum* and *C. pasteurianum* and cause by butyricum acid type of fermentation in acid or medium acid foods, with swelling of the container by the carbon dioxide and hydrogen gas produced.
- Because of the spores of clostridia have a comparatively low heat resistance, spoilage by these anaerobes take place most commonly in canned foods which have been processed at 100 degree C or less.
- Thus canned acid foods, such as pineapple, tomatoes, and pears are spoiled by *C. pasteurianum*. Such spoilage is more likely when the pH of the food is above 4.5. The putrefactive anaerobes grow best in the low acid canned foods, such as peas, corns, meat, fish, poultry, bit on rare occasion may spoil other foods.

Spoilage by Mesophilic Bacillus Species

- Spores of *B subtilis* and *B mesentericus* survive in low acid home canned foods that had been given a heat processing at 100 degree C.
- Commercial canned foods have been spoiled by *Bacillus* species, especially in poorly evacuated cans.
- Foods so spoiled have been mostly canned sea foods, meats and evaporated milks.
- Entrance may have been through a leak in the container. Spores of these bacteria have about the same heat resistance as those of *Clostridium pasteurianum*.

Spoilage by Non-Spore Forming Bacteria: Presence of non-spore formers in cans indicate post processing contamination or the bacteria entered the container through a leak.

- Vegetative cells of some kind of bacteria are fairly heat resistant in that they can withstand pasteurization. Among these thermoduric bacteria are the enterococci, *Streptococcus thermophilus*, some species of *Micrococcus* and *Lactobacillus* and *Microbacterium*. 
- Acid forming *Lactobacillus* and *Leuconostoc* species has been found growing in underprocessed tomato products, pears and other fruits.
- Micrococci have been reported in meat paste and in similar product with very poor heat penetration and *S. faecalis*, or *S. faecium* is often present in canned hams that are only partially sterilized and may be responsible for spoilage on storage.

**Spoilage by Fungi**

**Yeasts**
- Yeasts and their spores are not thermo tolerant, thus they are not found in suitably heat treated cans.
- Their presence indicates under processing or post pasteurization contamination through leakage.
- Fermentative yeasts are more prominent and they produce carbon dioxide, thus causing swelling of cans.
- Film yeasts too can grow on the surface of the food products.

**Moulds**
- *Aspergillus* and *Penicillium* are most spoiling organisms.
- These can grow at high sugar concentration.
- Acidification is considered method of preventing growth of moulds.
- Some of the moulds are resistant to heat.
- Moulds are more common in home canned foods where heating as well as sealing is not under total aseptic conditions.

*Fig. 4.11 Swelling of Can and a Normal Can*
Table 4.5 Classification of Canned Foods Based On pH Profile of the Organism

<table>
<thead>
<tr>
<th>pH</th>
<th>Low Acid Foods</th>
<th>Acid Foods</th>
<th>High Acid Foods</th>
</tr>
</thead>
</table>
| pH >4.6 | Meat, milk, many vegetables, etc.                                              | Vegetables, fruits, etc.                                                  | Fruits, pickled vegetables or other foods to which acid
                                                                                           |                                                                          |                                                       |
| pH <4.0 | Tomatoes, pears, etc.                                                          | Fruits, pickled vegetables or other foods to which acid                    | Spores can germinate, grow and cause spoilage.      |
|         |                                                                               | additives have been added                                                 |                                                      |
| Microbial |                                                                      |                                                                          |                                                     |
| Organism | C. botulinum, S. aureus enterotoxins                                         | Spores are usually due to nonspore forming mesophiles like yeasts,         |                                                      |
|         |                                                                               | molds and lactic acid bacteria.                                            |                                                      |
|         |                                                                               |                                                                          |                                                     |
| Sterilization |                                          |                                                                          |                                                      |
|         | Need sterilization under high temperature (116-130 °C)                      |                                                                          |                                                      |
|         |                                                                               |                                                                          |                                                      |

Check Your Progress

5. Give the type of cereal products.
6. What are the various spoilage factors that influence the quality of cereals?
7. What are the major factors involved in spoilage of stored grains by moulds?
8. What causes discoloration of the fish flesh during spoilage?

4.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Food contamination refers to the presence of harmful chemicals and microorganisms in food, which can cause consumer illness. This article addresses the chemical contamination of foods, as opposed to microbiological contamination, which can be found under foodborne illness.

2. Food spoilage is the process where a food product becomes unsuitable to ingest by the consumer. The cause of such a process is due to many outside factors as a side-effect of the type of product it is, as well as how the product is packaged and stored. Due to food spoilage, one-third of the worlds’ food produced for the consumption of humans is lost every
year. Bacteria and various fungi are the cause of spoilage and can create serious consequences for the consumers, but there are preventative measures that can be taken.

3. Generally, the following criteria are used for measuring the fitness of food:
   - Desired stage of maturity: Fruits should be consumed at a specific stage of maturity when they are just ripe. Similarly, sweet corn should be young enough to be tender and milky in character. Poultry should also be consumed young.
   - Pollution free: If fertilized with sewage, vegetables should not be consumed raw. Oysters that have been obtained from contaminated water should not be consumed. Food contaminated by flies or rodents should be spurned.
   - Microbial attack or enzymes action: Sometimes there is very little difference between harmless growth and spoilage by microorganisms. The same type of food change may be considered sometimes desirable and sometimes undesirable. For example, some people may consider sour milk as spoilt, but may easily consume cultured butter milk. Meat purification means spoilage, while putrefactive changes in Limburger cheese is considered normal in the ripening process.

4. The following are the common characteristics of spoilt food:
   - Slime: In this case, as bacteria touch one another, the food becomes slimy.
   - Sour: In this case, food goes sour when microorganisms produce acids.
   - Discolored: This occurs due to microbial growth.
   - Gas: Bacteria sometimes produce gas as a byproduct when there is a microbial growth.
   - Odour: Due to the breakdown of bacterial cells, there is a rotten smell.

5. Following are the type of cereal products:
   - Whole Cereals: Only the husk of the grain is removed, for example rice, wheat, gram, lentils, etc.
   - Milled Grain Products: Made by removing the bran and usually the germ of the seed and then crushing the kernel into various sized pieces, for example wheat flour, maida, semolina (rawa), etc.
   - Processed Cereals: Weaning food, breakfast cereals, breads, etc.
   - Ready Mixes: Cake and bakery products, pasta, macaroni.
6. Various spoilage factors influencing the quality of cereals are:
   - Physical: Physical losses are caused by spillages, which occur due to use of faulty packaging materials.
   - Physiological: Physiological losses include respiration and heating in grains, temperature, humidity and oxygen.
   - Biological: Biological losses occur due to micro-organisms, insects, rodents, natural microflora of harvested grains.

7. Major factors involved in spoilage of stored grains by moulds are:
   - Type and number of microorganisms.
   - Moisture content of more than 12-13%.
   - Storage temperature.
   - Physical damage.

8. Discoloration of the fish flesh may occur during spoilage:
   - Yellow to greenish yellow colors caused by Pseudomonas fluorescence, yellow micrococci.
   - Red or pink colors from growth of Sarcina, Micrococcus, Bacillus sp. or by moulds or yeasts.
   - Chocolate brown color by Asporogenous yeast.

4.5 SUMMARY

- Foods are not only of nutritional value to those who consume them but often are ideal culture media for microbial growth.
- Microorganisms can be used to transform raw foods into gastronomic delights, including chocolate, cheeses, pickles, sausages, and soy sauce.
- Microorganisms can degrade food quality and lead to spoilage and hence can serve as vehicles for disease transmission.
- Food spoilage is a major concern throughout the world. This can occur at any point in the course of food production process: growth, harvesting, transport, storage, or final preparation.
- When foods become harmful and unsuitable for consumption due to damage of original nutritional value, texture and flavour, it is known as food spoilage.
- Yeasts, moulds and bacteria cause this type of spoilage. Growth of yeasts causes fermentation which results in yeast metabolism.
- True yeast and false yeast are the two classifications of yeasts.
- True yeast metabolizes sugar and as a result, alcohol and carbon dioxide are produced. This is known as fermentation.
• False yeast grows as a dry film on a food surface. Also, it occurs in foods that have an environment of high sugar or high acid.

• Moulds grow in filaments thereby forming a tough mass which is visible as ‘mould growth’.

• Spores are formed by the moulds which after drying float through the air to find optimal conditions to start the growth cycle again.

• Bacteria are microorganisms that are found in round, rod or spiral shapes. Different kinds of bacteria cause different kinds of spoilage.

• If uncooked foods are not used while fresh, enzymes cause undesirable changes in color, texture and flavor. Enzymes can be destroyed easily by heat processing.

• Atmospheric oxygen on reacting with some components of food may cause rancidity or color changes.

• Diseases in fruits and vegetables are generally caused because of microbial spoilage.

• Owing to the low water activity in cereals like wheat, rice, rye, corn they remain comparatively protected from the attack of microbes, if they are stored properly.

• The flour made from these cereals also remains protected due to the action of bleaching agents used during milling operation.

• Microbial spoilage occurs quite readily in dairy products like butter, milk, cream, cheese.

• Natural deterioration of organic matter is known as food spoilage. Nature’s every component is broken down in simpler parts so that it can again become a part of soil.

• The main or biggest symptom of food spoilage is bad odour. Thus, it is believed that the foods that have a bad odour must be thrown out.

• If foods having bad odour are kept in the refrigerator for some time, they transfer their odour to other foods, making them disagreeable to eat.

• Smoked salmon’s smell permeates into cake and nobody prefers to eat a cake smelling like a salmon.

• Oxidizing enzymes react with oxygen and cause the process of food spoilage, for example, vegetable enzymes like catase and peroxidase cause browning of some foods, such as, apples and potatoes.

• Enzymes are basically substances which increase the speed of chemical and enzymatic reactions with oxygen in the organic matter, thereby hastening degeneration of the matter.
Microbes play an important role in the balance of nature. We find that some bacteria enjoy a symbiotic relationship with living organisms and in some cases they develop parasitical relationship with them.

Temperature is probably the single most important environmental variable which is under our control. In order to prevent the food from spoilage, it is imperative to control the temperature.

Temperature is professionally controlled, these days. It has been observed that over freezing can result in cracking of the surfaces thereby puncturing the cells and causing the flesh to become soft and pulpy.

The kind of microbial spoilage of foods depends on the kinds and numbers of these agents present in the environment. A variety of bacteria, yeasts, molds and enzymes are present in most of the raw foods. Owing to specific environmental conditions, only a small proportion of them are able to grow rapidly and cause food spoilage.

The kinds and numbers of microorganisms that will be present in the food depend on the kind and extent of contamination, earlier opportunities for growth of a particular organism, and earlier treatments that the food has received.

Preservation of food entails keeping the food in such a state that it does not get spoilt for a long time, thereby making it palatable for an extended period of time.

The major causes of food deterioration are microbes, enzymatic action, chemical reactions, physical changes, time, insects and rodents.

Changes in appearance will not cause a food to be spoilt but may cause deteriorative changes in the food items making them unsuitable for human consumption.

Sorting spoiled fruits or vegetables or trimming spoiled parts removes microorganisms, but additional handling may result in mechanical damage and therefore, greater susceptibility to decay.

Inclusion of decayed parts of fruits increases the numbers of microorganisms in fruit juices.

Due to low moisture content grains and flours usually have long shelf life if these are properly harvested or stored under proper conditions where microbial growth is not supported.

Moulds can rapidly grow on grains and corn when these products are stored in at high temperature and humidity.
The process of flour making such as washing and the milling reduce the microbial content and hence microbial load. Moisture content of less than 15% does not allow growth of moulds.

The chief causative organism is mucoid variant of *Bacillus subtilis* or *B. licheniformis*, with their spores surviving baking temperatures (not more than 100°C). These spores can germinate once they get favourable conditions.

Chalky bread spoilage is characterized by development of white chalk like spots, which are caused by growth of yeast like fungi *Endomycopsis fibuligera* and *Trichonosporon* variable.

Sugar is involved for color stabilization, flavoring, and substrate for lactic fermentation, also moderating the harsh flavor of sodium chloride. Phosphates are used in most pumped meats to increase water binding.

Spoilage organisms can grow on processed and cooked cured meats, so they are best stored chilled, under a vacuum or modified atmosphere.

Canning is a method of preserving food in which the food contents are processed and sealed in an airtight container made up of tin (tin can), wrought iron canisters.

### 4.6 KEY WORDS

- **Food contamination**: Food contamination refers to the presence of harmful chemicals and microorganisms in food, which can cause consumer illness.
- **Food spoilage**: Food spoilage is the process where a food product becomes unsuitable to ingest by the consumer.
- **Canning**: Canning is a method of preserving food in which the food contents are processed and sealed in an airtight container made up of tin, wrought iron canisters.

### 4.7 SELF ASSESSMENT QUESTIONS AND EXERCISES

**Short Answer Questions**

1. What is contamination?
2. What is spoilage?
3. What are the common types of spoilage?
4. Give a short note on fitness or unfitness of food for contamination.
Contamination and Spoilage

5. Briefly describe the contamination and spoilage of seafoods.
6. Write a short note on microbial spoilage of canned food.

Long Answer Questions

1. Write a note on contamination and spoilage.
2. Write a note on contamination and spoilage of cereals.
3. How are fruits and vegetables contaminated?
4. Give a detailed note on contamination of meat and meat products.
5. What are the factors that effect seafoods?
6. Discuss about the contamination of canned foods.

4.8 FURTHER READINGS

UNIT 5 FOOD POISONING AND FOOD BORNE INFECTIONS

Structure
5.0 Introduction
5.1 Objectives
5.2 Food Poisoning and Food Borne Infections
  5.2.1 Bacterial Diseases
  5.2.2 Viral Diseases
  5.2.3 Fungal Diseases/Mycotoxins
  5.2.4 Protozoal Diseases
  5.2.5 Toxic Metals and Chemicals
5.3 Answers to Check Your Progress Questions
5.4 Summary
5.5 Key Words
5.6 Self Assessment Questions and Exercises
5.7 Further Readings

5.0 INTRODUCTION

Food poisoning is when someone gets sick from eating food or drink that has gone bad or is contaminated. There are two kinds of food poisoning: poisoning by toxic agent or by infectious agent. Food infection is when the food contains bacteria or other microbes which infect the body after it is eaten. Food intoxication is when the food contains toxins, including bacterially produced exotoxins, which can happen even when the microbe that produced the toxin is no longer present or able to cause infection. Even though it is commonly called food poisoning, most cases are caused by a variety of pathogenic bacteria, viruses, prions or parasites that contaminate food, rather than chemical or natural toxins which we usually call poison. According to the Centers for Disease Control and Prevention, about 76 million people in the United States become ill from the food they eat, and about 5,000 of them die every year.

Most cases of food poisoning occur when people eat food or drink water containing bacteria, bacterial toxins, parasites, or viruses. Food poisoning can also occur when non-infectious poisons or heavy metals (find their way into people’s stomachs.

Foodborne illness is any illness resulting from the spoilage of contaminated food, pathogenic bacteria, viruses, or parasites that contaminate food, as well as toxins such as poisonous mushrooms and various species of beans that have not been boiled for at least 10 minutes.

Symptoms vary depending on the cause, and are described below in this article. A few broad generalizations can be made, for example the incubation...
period ranges from hours to days, depending on the cause and on quantity of consumption. The incubation period tends to cause sufferers to not associate the symptoms with the item consumed, and so to cause sufferers to attribute the symptoms to gastroenteritis for example.

Symptoms often include vomiting, fever, and aches, and may include diarrhea. Bouts of vomiting can be repeated with an extended delay in between, because even if infected food was eliminated from the stomach in the first bout, microbes, like bacteria, can pass through the stomach into the intestine and begin to multiply. Some types of microbes stay in the intestine, some produce a toxin that is absorbed into the bloodstream, and some can directly invade deeper body tissues.

In this unit, you will study about food poisoning and food borne infections, i.e., bacterial, viral, fungal and protozoa, bacterial and fungal toxins in detail.

5.1 OBJECTIVES

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

- Understand what food poisoning and food borne infections, i.e., bacterial, viral, fungal and protozoa
- Discuss the bacterial and fungal toxins

5.2 FOOD POISONING AND FOOD BORNE INFECTIONS

Foodborne illness, more commonly referred to as food poisoning, is the result of eating contaminated, spoiled, or toxic food. The most common symptoms of food poisoning include nausea, vomiting, and diarrhea.

In other words, we can say that food poisoning is a food borne disease. Ingestion of food that contains a toxin, chemical or infectious agent (like a bacterium, virus, parasite, or prion) may cause adverse symptoms in the body. Those symptoms may be related only to the gastrointestinal tract causing vomiting or diarrhea or they may involve other organs such as the kidney, brain, or muscle. Typically most foodborne diseases cause vomiting and diarrhea that tend to be short lived and resolve on their own, but dehydration and electrolyte abnormalities may develop. The Center for Disease Control and Prevention (CDC) estimates approximately 48 million people become ill from food-related diseases each year resulting in 128,000 hospitalizations, and 3,000 deaths.

A foodborne infection is an inflammation of the stomach and bowels. The infection can happen when you eat or drink something that is contaminated by a bacteria, virus or parasite. Often the inflammation leads to diarrhoea, nausea, vomiting, abdominal pain, abdominal cramps and sometimes fever. A foodborne infection can last between one and three days.
Many foodborne infections occur at people’s homes, simply due to poor hygiene. It’s as easy as this: preparing food without hand washing after visiting the toilet or food. Cross-contamination is also a risk, for instance if raw meat and lettuce are both chopped on the same cutting board. Even using the same knife to chop both could cause contamination by foodborne pathogens. Eating meat or fish that is not cooked all the way through, or eating raw shellfish, increases the risk of food-borne infections.

A food-borne infection involves the ingestion of the pathogen such as Salmonella, Campylobacter, Listeria, and E. coli, followed by growth in the host, including tissue invasion and/or the release of toxins. Major food borne infections are Salmonellosis, Listeriosis, Shigellosis, Yersiniosis, etc. Proper hygiene will prevent contamination and further spreading. For instance, wash your hands before preparing dinner, and after each visit to the toilet. Also pay particular attention to separating your prepared and unprepared food. Put food in the refrigerator within two hours, chop your meat and vegetables on separate cutting boards and wash tea towels at 60 degrees.

Food-Borne Intoxications

If the pathogen grows in the food before consumption and forms toxins that affect the food consumer without further microbial growth, the disease is a food-borne intoxication. Examples are intoxications caused by Staphylococcus, Clostridium, and Bacillus. Intoxication produces symptoms shortly after the food is consumed because growth of the disease-causing microorganism is not required. Toxins produced in the food can be associated with microbial cells or can be released from the cells. Most Staphylococcus aureus strains cause a staphylococcal enteritis related to the synthesis of extracellular toxins. These are heat-resistant proteins, so heating does not usually render the food safe. The effects of the toxins are quickly felt, with disease symptoms occurring within 2 to 6 hours. The main reservoir of S. aureus is the human nasal cavity. Frequently S. aureus is transmitted to a person’s hands and then is introduced into food during preparation. Growth and enterotoxin production usually occur when contaminated foods are held at room temperature for several hours. Three Gram-Positive rods are known to cause food intoxications: Clostridium botulinum, C. perfringens, and Bacillus cereus.

Some food-borne diseases are not caused by bacteria or their toxins but result from mycotoxins, viruses, and protozoa or from the consumption of food contaminated with toxic substances. Below are some of the food borne diseases caused by bacteria, viral, fungi and protozoa.

5.2.1 Bacterial Diseases

A food-borne illness is a general term applied to all types of illnesses caused by an organism, substance or material of any kind which is present in food and gains entrance into the body when such food is consumed. Food poisoning or food intoxication is an illness caused by toxins present in contaminated food. The toxin
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may be a poisonous chemical toxin which is accidentally or intentionally added, a naturally occurring poison such as solanine in green potatoes or a toxic metabolite excreted by bacteria.

**Botulism**: Botulism is an uncommon type of food poisoning. It is produced by anaerobic spore-forming bacteria found in the soil. The organism produces a toxin which is extremely poisonous and affects the nervous system resulting in the death of approximately two-thirds of the affected victims.

As these bacteria are present in the soil, they contaminate vegetables. When they are present in marine soil and beds of fresh water lakes, fish also get contaminated. Since the organism is a strict anaerobe, it is unable to grow unless oxygen is excluded. Therefore, it is usually able to grow in canned, bottled and vacuum-packed foods. The spores of this organism are extremely heat resistant. Some spores can survive boiling for six hours. However, the organism cannot grow if the pH is below 4.5, so canned acidic foods are safe. In addition, the toxin is easily destroyed by heat. Heating the food to 80°C for 15 minutes destroys the toxin.

**Bacillus Cereus Food Poisoning**: Bacillus cereus is a spore-forming aerobic rod-shaped bacteria which causes a toxic type of food-borne illness. It is found in soil, dust, water and on cereal grains. It is a common type of food poisoning. The food usually affected is rice, especially boiled rice which is prepared and used on the following day. The spores survive boiling. They germinate, multiply and release a toxin in rice if it is not refrigerated. If the rice is not reheated thoroughly the toxin is not destroyed and consumption of the rice will cause food poisoning (Refer Figure 5.1).

Outbreaks sometimes follow celebrations where rice was not washed properly in clean water or was boiled in advance and cooled slowly.

![Fig. 5.1 Bacillus Cereus Infected Rice](image-url)
**Perfringens Food Poisoning**

Clostridium perfringens is a spore-forming anaerobe found in the human and animal intestinal tract, soil, dust, contaminated raw meat, poultry and some dried foods. The illness is caused by large numbers of rod-shaped microorganisms growing in food. The spores can survive normal cooking temperatures and multiply rapidly in cooked meat which is cooled slowly or stored in a warm place. The toxin is released in the intestine after the living microorganisms have been ingested. Figure 5.2 shows the entry of *Clostridium perfringens* in human beings.

![Fig. 5.2 Entry of Clostridium perfringens in Human Beings](image)

**Salmonellosis:** *Salmonellosis* is the most common cause of bacterial food-borne disease and the most serious. Organisms of the salmonella group cause infection in the intestine. Many species are infectious. These rod-shaped bacteria are aerobic and non-spore producing. They are present in the intestine of humans and animals and are excreted in the faeces. Illness occurs when living organisms are ingested in large numbers. Even a small number of organisms in food can result in infection. Figure 5.3 shows the mechanism of entry of *salmonella* into the intestinal mucosa.

![Fig. 5.3 Invasion of Intestinal Mucosa by Salmonella](image)
Typhoid and Paratyphoid (Enteric Fever): Typhoid fever is caused by Salmonella typhi, whereas paratyphoid fever is caused by Salmonella enteritidis. Both organisms are excreted in the faeces and urine of patients suffering from the disease or are excreted by healthy carriers. Outbreaks of both diseases are caused by water contaminated by sewage and food contaminated by food handlers directly or indirectly. Typhoid is a common infection seen in India, especially in areas where hygiene standards are poor.

Bacillary Dysentery or Shigellosis: Shigellosis is caused by rod-shaped bacteria that cause infection in human intestine. The main cause for spread of this infection is poor personal hygiene and faulty sewage disposal.

Cholera: Cholera is caused by the comma-shaped bacteria Vibrio cholerae which is present in water contaminated by faeces of people suffering from cholera. When the bacteria enter the body, they multiply rapidly in the small intestine and violent diarrhoea sets in. This infection occurs mainly in Asia and Southern Europe (Refer Figure 5.4).

Enteropathogenic Infection: Although Escherichia coli is a normal inhabitant of the intestinal tract of humans, many strains cause acute diarrhoea in infants and some can infect adults also. It is also often the cause of a gastrointestinal upset called travellers’ diarrhoea. The disease causing strains are enteropathogenic, i.e., they cause disease in the enteron or intestine. They are present in the human and animal intestine and are excreted in faeces.

5.2.2 Viral Diseases

In recent years viruses have been increasingly recognized as an important cause of foodborne illness. Viral infections make up perhaps one third of cases of food poisoning in developed countries. Virus contamination is based on transmission by waters or by lack of hygiene in food preparation and direct contamination by food processors and handlers. Since these are spread by the faecal–oral rout, food is one potential means of transmission.
Major Food Borne Viral Diseases

Infectious Hepatitis

**Etiological Agent:** Hepatitis A virus.

**Characteristics of Agent:** Small round virus, member of Picornaviridae, around 28nm in diameter, containing single-stranded RNA. Multiplies in the gut epithelium before being carried by the blood to the liver. In the later part of incubation, the virus is shed in the faeces. Relatively acid-resistant.

**Spread/ Mode of Transmission:** They are transmitted by the fecal–oral route. Person-to-person spread is not common. One of the ways people get infected with hepatitis viruses is by consuming fecal contaminated food and water. Following ingestion of hepatitis viruses through contaminated food, an individual may or may not develop symptoms. The incubation period for infectious hepatitis ranges from 15 to 45 days. During this period, the virus multiply in cells of the gut epithelium before it is carried by blood to the liver and other organs and tissues. Virus replication can also occur in the small intestine, gall bladder, lymph nodes, and salivary glands. In the later part of the incubation period the virus is shed in the faeces.

**Incubation Period:** 10-15 days, mean 25 days.

**Symptoms:** Loss of appetite, fever, malaise, abdominal discomfort, nausea and vomiting, followed by symptoms of liver damage (passage of dark urine, pale stools, jaundice).

**Sequelae:** Acute liver failure, particularly in older persons.

**Duration:** Varies with clinical severity: recovery within a few weeks when mild, several months when severe.

**Reservoir/Source:** Humans (sewage and contaminated water).

**Food Vehicles:** Raw fruits and vegetables, raw milk and milk products, Salad vegetables (such as lettuce), bakery products, shellfish, raw oysters and clams. The infectious hepatitis virus has been shown to be stable during refrigerated storage of oyster, iced drinks, cold meat cuts. Contamination can occur by food handlers.

**Diagnosis:** Diagnosis is based on the epidemiological characteristics of an outbreak and serological tests. Confirmation requires identification of 28nm virus-like particles by immune electron microscopy in feces of acutely ill patients and by molecular detection of genomic RNA in serum or feces.

**Prevention and Control:**
- Industrial: Treatment of water supply: safe sewage disposal.
- Food Service Establishment/ Household: Good personal hygiene, particularly thorough hand-washing with soap and water before handling foods and abstinence from handling food when infected; washing raw fruits and vegetables under running water.
water, and good hygiene practices during production and processing, thoroughly cooking meat, shellfish; sufficient heating of milk and foods.

An effective vaccine is available and vaccination of professional food-handlers and travellers should be considered. Immune-serum globulin is effective in preventing illness if administered within 14 days of exposure to hepatitis A, and can be used for pre-exposure prophylaxis in travellers who cannot be vaccinated.

**Exclusion from Work/School:** All cases (including those in risk groups 1-4) for 7 days after onset of jaundice and/or symptoms.

### Viral Gastroenteritis

- **Rotavirus**
- **Norovirus**

#### I. Gastroenteritis by Rotavirus

**Name of Illness:** Gastroenteritis (inflammation of the stomach and intestines). They are known to be associated with travelers’ diarrhea.

**Etiological Agent:** Rotavirus.

**Characteristics of Agent:** Rotaviruses belong to the family Reoviridae; they are about 70 nm in diameter, are non-enveloped, and contain double-stranded RNA (dsRNA).

**Symptoms:** Watery diarrhea, fever, abdominal pain and vomiting, dehydration.

**Incubation Period:** 1-3 days.

**Name of Illness:** Gastroenteritis (inflammation of the stomach and intestines). They are known to be associated with travelers’ diarrhea.

**Etiological Agent:** Rotavirus.

**Characteristics of Agent:** Rotaviruses belong to the family Reoviridae; they are about 70 nm in diameter, are nonenveloped, and contain double-stranded RNA (dsRNA).

**Symptoms:** Watery diarrhea, fever, abdominal pain and vomiting, dehydration.

**Incubation Period:** 1-3 days.

**Spread/Mode of Transmission:** Rotaviruses are transmitted by the fecal-oral route; this means that the virus must be passed by an infected person and then enter a susceptible person’s mouth to cause infection. Person-to-person spread can also possible. Rotaviruses cause an estimated one-third of all hospitalizations for diarrhea in children below age 5. Rotavirus may activate neurons of the enteric nervous system that stimulate secretion of fluids and solutes. After an incubation period of 1–3 days, rotavirus infects the mature absorptive enterocytes in the ileum, and causes watery diarrhea, fever, abdominal pain and vomiting, which generally lasts 3–8 days. In babies and young children, it can lead to dehydration. Severe diarrhea without fluid and electrolyte replacement may result in severe
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dehydration, and in severe cases, death. Severe dehydration may require hospitalization for treatment with Intravenous (IV) fluids (fluids given to the patient directly through their veins). The best way to protect against dehydration is to drink plenty of liquids (oral rehydration therapy).

**Duration:** Varies with clinical severity: recovery within a few weeks when mild, several months when severe.

**Food Vehicles:** Foods such as shellfish, soft cheese, vegetables, and fruits can contaminate with viruses from water. Infected food handlers may also contaminate foods.

**Diagnosis:** Rotaviral infections can be diagnosed by Enzyme Immunoassays (EIA), immune electron microscopy, RT-PCR, Enzyme-Linked Immunosorbent Assay (ELISA), and latex agglutination methods.

**Prevention and Control:** Rotaviruses are rapidly inactivated at 56°C. Rotaviruses are inactivated by UV light and disinfectants, including chlorine, H₂O₂, and ethanol.

**Occurrence:** Worldwide. Incidence for rotavirus more prevalent than others. Rotavirus infections make up 15–25% of diarrhoeal disease cases identified in children seen at treatment centres in developing countries.

**II. Gastroenteritis by Norovirus**

**Name of Illness:** Gastroenteritis. They are known to be associated with travelers' diarrhea. Norovirus causes inflammation of the stomach, intestines or both, whereby, Noroviruses reduces the absorptive capacity of the villi contributing diarrhea, nausea, and vomiting.

**Etiological Agent:** Norovirus.

**Characteristics of Agent:** as Small, Round, Structured Viruses (SRSVs), which include norovirus (Norwalk virus).

**Symptoms:** After incubation period Nausea is the first symptom, usually accompanied by vomiting, non bloody diarrhea, and abdominal cramps.

**Spread/ Mode of Transmission:** Spread by faecal–oral route. Food and drinking-water may be contaminated either at source when exposed to sewage/wastewater in the environment or used for irrigation, or by an infected food-handler.

**Incubation Period:** Noroviruses gastroenteritis is characterized by an incubation period 48h after ingestion of viruses through contaminated food.

**Duration:** Illness lasts after 24–48h. The viruses are excreted in the feces of infected persons.

**Reservoir/Source:** Humans are the only known reservoir of the Norovirus.

**Food Vehicles:** Foods commonly involved in outbreaks include leafy greens (such as lettuce, spinach), fresh fruits, fish, shellfish (such as oysters), clams, and mussels cached from coastal water containing sewage canals carry virus Salad vegetables fertilized with human excrete can contaminate viruses. Norovirus may be thermally
stable and can present in inadequately cooked shellfish harvested from contaminated estuaries is common cause of Norovirus outbreaks. However, any food served raw or handled after being cooked can get contaminated. The use of contaminated water in food processing and improper sanitation lead to food contamination.

Secondary contamination can occur with salad during preparation from food handler.

Filter-feeding shellfish most common food contaminated at source, but a wide range of different cooked and uncooked foods have been implicated in secondary contamination by food handlers.

**Diagnosis:** They have yet to be cultured in the laboratory, the method of choice is RT–PCR.

**Prevention and Control:** If properly cooked, viruses are inactivated. This virus is relatively resistant to physical and chemical inactivation. Viruses can be inactivated by light and ozone.

Industrial: Hygienic sewage disposal; treatment of drinking-water; treatment of wastewater used for irrigation; thermal processing; good hygiene practices during production and processing.

Food service establishment/household: Good personal hygiene (hand-washing with soap and water); abstinence from handling food when ill, especially when diarrhoea and vomiting present. Vaccines against rotavirus are now available.

### III. Poliomyelitis

**Name of Illness:** Poliomyelitis.

**Etiological Agent:** Poliovirus.

**Characteristics of Agent:** Small round virus, member of Picornaviridae, contains single-stranded RNA, withstands pH 3–5. Virus infects gastrointestinal tract, spreads to the regional lymph nodes and in a minority of cases, to the nervous system.

**Symptoms:** Like other enterovirus infections, poliovirus is more likely to produce an asymptomatic infection in very young children. Poliomyelitis may be a transient viremia with an incubation period of 3-14 days after ingestion of infected foods and characterized by headache, fever and malaise.

In minority of cases it can progress to a second stage where the virus invades the meninges causing back pain and headaches. In the worst cases the virus may spread to neurons in the spinal chord causing cell destruction and various degrees of paralysis. More severe illness is characterized by severe muscle pain and stiffness of the neck and back with or without flaccid paralysis. Flaccid paralysis occurs in~1% of poliovirus infections, most often in the legs, sometimes in the arms. Paralysis persisting longer than 60 days is likely to be permanent. Paralysis of the muscles used in respiration and/or swallowing is life-threatening.
**Incubation Period:** 3-14 days, 5-35 days.

**Sequelae:** Permanent paralysis.

**Duration:** Maximum extent of paralysis generally reached within 3-4 days. Paralysis persisting longer than 60 days is likely to be permanent.

**Reservoir/Source:** Humans, most frequently asymptomatic persons.

**Food Vehicle:** Contaminated milk (both pasteurized and raw).

**Treatment:** Poliomyelitis is now virtually eradicated in developed countries due to the availability of very effective live and inactivated vaccines.

**Prevention and Control:** Infection had been controlled by improvements in hygiene Vaccination.

**Food-Specific Control Measures**

- **Industrial:** Treatment of drinking-water; effective sewage disposal system; thermal processing; good hygiene practices during production and processing.
- **Foodservice establishment/household:** Safe food preparation practices, including careful hand-washing with soap and water; thorough cooking and reheating of food before consumption and thorough washing of all fruits and vegetables.

**Occurrence:** Poliomyelitis has been almost entirely eliminated in industrialized countries and the Americas by effective immunization. Incidence in developing countries depending on immunization coverage.

**Other Comments:** Risk of transmission greatest several days before and after onset of symptoms. Infants and children under 5 years of age most frequently affected. Immunization of the elderly is recommended, particularly when travelling abroad.

### 5.2.3 Fungal Diseases/Mycotoxins

A very large number of molds produce toxic substances designated mycotoxins. Some are mutagenic and carcinogenic, some display specific organ toxicity. At least 14 mycotoxins are known to be carcinogens, with the aflatoxins being the most potent.

Mycotoxins are produced as secondary metabolites by fungi. Secondary metabolites are formed during the end of the exponential growth phase and have no apparent significance to the producing organism relative to growth or metabolism. However, primary metabolites are those compounds that are essential for growth.

The syndrome resulting from the ingestion of toxin in a mold contaminated food is referred to as **mycotoxicosis**. The first documented case of mycotoxicosis attributed to a fungus-containing food was that of rye ergot. *Claviceps purpurea* parasitizes the rye and elaborately many lysergic acid derivatives which are responsible for the syndrome.
Fungal Diseases

I. Aflatoxins

Types of Aflatoxins (Chemistry): Aflatoxins are clearly the most widely studied of all mycotoxins. Toxin produced by Aspergillus flavus was designated aflatoxin (Aspergillus flavus toxin–Aflatoxin).

The two major metabolites of aflatoxins have been designated as AFB1 and AFG1 because they fluoresce blue (B1) and green (G1) when exposed to long wave ultraviolet light. Aflatoxins AFB2 and AFG2 are the dihydro derivatives of AFB1 and AFG1. Aflatoxins AFM1, AFM2 and AFP1 are the hydroxylated derivatives of AFB1 and AFB2. AFM1, AFM2 are excreted in the urine, feces, and milk as metabolic products of AFB1 and AFB2 following their consumption by mammals. They are highly oxygenated heterocyclic compounds.

1. AFB1: produced by all aflatoxin-positive strains and it is the most potent of all. AFB1 bind to both nuclear DNA as well as to liver mitochondrial DNA, but has been shown to bind covalently to liver mitochondrial DNA, preferentially to nuclear DNA.
   - AFM1 is a hydroxylated product of AFB1, and it is excreted in the milk.
   - AFL, AFLH1, AFQ1, and AFP1 are excreted in the urine, feces, and milk as metabolic products from AFB1

2. AFB2: AFB2 is the 2,3-dihydro form of AFB1. AFB2 form reduces mutagenicity by 200- to 500-fold.
   - AFM2 are the hydroxylated derivatives of AFB2, excreted in milk.

3. AFG1: green fluorescing aflatoxin.

4. AFG2: AFG2 is the 2,3-dihydro form of AFG1.

Order of Toxicity: The toxicity of the six most potent aflatoxins decreases in the following order: B1>M1>G1>B2>M2=G2.

When Viewed Under Ultraviolet (UV) Light, six of the toxins fluoresce as noted: B1 and B2—fluoresce blue, G1—fluoresce green, G2—green-blue, M1—blue-violet, M2—violet.

Relative Toxicity and Mode of Action: Cyclopiazonic acid is produced by some A. flavus strains and it is thought to contribute to the toxicity of aflatoxin. For the expression of mutagenicity, mammalian metabolizing systems are essential for aflatoxins, especially AFB1. Also essential is their binding with DNA. Although nuclear DNA is normally affected, AFB1 has been shown to bind covalently to liver mitochondrial DNA, preferentially to nuclear DNA. Cellular macromolecules other than nucleic acids are possible sites for aflatoxins. The site of the aflatoxin molecule responsible for mutagenicity is the C2–C3 double bond in the dihydrofurofuran moiety. Its reduction to the 2,3-dihydro (AFB2) form reduces mutagenicity by 200- to 500-fold.
Following binding to DNA, point mutations are the predominant genetic lesions induced by aflatoxins, although frameshift mutations are known to occur. The mutagenesis of AFB1 has been shown to be potentiated twofold by Butylated Hydroxy Anisole (BHA) and Butylated Hydroxy Toluene (BHT). Aflatoxins are carcinogenic to humans. Among conditions believed to result from aflatoxins is i) the EFDV syndrome of Thailand, ii) Reye’s syndrome of Thailand and New Zealand, and iii) an acute hepatoma condition in a Ugandan child. The risk of liver cancer from aflatoxin consumption is 30 times higher in individuals who have been exposed to hepatitis B (HbsAg+) than in HbsAg- individuals.

**Producing Mold:** *Aspergillus flavus* produces AFB1 and AFB2; and *A. parasiticus* produces all four of the major aflatoxins (B1, G1, B2, and G2). AFB1 is produced by all aflatoxin-positive strains, and it is the most potent of all. Other known aflatoxin producers include *A. nomius, A. bombycis, A. pseudomaritii,* and *A. ochraceoroseus* among the aspergilli; and *Penicillium.*

**Food Vehicles:** They have been isolated from a wide variety of foods, including milk, beer, cocoa, raisins, soybean meal, wheat, corn, barley, cottonseed, millet, oats, peanuts, peanut meal, peanut butter, rice, sorghum, peas, sweet potatoes.

**II. Alternaria Toxin**

**Toxicity:** Alternariol, alternariol monomethyl ether, tenuene, tenuazonic acid, and altertoxin-I are some of the toxins produced by several Alternaria species.

**Producing Mold:** Several species of Alternaria (including *A. citri, A. alternata, A. solani,* and *A. tenuissima*)

**Food Vehicles:**
- On slices of apples, tomatoes, or crushed blueberries incubated for 21 days at 21°C, Alternaria produced toxins at levels up to 137 mg/100 g.
- Tenuazonic acid was the main toxin produced in tomatoes, with levels as high as 13.9 mg/100 g.
- On oranges and lemons, *A. citri* produced tenuazonic acid, alternariol, and alternariol monomethyl ether at a mean concentration of 1.15–2.66 mg/100 g.

**III. Ochratoxins**

**Toxicity:** The ochratoxins consist of a group of at least seven structurally related secondary metabolites of which Ochratoxin A (OA) is the best known and the most toxic.

Mold producing OA cause Mold nephrosis, a disease in pigs. A moldy alfalfa-grass was considered to be responsible for a high abortion rate in dairy cows. Its oral LD50 in rats is 20–22 mg/kg, and it is both hepatotoxic and nephrotoxic.
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Chemistry: Ochratoxin A (OA) is structurally a chlorinated isocoumarin derivative with an amide bond to phenylalanine. A dechlorinated analog of this compound is named Ochratoxin B (OB). In addition, there are other minor components including a methyl and ethyl ester of A and B and 4-hydroxyochratoxin A. Ochratoxin A fluoresces green under ultraviolet light, and ochratoxin B fluoresces blue.

Producing Mold: OA is produced by a large number of storage fungi, including Aspergillus ochraceus, A. alliaceus, A. petrakii, A. niger and A. carbonarius. The latter was found in Spain to be the main producer of OA with 97% of 91 isolates found to be OA producers. Among penicillia that produce OA are P. viridicatum, P. cyclopium, P. commune.

Food Vehicles: Corn, wheat, barley, white beans, peanuts, dough, bread, hen’s eggs, dried vine fruits (currants, raisins, and sultanas).

IV. Citrinin

Toxicity: Under long-wave UV light, it fluoresces lemon yellow. It is a known carcinogen.

Producing Mold: Penicillium citrinum, P. viridicatum, and other fungi. Of seven strains of P. viridicatum recovered from country-cured hams, all produced citrinin in potato dextrose broth.

Food Vehicles: Corn, wheat, dried beans, cocoa beans, soybeans, oats, barley, white beans, peanuts, dough, bread, hen’s eggs, citrus fruits, Brazil nuts, moldy tobacco, country-cured ham, coffee beans, polished rice, moldy bread.

V. Patulin (Clavicin, Expansin)

Toxicity: First isolated and described as antibiotic. Its biological properties are similar to those of penicillic acid. Some patulin-producing fungi can produce the compound below 2°C.

Patulin causes chromosomal aberrations in animal and plant cells and is a carcinogen. Originally isolated as an antibiotic, it is effective against many bacterial species. Concentrations as low as 0.1% completely inhibit E. coli and S. aureus. It also exhibits strong fungicidal activity and is toxic to seeds and seedlings of higher plants including sugar beets, corn, wheat, peas, tomato, cucumber, and flax.

Oral and intravenous injection of patulin in concentrations of 0.3 to 2.5 mg per gram of body weight are fatal to mice and rats. Pathological changes included edema of the brain, hemorrhaging of the lungs, and capillary damage of the liver, spleen, and kidney. Carcinogenic and mutagenic properties of patulin have been observed in mice at sublethal doses. Since many other animals are known to be sensitive to patulin, it is considered a potential human carcinogen.

Chemistry: Patulin in the pure state is a white crystal with a melting point of 110.5°C and a molecular weight of 154. It is an unsaturated lactone with the chemical name of 4-hydroxy-4H-furo[3, 2c]pyran-2(6H)-one. It is sensitive to SO2 and is unstable in alkali but stable in acid.
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Producing Mold: Produced by a large number of penicillia including *P. claviforme, P. expansum, P. patulum, P. melini*, by some aspergilli like *A. clavatus, A. terreus, A. giganteus* and by *Byssochlamys nivea* and *B. fulva*.

Food Vehicles: Moldy bread, sausage, fruits (including bananas, pears, pineapples, grapes, and peaches), apple juice, apple cider, apple sap.

VI. Penicillic Acid

Toxicity: This mycotoxin has biological properties similar to patulin. It is a proven carcinogen.

Producing Mold: It is produced by a large number of fungi, including many penicillia (*P. puberulum, P. cyclopium, P. thomii, P madriti*) as well as members of the Aspergilli (*A. ochraceus, A. mellens, A. sulphureus*). One of the best producers is *P. cyclopium*.

Food Vehicles: It has been found in dried beans, tobacco, corn, beans.

VII. Sterigmatocystin

Toxicity: At least eight derivatives are known. The LD50 for rats by intraperitoneal injection is 60–65 mg/kg. Under UV light, the toxin fluoresce dark brick-red. Structurally and biologically related to the aflatoxins and causes hepato carcinogenic activity in animals, but are not as potent as aflatoxins. They act by inhibiting DNA synthesis.

Producing Mold: Among the producing organisms are *Aspergillus versicolor, A. nidulans, A. rugulosus, and Penicillium luteum*.

Food Vehicles: Although not often found in natural products, they have been found in wheat, oats, Dutch cheese, and coffee beans.

VIII. Fumonisins

Toxicity: There are at least 15 fumonisins, with the best known being FB1, FB2, FB3,FB4, FA1, FA2, and FA3. The major ones are FB1–FB3, and the others are considered to be minor and less well characterized. Of the three major toxins, FB1 (also designated macrofusine) is produced in the largest quantities by producing strains.

Producing Mold: The species demonstrated to produce fumonisins include *Fusarium* spp. including *F. sacchari, F. subglutinans, F. thapsinum, F. globosum, F. anthophilum, F. dlamini, F. napiforme, F. nygami, F. moniliforme, and F. proliferatum*. *F. moniliforme* (formerly *F. verticilliodes; Gibberella fujikuroi*)

Food Vehicles: The fumonisins are produced by *Fusarium* spp. on corn and other grains. The prevalence of *F. moniliformis* significantly higher in corn from areas where a high rate of human esophageal cancer occurred than in low esophageal cancer rate areas.
IX. Sambutoxin

**Toxicity:** Sambutoxin causes hemorrhage in the stomach and intestines of rats, and the animals refuse feed and lose weight.

**Producing Mold:** produced primarily by strains of *Fusarium sambucinum* and *F. oxysporum*.

**Food vehicles:** The toxin was found in potatoes from parts of Iran that had a high incidence of esophageal cancer.

X. Zearalenone

**Toxicity:** There are at least five naturally occurring zearalenones. The toxins fluoresce blue–green under long-wave UV and greenish under short-wave UV. They possess estrogenic properties and promote estrus in mice and hyperestrogenism in swine. Although they are nonmutagenic in the Ames assay, they produce a positive response in the Bacillus subtilis Rec assay.

**Producing Mold:** Produced by *Fusarium* spp., mainly *F. graminearum* (formerly *F. roseum* = *Gibberella zeae*) and *F. tricinctum*.

**Food Vehicles:** These organisms invade field corn at the silking stage, especially during heavy rainfall. If the moisture levels remain high enough following harvesting, the fungi grow and produce toxin. Other crops, such as wheat, oats, barley, and sesame, may be affected in addition to corn.

XI. Roquefortine

**Toxicity:** A toxic substance has recently been detected in commercial blue cheese from several countries. Heavily molded portions of the cheese contain higher concentrations than the white portions suggesting that *Penicillium roqueforti* may be producing a mycotoxin during curing of Roquefort cheese (blue cheese). The toxic factor named roquefortine, acts as a neurotoxin when injected into mice and leads to convulsive seizures.

**Producing Mold:** *Penicillium roqueforti*.

**Food Vehicles:** Blue cheese, Roquefort cheese, Stilton cheese.

XII. Luteoskyrin

**Toxicity:** *Penicillium islandicum* produce two metabolites, luteoskyrin and cyclochlorotine, which are hepatotoxic to some animals. Although luteoskyrin is not as carcinogenic as aflatoxin B1, mice seem to be more sensitive to luteoskyrin than to aflatoxin b1. There are no known acute human intoxications attributed to luteoskyrin and cyclochlorotine.

**Producing Mold:** *Penicillium islandicum*.

**Food Vehicles:** Mold, rice flour.

XIII. Alimentary Toxic Aleukia (ATA)

**Toxicity:** Outbreaks of this mycotoxicosis have occurred in the Soviet Union, where between 1942-47 casualties approached 1000 per 10000 population in
certain regions. The syndrome in humans is caused by the toxic metabolites fusariogenin, epicladosporic acid, and fagicladosporic acid. ATA is not a problem if grain is harvested and stored properly. Previous outbreaks have all resulted from the consumption of overwintered grain, i.e., grain that has been allowed to remain in the fields during the winter and harvested late.

**Producing Mold:** Species of *Cladosporium, Penicillium, Fusarium, Mucor, Alternaria.*

**Food Vehicles:** Grain.

5.2.4 Protozoal Diseases

Below are some of the protozoal diseases that are caused by protozoa:

1. Cryptosporidiosis

**Name of Illness:** Cryptosporidiosis.

**Etiological Agent:** Protozoa: *Cryptosporidium parvum*

**Characteristics of Agent:** *C. parvum* is an obligate intracellular coccidian parasite that carries out its life cycle in one host. The oocysts of *C. parvum* are spherical to ovoid and average 4-6µm in size. The oocysts are highly resistant in the natural environment and may remain viable for several months when kept cold and moist. Oocysts are very resistant to chlorination but killed by conventional cooking procedures. They have been reported to be destroyed by treatments with 50% or more ammonia and 10% or more formalin for 30 minutes. Temperatures above 60°C and below -20°C may kill *C. parvum* oocysts. The organism is destroyed by High-Temperature, Short Time (HTST) milk pasteurization. Holding oocysts at 45°C for 5–20 minutes has been reported to destroy their infectivity.

**Life Cycle:** Following ingestion of the thick-walled oocysts, they excyst in the small intestine and free sporozoites penetrate the microvillous region of host enterocytes, where sexual reproduction leads to the development of zygotes. They invade host cells by disrupting their own membrane as well as that of the host. Host cell actin polymerization at the interface between the parasite and the host cell cytoplasm has been found to be necessary for infection. About 80% of the zygotes form thick-walled oocysts that sporulate within host cells. The environmentally resistant oocysts are shed in feces, and the infection is transmitted to other hosts when they are ingested.

**Sequelae:** In humans, the disease is self-limiting in immunocompetent individuals, but it is a serious infection in the immunocompromised, such as AIDS patients.

**Incubation Period:** 6–14 days.

**Symptoms:** In immunocompetent individuals, the organism primarily parasitizes the intestinal epithelium and causes diarrhea, which is profuse and watery, with as many as 71 stools per day and up to 171 per day reported. Diarrhea is sometimes accompanied by mucus but rarely blood, abdominal pain, nausea, vomiting, sometimes accompanied by influenza-like illness with fever.
**NOTES**

**Duration:** Symptoms may last for more than 30 days in the immunocompromised but generally less than 20 days (range: 4–21 days) in the immunocompetent.

**Reservoir/Source:** Humans, wild and domestic animals, for example cattle

**Food Vehicles:** Foods include raw milk, drinking-water and apple cider.

**Spread/Mode of Transmission:** Spread through the faecal-oral route, person to person or consumption of fecally contaminated food and water, bathing in contaminated pools. Human cryptosporidiosis may be acquired by at least one of five known transmission routes: zoonotic, person-to-person, water, nosocomial (hospital acquired), or food. Zoonotic transmission (from vertebrate animals to humans) is most likely where infected animals (such as calves) deposit fecal matter to which humans are exposed. The disease may be contracted by drinking untreated water.

**Diagnosis:** Diagnosis of cryptosporidiosis requires the identification of oocysts in stools of victims. Staining methods such as acid-fast, negative staining, and sugar flotation are used. Another diagnostic method is a direct immunofluorescence test used for the detection of oocysts in feces, which employs a monoclonal antibody against an oocyst wall antigen.

**Treatement:** Spiramycin, fluconazole, and amphotericin B show some promise. More recently, the aminoglycoside antibiotics paromomycin and geneticin were found to inhibit the growth of intracellular *C. parvum*.

**Prevention and Control**

**Industrial:** Pasteurization/sterilization of milk; filtration and disinfection of water; sanitary disposal of excreta, sewage and waste water; thermal processing; good hygiene practices during production and processing.

**Food service establishment/household:** Boiling of water when safe water is not available; boiling of milk; thorough cooking of food; thorough hand-washing.

**Occurrence:** Cryptosporidiosis is one of the leading causes of diarrhoeal disease in infants and young children.

**Other Comments:** Children under the age of 5 years are at higher risk of infection. Immunocompromised individuals may suffer from longer and more severe infection; maybe fatal in AIDS patients.

II. **Cyclosporiasis**

**Name of Illness:** Cyclosporiasis

**Etiological Agent:** *Cyclospora cayetanensis*, is a coccidian that is closely related to the cryptosporidia

**Life Cycle:** The *C. cayetanensis* oocysts measure approximately 8–10µm in diameter, and they contain two sporocysts (about 4µm wide and 6µm long). Each sporocyst contains two crescent-shaped sporozoites about 1µm wide and 9µm long. The oocysts are acid-fast and sensitive to drying, but resistant to chlorine.
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The oocysts sporulate between 5 and 13 days in culture, and best at 22 or 30°C but not at either 4 or 37°C.

**Symptoms:** The disease symptoms mimic those of cryptosporidiosis. Diarrhea, loss of appetite, fatigue, and weight loss. Diarrhea is prolonged but self-limiting, lasting a mean of 43±24 days, and it is more severe in Human Immunodeficiency Virus (HIV)-infected individuals.

**Spread/Mode of Transmission:** Person-to-person transmission of this disease is unlikely since the excreted oocysts must sporulate to become infectious.

**Diagnosis:** The oocysts in stools can be identified by wetmount, phase microscopy, acid-fast staining, or epifluorescence microscopy. Confirmation or detection can be made by Polymerase Chain Reaction (PCR).

**Treatment:** Unlike cryptosporidia, this protozoan is susceptible to Bactrim (trimethoprim-sulfamethoxazole).

### III. Giardiasis

**Name of Illness:** Giardiasis

**Etiological Agent:** Protozoa: *Giardia lamblia*

**Characteristics of Agent:** *Giardia lamblia* is a flagellate protozoan. The protozoal cells (vegetative trophozoites) produce cysts (cyst stage), which are the primary forms in water and foods. The cysts are pear shaped (or oval), with a size range of 8–20µm in length and 5–12µm in width. The trophozoites have eight flagella that arise on the ventral surface near the paired nuclei and give rise to “falling-leaf” motility. Cysts are resistant to the chlorination process used in most water-treatment systems but killed by conventional cooking procedures.

**Incubation Period:** 1–4 weeks.

**Symptoms:** Diarrhoea (which may be chronic and relapsing), abdominal cramps, abdominal distention, fatigue, weight loss, dehydration fever, vomiting, anorexia and nausea. Symptoms may be caused by a protein toxin.

**Sequelae:** Cholangitis, dystrophy, joint symptoms, lymphoid hyperplasia.

**Duration:** Asymptomatic cyst passage is the most benign manifestation of *G. lamblia* infection in humans, but when clinical giardiasis occurs, symptoms may last from several months to a year or more.

**Reservoir/Source:** Humans and animals.

**Life Cycle:** Upon ingestion, Giardia cysts release the active trophozoite which adheres to the gut wall and give rise to clinical giardiasis in some individuals. Excystation of the trophozoites occurs somewhere in the upper small intestine. The trophozoites obtain their nutrients by absorption. Growth of the organism is favored by the high bile content in the duodenum and upper jejunum. Occasionally, bile ducts are invaded, leading to cholecystitis. Compared to some of the other intestinal protozoal parasites, *Giardia* trophozoites do not penetrate deeply in parenetal tissues.
NOTES

Spread /Mode of Transmission: Infected individuals excrete Giardia cysts in large numbers. Giardiasis is a highly contagious disease. It has been documented in daycare centers where unsanitary conditions prevailed. Illness is spread by faecal-oral route, person-to-person contact or faecally contaminated food and water. Anything that comes into contact with feces from infected humans or animals can become contaminated with the *Giardia* parasite. People become infected when they swallow the parasite picked up from surfaces (such as bathroom handles, changing tables, diaper pails, or toys) that contain stool from an infected person or animal.

- Eating uncooked food that contains *Giardia* organisms.
- Having contact with someone who is ill with giardiasis.
- Traveling to countries where giardiasis is common.
- Infection also associated with drinking-water from surface waters and shallow wells.

Food Vehicles: Cysts have been isolated from lettuces and fruits such as strawberries. Foods involved include water, home-canned salmon and noodle salad. The consumption of tapwater and the eating of ice cream or raw vegetables are significantly associated with the illness.

Diagnosis: Giardiasis is diagnosed by the demonstration of trophozoites in stool specimen by microscopic examinations using either wet mounts or stained specimens. *G. lamblia* can be grown in axenic culture, but this does not lend itself to rapid diagnosis. Effective Enzyme-Linked Immunosorbent Assay (ELISA) tests have been developed. Both circulating antibodies and T lymphocytes are elicited during infection by *G. lamblia*. Because no enterotoxin has been demonstrated, diarrhea is caused by other factors.

Treatment: The drug of choice for the treatment of giardiasis is quinacrine, an acridine derivative. Also effective are metronidazole and tinidazole.

Prevention and Control

Industrial: Filtration and disinfection of water supply; sanitary disposal of excreta and sewage water; treatment of irrigation water; thermal processing; good hygiene practices during production and processing.

Food service establishment/household: Boiling of water when safe water is not available; thorough washing of fruit and vegetables; thorough cooking of foods; thorough hand-washing.

Consumers, and more specifically campers, should avoid drinking surface water unless it has been boiled or filtered.


Other Comments: Number of asymptomatic carriers high. Children are affected more frequently than adults. Tourists are particularly at risk. Illness is prolonged and more serious in AIDS patients.
IV. Toxoplasmosis

Name of Illness: Toxoplasmosis and congenital toxoplasmosis.

Etiological Agent: Protozoa: *Toxoplasma gondii*, a coccidian protozoan that is an obligate intracellular parasite.

Characteristics of Agent: The generic name is based on the characteristic shape of the sporozoites, i.e., crescent shaped and measure about 3×7µm. (Gr. toxo, ‘arc’). It was first isolated in 1908 from an African rodent, the gondi—hence, its species name. Oocysts are ovoid shaped, measure 10–12µm in diameter, and possess a thick wall.

Disease: The infection is self-limiting. In these cases, the organism encysts and becomes latent. However, when the immunocompetent state is abated, life-threatening toxoplasmosis results from the breaking out of the latent infection.

Incubation Period: The incubation period in adults is 6–10 days while in infants it is congenital.

Symptoms: In most individuals, toxoplasmosis is symptomless, but when symptoms occur, they consist of fever with rash, headache, muscle aches and pain, and swelling of the lymph nodes. The muscle pain, which is rather severe, may last up to a month or more.

Sequelae: *T. gondii* infections are asymptomatic in the vast majority of human cases (immunocompetents), but in congenital infections and in immunocompromised hosts, such as patients with Acquired Immuno Deficiency Syndrome (AIDS), the disease is much more severe. Cerebral toxoplasmosis is a particular threat for AIDS patients. In pregnant mothers with newly contracted toxoplasmosis, the tachyzoites are reported to cross the placenta about 45% of the time and may cause abortion or stillbirth, chorioretinitis, brain damage.

Duration: Infections often asymptomatic or present acute diseases with lymphadenopathy and lymphocytosis persisting for days or weeks.

Reservoir/Source: Domestic and wild cats are the only definitive hosts for the intestinal or sexual phase of this organism, making them the primary sources of human toxoplasmosis. Normally, the disease is transmitted from cat to cat, but virtually all vertebrate animals are susceptible to the oocysts shed by cats. As few as 100 oocysts can produce clinical toxoplasmosis in humans, and the oocysts can survive over a year in warm, moist environments. Pigs are the major animal food source to humans.

Life Cycle: The disease is initiated upon the ingestion of oocysts (if from cat feces), which pass to the intestine where digestive enzymes affect the release of the eight motile sporozoites. They cannot survive for long outside animal host tissues, nor can they survive the activities of the stomach. When freed in the intestines, these forms pass through intestinal walls and multiply rapidly in many other parts of the body, giving rise to clinical symptoms. The most rapidly multiplying forms are designated tachyzoites (Gr.tachy, ‘rapid’), and in immunocompetent individuals,
they eventually give rise to clusters that are surrounded by a protective wall. This is a tissue cyst, and the protozoa inside are designated bradyzoites (Gr. bradus, ‘slow’). These cysts are 10–200µm in diameter and the bradyzoites are smaller in size than the more active tachyzoites. Bradyzoites may persist in the body for the lifetime of an individual, but if the cysts are mechanically broken or break down under immunosuppression, bradyzoites are freed and begin to multiply rapidly as tachyzoites and thus bring on another active infection. The development of a cyst wall around bradyzoites coincides with the development of permanent host immunity. The cysts are normally intracellular in host cells.

**Food Vehicles:** Food involved include raw or uncooked meat, vegetables and goat’s milk.

**Spread/Mode of Transmission:** Infections occur through ingestion of oocysts. Children may acquire the infection by playing in sand polluted with cat excreta. Oocysts shed by cats can sporulate and become infective 1–5 days later and may remain infective in water or soil for a year. Infection may also acquired by eating raw or undercooked meat containing the cysts or food and water contaminated with feline faeces. Transplacental infection may occur when the infection is acquired during pregnancy.

**Prevention and Control:**

- **Industrial:** The cysts of *T. gondii* can be destroyed by heating meats above 60°C or by irradiating at a level of 30 krad (0.3 kGy) or higher. The organism may be destroyed by freezing, but because the results are variable, freezing should not be relied on to inactivate oocysts.

- **Food service establishments, household:** Thorough cooking of meat; by avoiding the consumption of meat and meat products that contain viable tissue cyst, good personal hygiene (particularly after contact with cats and before food preparation), avoiding environmental contamination with cat feces and safe disposal of cat feces.

  Consumers, particularly pregnant women if not immune, should be advised to avoid raw or undercooked meat, wash vegetables carefully and wash hands after contact with cats.

**Diagnosis:** Unlike certain other intestinal protozoal diseases, toxoplasmosis cannot be diagnosed by demonstrating oocysts in stools, as these forms occur only in cat feces. A fourfold rise in Immuno Globulin G (IgG) antibody titer between acute and convalescent serum specimens is indicative of acute infection. A more rapid confirmation of acute infection can be made by the detection of Immuno Globulin M (IgM) antibodies, which appear during the first week of infection and peak during the second to fourth weeks. Among other diagnostic methods are the methylene blue dye test, indirect haemagglutination, indirect immunofluorescence and immunoelectrophoresis. With the indirect haemagglutination test, antibody titers above 1:256 are generally indicative of active infection.
Food Poisoning and Food Borne Infections

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Treatment: Antimicrobial therapy for toxoplasmosis consists of sulfonamides, pyrimethamine, pyrimethamine plus clindamycin, or fluconazole. Pyrimethamine is a folic acid antagonist that inhibits dihydrofolate reductase.

Occurrence: Worldwide.

Other Comments: T. gondii cysts remain in the tissue and may be reactivated if the immune system becomes compromised. In immunosuppressed individuals, the infection may be fulminant and fatal.

V. Amebiasis

Name of Illness: Amoebiasis (amoebic dysentery).


Characteristics of Agent: The organism is unusual in being anaerobic, and the trophozoites (amoeba stages) lack mitochondria. It is an aerotolerant anaerobe that requires glucose or galactose as its main respiratory substrate. The trophozoites of *E. histolytica* range in size from 10 to 60µm, whereas the cysts usually range between 10 and 20µm.

The trophozoites are motile; the cysts are not. In warm stools from a case of active dysentery, *E. histolytica* is actively motile and usually contains red blood cells that the protozoan ingests by pseudopodia. Although the trophozoites do not persist under environmental conditions, the encysted forms can survive as long as 3 months in sewage sludge.

A person with this disease may pass up to 4.5×10^7 cysts each day. Amebaoid, aerotolerant anaerobe that survives in the environment in an encysted form. Cysts remain viable and infective in faeces for several days, in soil for at least 8 days at 28-34°C (and for >1month at 10°C). Relatively resistant to chlorine.

Life cycle: In its trophozoite stage, the organism induces infection in the form of abscesses in intestinal mucosal cells and ulcers in the colon. Its adherence to host-cell glycoproteins is mediated by a galactose-specific lectin. It reproduces by binary fission in the large intestine. It encysts in the ileum, and cysts may occur free in the lumen. The organism produces an enterotoxic protein.

Incubation Period: 2-4 weeks (range several days to several months).

Symptoms: Symptoms may persist for several months. Its onset is often insidious, with loose stools and generally no fever. Mucus and blood are characteristic of stools from patients. Later symptoms consist of pronounced abdominal pain, fever, severe diarrhea, vomiting, and lumbago, and somewhat resemble those of shigellosis. Weight loss is common, and all patients have heme-positive stools.

Sequelae: Liver abscess.

Duration: Amebiasis may last in some individuals for many years, in contrast to giardiasis, where disease symptoms rarely exceed 3 months.
Food Poisoning and Food-Borne Infections

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Reservoir/Source: Mainly humans, but also dogs and rats. The organism is also found in night soil and sewage used for irrigation.

Food Vehicles: Water contaminated with sewage, moist food contaminated with human feces.

Spread/Mode of Transmission: Often transmitted by the fecal–oral route, although transmission is known to occur by water, food handlers, and foods.

The possible transmission of cysts to foods becomes a real possibility when poor personal restroom hygiene is practiced. It is estimated that 10% of the world’s population is infected with *E. histolytica* and that up to 100 million cases of amebic colitis or liver abscesses occur each year.

Diagnosis: Amebiasis is diagnosed by demonstrating trophozoites and cysts in stools or mucosal scrapings. Immunological methods such as indirect hemagglutination, indirect immunofluorescence, latex agglutination, and ELISA are useful. The sensitivity of these tests is high with extra intestinal amebiasis, and a titer of 1:64 by indirect hemagglutination is considered significant.

Treatment: This syndrome can be treated with the amebicidal drugs metronidazole and chloroquine. Resistance is mediated by cell immunity. Lymphocytes from patients in the presence of *E. histolytica* antigens have been shown to produce ß-interferon, which activates macrophages that display amebicidal properties.

5.2.5 Toxic Metals and Chemicals

Metals, when consumed in excess of the requirements, could cause toxicity. These contaminants may be present in the environment and may accumulate during different stages of food preparation. By the time food is ready to be consumed, it can reach levels which are toxic to humans.

Selenium: When soil contains excessive amounts of selenium, it may enter the food and cause gastrointestinal disturbances, loss of appetite and stunted growth.

Zinc: If acidic foods are kept in galvanized metal containers for long periods of time, they may contain toxic levels of zinc. High consumption of zinc can cause anaemia, growth depression, dizziness, drowsiness, nausea and diarrhoea.

Arsenic: Exposure to arsenic over years causes cutaneous lesions, cancer of the lungs and many other symptoms. Many insecticides, weed killers and rat poisons contain arsenic. It is also present in water from polluted streams. Fruits, vegetables and shellfish are likely to be contaminated with arsenic.

Lead: Lead can contaminate food through various sources such as:

- Lead pipes that convey drinking water; soft water and alcoholic drinks dissolve more lead than hard water does.
- Mineral pigments in food; lead chromate may be used as an adulterant in turmeric powder.
- Some pesticides contain lead arsenate.
Food Poisoning and Food Borne Infections

- Exposure to dust containing lead
- Contact with machines

Food Allergies

An allergy is defined as a special reaction of an individual to some ingredient in food. Some people show abnormal sensitivity to foods that are harmless to a non-allergic person. The substance that causes the allergy is called an allergen. Allergens may be present in foods such as eggs, wheat fish, shellfish, chocolate, strawberries and cow’s milk.

Infections and Intoxications

Below are some infections and intoxications:

Gastrointestinal Infections: Gastrointestinal disturbances resulting from the ingestion of food can have a variety of causes, for example, overeating, allergies, nutritional deficiencies, actual poisoning by chemicals, toxic plants, or animals, toxins produced by bacteria, infestation by animal parasites, and infection by microorganisms. These illnesses often are grouped together because they have rather similar symptoms at times and sometimes are mistaken for each other.

Food Intoxication: Food poisonings can be the result of either chemical poisoning or the ingestion of a toxicant. The toxicant might be found naturally in certain plants or animals or be a toxic metabolic product excreted by a microorganism. Bacterial food intoxication therefore refers to food-borne illnesses caused by the presence of a bacterial toxin formed in the food. A bacterial food infection refers to food-borne illnesses caused by the entrance of bacteria into the body through ingestion of contaminated foods and the reaction of the body to their presence or to their metabolites.

Infection by Clostridium: Some workers suggest that Clostridium perfringens food-borne illness and Bacillus cereus gastroenteritis should be listed as food intoxications rather than as food infections since toxins might be released by B. cereus as a result of autolysis of the cells in the food. If the toxins were released in food, they would be better classified as food intoxicants. However, as is discussed in the following sections, a large number of viable cells must be consumed in both cases, which implies the release of toxin in-vivo rather than in the food, the controversial status of these two food-borne illnesses is recognized. Additionally, the whole categorization into food infections and food intoxications is not as straightforward as it once was. S. aureus produces an enterotoxin in the food and is frequently referred to as the classical food intoxication. However, several Gram-Positive Bacteria, including S. aureus, C. perfringens, and B. cereus, can colonize the intestinal mucosa and be responsible for chronic diarrhoea.
Neurotoxin Infection: Botulism is a disease caused by the ingestion of food containing the neurotoxin produced by *Clostridium botulinum*. This rod-shaped soil bacterium is saprophytic, spore forming, gas forming and anaerobic. Seven types are distinguished on the basis of the serological specificity of their toxins; the predominant (or only) toxin from these types is designated by the same capital letter (Type A to Type G).

Toxin production by *C. botulinum* depends on the ability of the cells to grow in a food and to autolyse there, for the types A, B, E, and F toxins apparently are synthesized as large, comparatively inactive proteins which become fully toxic after some hydrolysis. Therefore, the factors that influence spore germination, growth, and hence toxin production are of special interest. These factors include the composition of the food or medium, especially its nutritive properties (for example, glucose or maltose is known to be essential for toxin production), moisture content, pH, OR potential and salt content, and the temperature and time of storage of the food. It is the combination of these factors that determines whether growth can take place and the rate and extent of that growth. Thus, the nutritive properties of the food are likely to determine the minimal pH or temperature and the maximal concentration of sodium chloride for growth and toxin production. Results will differ with the serological type of organism and the particular strain.

Chemical Concentration: Experiments on dehydrated meat have shown that toxin was produced more slowly when the moisture content was 40 per cent than when it was 60 per cent and that reduction to 30 per cent prevented toxin production. The concentrations of sodium chloride necessary to prevent growth and toxin production in foods depend on the composition of the food and its temperature. The presence of sodium nitrate in sausages or of disodium phosphate in cheese spread reduces the level of sodium chloride necessary to prevent toxin production. More salt is needed at a higher temperature, such as 37 ºC, than at a lower one, say, 15 ºC. Under favourable conditions for growth 8 per cent or more of salt is needed to inhibit *C. botulinum*.

pH: A pH near neutrality favours the growth of *C. botulinum*. The minimal pH at which growth and toxin production will take place depends on the kind of food and its temperature. A pH of 4.5 or lower will prevent toxin production in most foods, but the lowest pH for spore germination is considerably higher. Minimal pH values reported are 4.87 for vegetative cells and 5.01 for spore germination in a veal infusion broth, 4.8 to 5.0 in bread, and 4.8 in pineapple-rice pudding. A maximal pH of 8.89 was found for vegetative growth. *C. botulinum* has been found growing and producing toxin in foods that normally are too acid for it, when other microorganisms also were growing in the food and presumably raising the pH locally or generally.
Canned Food Products: There have been outbreaks of botulism from inadequately heat-processed canned high-acid foods (pH less than 4.5) including tomatoes, tomato juice and blackberries. Possible explanations for these outbreaks have included (i) growth of other organisms which could raise the pH of a food so that *C. botulinum* could grow, (ii) growth of *C. botulinum* followed by growth of other organisms which lowered the pH of a food that originally had a pH higher than 4.5, and (iii) variation or stratification of the pH in an acidulated product to permit growth of *C. botulinum*. Tanaka (1982) has reported toxin production in media at a pH lower than 4.6.

Temperature: Temperature is an important factor in determining whether toxin production will take place and what the rate of production will be. Vegetative growth will take place at a lower temperature than the minimum for spore germination. Different strains of *C. botulinum* types A and B vary in their temperature requirements. A few strains have been reported able to grow at 10–11°C, but about 15°C has been claimed to be the lowest temperature for germination of spores. The maximal temperature for growth is about 48°C for these types and about 45°C for Type E. Type E organisms produce gas and toxin within thirty-one to forty-five days at temperatures as low as 3.3°C. The optimal temperature for toxin production and growth of the proteolytic strains is about 35°C, while 26 to 28°C is usually given for the optimal temperature for the non-proteolytic strains. Obviously, the slower the rate of toxin production, the longer it will take to obtain appreciable amounts. The toxin of *C. botulinum*, a protein that has been purified and crystallized, is so powerful that only a tiny amount is sufficient to cause death. It is absorbed mostly in the small intestine and causes paralysis of involuntary muscles of the body. An important characteristic is its comparative thermo ability. The heat treatment necessary to destroy it depends on the type of organism producing the toxin and the medium in which it is heated. This should not imply that thorough cooking of a highly suspect food would be a worthwhile risk.

Cooked Foods: Investigators have shown that spores of *C. botulinum* will survive long storage periods in raw and precooked frozen foods and can grow and produce toxin if these foods are held long enough at a high enough temperature after thawing. Similarly, temperature abuse of foods that may support the growth of *C. botulinum* and which may be contaminated must be avoided. Diseased people are so susceptible to botulism that if appreciable amounts of toxin are present, consumption of very small pieces of food, a pod of string bean or a few peas, can cause illness and death.

Conditions Necessary for Intoxication

The following conditions are necessary for an outbreak of botulism:

- Presence of spores of *C. botulinum* of types A, B, or E in the food being canned or being processed in some other way.
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- A food in which the spores can germinate and the clostridia can grow and produce toxin.
- Survival of the spores of the organism, for example, because of inadequate heating and canning or inadequate processing otherwise.
- Environmental conditions after processing that will permit germination of the spores and growth and toxin production by the organism.
- Insufficient cooking of the food to inactivate the toxin.
- Ingestion of the toxin-bearing food.

Prevention of Intoxication

The methods and precautions for the prevention of botulism include (i) use of approved heat processes for canned foods, (ii) rejection of all gassy (swollen) or otherwise spoiled canned foods, (iii) refusal even to taste a doubtful food, (iv) avoidance of foods that have been cooked, held, and not well reheated, and (v) boiling a suspected food for at least 15 min. To this list might be added avoidance of raw or precooked foods that have been frozen, thawed, and held at room temperatures. To prevent botulism from smoked fish it has been recommended that (i) good sanitation be maintained throughout production and handling, (ii) during smoking or thereafter the fish be heated to at least 82°C for 30 min in the coldest part, (iii) the fish be frozen immediately after packaging and kept frozen, and (iv) all packages be marked ‘Perishable—Keep Frozen’.

![Fig. 5.6 Measures to Prevent Bacterial Contamination](image)

**Toxicity:** Originally isolated as an antibiotic, it is effective against many bacterial species. Application of patulin in very low concentration (0.1 per cent) completely inhibits *E. coli* and *S. aureus*. It also exhibits strong fungistatic activity and is toxic to seeds and seedlings of higher plants including sugar beets, corn, wheat, peas, tomato, cucumber and flax. Oral and intravenous injections of patulin in
concentrations of 0.3 to 2.5 mg per gram of body weight are fatal to mice and rats. Pathological changes included oedema of the brain, hemorrhaging of the lungs, and capillary damage of the liver, spleen and kidney. Carcinogenic and mutagenic properties of patulin have been observed in mice at sub lethal doses. Since many other animals are known to be sensitive to patulin, it is considered a potential human carcinogen.

**Significance in Foods**

Many patulin-producing strains have been isolated from food and animal feeds. The presence of patulin has been detected in apple cider and apple juice. Its occurrence in apple products may be related to the fact that over 60 per cent of sampled rotted apples have yielded patulin-producing strains of Penicillium expansum. In most other products patulin-producing molds represent only a small percentage of the total isolates. Another explanation for the inability to detect patulin chemically in foodstuffs which support good growth of patulin-producing fungi might be the observed inhibitory effect of several food compounds on patulin. For example, peptone, glycine, methionine, p-aminobenzoic acid, asparagine, sodium sulphate, sodium thiosulphate, and casein hydrolysate have an inhibitory action on patulin. It is possible, therefore, that some foods which will support patulin-producing fungi do not constitute a problem because of their inherent composition. Of additional interest to the food microbiologist is that several patulin-producing fungi when grown on a synthetic medium will elaborate patulin at refrigeration temperatures. The resistance of patulin to heat also has been noted. This mycotoxin resists boiling; for example, it is stable at 100 °C for 15 min.

The carcinogenic property of patulin in animals, the isolation of patulin-producing fungi from several foodstuffs, its inactivation in some foods, the potential for toxin production at refrigeration temperatures, and its stability to heat suggest that patulin will be the subject of further study and concern in the food industry.

**Toxicity**

Ochratoxin A is toxic to ducklings, rats, chicks, trout, and other animals. It is about one-third as toxic as aflatoxin B, to rats. The other derivatives or analogues are all equally toxic or less toxic than Ochratoxin A. They have been shown to produce lesions in the kidneys of rats and lesions in trout and chicks.

Citrinin, another mycotoxin, also has a pathological effect on the kidneys of animals. Mold nephrosis, a disease in pigs, has occurred at high rates when the animals were fed grain which had been harvested wet. There is speculation that molds producing ochratoxin A and citrinin are responsible for this syndrome. If these mycotoxin-producing molds are isolated and grown on synthetic media, citrinin is produced. Consumption of the citrinin by rats, rabbits, guinea pigs, and pigs results in kidney damage similar to that observed in pigs with mold nephrosis. A moldy alfalfa grass mixture was considered to be responsible for high abortion rate in dairy cows. Although ochratoxin A was not isolated, an ochratoxin-producing Aspergillus strain was identified.
Food Poisoning and Food Borne Infections

<table>
<thead>
<tr>
<th>NOTES</th>
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Check Your Progress

1. What is food borne illness?
2. What is Bacillus cereus?
3. What is Salmonellosis?
4. Give the characters of Hepatitis A virus.
5. How does transmission of rotaviruses occur?
6. How are Mycotoxins produced?
7. What is the ecological agent of Cryptosporidiosis and give the characters of Cryptosporidiosis agent.

5.3 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Foodborne illness, more commonly referred to as food poisoning, is the result of eating contaminated, spoiled, or toxic food. The most common symptoms of food poisoning include nausea, vomiting, and diarrhea.

2. Bacillus cereus is a spore-forming aerobic rod-shaped bacteria which causes a toxic type of food-borne illness. It is found in soil, dust, water and on cereal grains. It is a common type of food poisoning. The food usually affected is rice, especially boiled rice which is prepared and used on the following day. The spores survive boiling. They germinate, multiply and release a toxin in rice if it is not refrigerated. If the rice is not reheated thoroughly the toxin is not destroyed and consumption of the rice will cause food poisoning.

3. Salmonellosis is the most common cause of bacterial food-borne disease and the most serious. Organisms of the salmonella group cause infection in the intestine. Many species are infectious. These rod-shaped bacteria are aerobic and non-spore producing. They are present in the intestine of humans and animals and are excreted in the faeces. Illness occurs when living organisms are ingested in large numbers. Even a small number of organisms in food can result in infection.

4. Characteristics of Hepatitis A virus: Small round virus, member of Picornaviridae, around 28nm in diameter, containing single-stranded RNA. Multiplies in the gut epithelium before being carried by the blood to the liver. In the later part of incubation, the virus is shed in the faeces. Relatively acid-resistant.

5. Spread/Mode of Transmission of rotaviruses are transmitted by the fecal–oral route; this means that the virus must be passed by an infected person.
and then enter a susceptible person’s mouth to cause infection. Person-to-
person spread can also possible. Rotaviruses cause an estimated one-third
of all hospitalizations for diarrhea in children below age 5. Rotavirus may
activate neurons of the enteric nervous system that stimulate secretion of
fluids and solutes. After an incubation period of 1–3 days, rotavirus infects
the mature absorptive enterocytes in the ileum, and causes watery diarrhea,
fever, abdominal pain and vomiting, which generally lasts 3–8 days. In babies
and young children, it can lead to dehydration. Severe diarrhea without
fluid and electrolyte replacement may result in severe dehydration, and in
several cases, death. Severe dehydration may require hospitalization for
treatment with Intravenous (IV) fluids (fluids given to the patient directly
through their veins). The best way to protect against dehydration is to drink
plenty of liquids (oral rehydration therapy).

6. Mycotoxins are produced as secondary metabolites by fungi. Secondary
metabolites are formed during the end of the exponential growth phase and
have no apparent significance to the producing organism relative to growth
or metabolism. However, primary metabolites are those compounds that
are essential for growth.

7. Cryptosporidiosis
Etiological agent Protozoa: Cryptosporidium parvum.
Characteristics of Agent: C. parvum is an obligate intracellular coccidian
parasite that carries out its life cycle in one host. The oocysts of C. parvum
are spherical to ovoid and average 4-6µm in size. The oocysts are highly
resistant in the natural environment and may remain viable for several months
when kept cold and moist. Oocysts are very resistant to chlorination but
killed by conventional cooking procedures. They has been reported to be
destroyed by treatments with 50% or more ammonia and 10% or more
formalin for 30 minutes. Temperatures above 60°C and below −20°C may
kill C. parvum oocysts. The organism is destroyed by High-Temperature,
Short Time (HTST) milk pasteurization. Holding oocysts at 45°C for 5–20
minutes has been reported to destroy their infectivity.

5.4 SUMMARY
- Foodborne illness, more commonly referred to as food poisoning, is the
result of eating contaminated, spoiled, or toxic food.
- The most common symptoms of food poisoning include nausea, vomiting,
and diarrhea.
- A foodborne infection is an inflammation of the stomach and bowels. The
infection can happen when you eat or drink something that is contaminated
by a bacteria, virus or parasite.
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- Often the inflammation leads to diarrhoea, nausea, vomiting, abdominal pain, abdominal cramps and sometimes fever. A foodborne infection can last between one and three days.
- Cross-contamination is also a risk, for instance if raw meat and lettuce are both chopped on the same cutting board.
- A food-borne infection involves the ingestion of the pathogen such as Salmonella, Campylobacter, Listeria, and E. coli, followed by growth in the host, including tissue invasion and/or the release of toxins.
- If the pathogen grows in the food before consumption and forms toxins that affect the food consumer without further microbial growth, the disease is a food-borne intoxication.
- Toxins produced in the food can be associated with microbial cells or can be released from the cells.
- Botulism is an uncommon type of food poisoning. It is produced by anaerobic spore-forming bacteria found in the soil. The organism produces a toxin which is extremely poisonous and affects the nervous system resulting in the death of approximately two-thirds of the affected victims.
- *Bacillus cereus* is a spore-forming aerobic rod-shaped bacteria which causes a toxic type of food-borne illness. It is found in soil, dust, water and on cereal grains. It is a common type of food poisoning.
- *Clostridium perfringens* is a spore-forming anaerobe found in the human and animal intestinal tract, soil, dust, contaminated raw meat, poultry and some dried foods.
- *Salmonellosis* is the most common cause of bacterial food-borne disease and the most serious. Organisms of the salmonella group cause infection in the intestine.
- Typhoid fever is caused by Salmonella hyphi, whereas paratyphoid fever is caused by *Salmonella enteritidis*.
- Cholera is caused by the comma-shaped bacteria Vibrio cholerae which is present in water contaminated by faeces of people suffering from cholera.
- Gastroenteritis are known to be associated with travelers’ diarrhea. Norovirus causes inflammation of the stomach, intestines or both, whereby, Noroviruses reduces the absorptive capacity of the villi contributing diarrhea, nausea, and vomiting.
- *Noroviruses gastroenteritis* is characterized by an incubation period of 48h after ingestion of viruses through contaminated food.
- Poliomyelitis has been almost entirely eliminated in industrialized countries and the Americas by effective immunization. Incidence in developing countries depending on immunization coverage.
A very large number of molds produce toxic substances designated mycotoxins. Some are mutagenic and carcinogenic, some display specific organ toxicity. At least 14 mycotoxins are known to be carcinogens, with the aflatoxins being the most potent.

Mycotoxins are produced as secondary metabolites by fungi. Secondary metabolites are formed during the end of the exponential growth phase and have no apparent significance to the producing organism relative to growth or metabolism.

The ochratoxins consist of a group of at least seven structurally related secondary metabolites of which Ochratoxin A (OA) is the best known and the most toxic.

Ochratoxin A (OA) is structurally a chlorinated isocoumarin derivative with an amide bond to phenylalanine. A dechlorinated analog of this compound is named Ochratoxin B (OB).

In addition, there are other minor components including a methyl and ethyl ester of A and B and 4-hydroxyochratoxin A. Ochratoxin A fluoresces green under ultraviolet light, and ochratoxin B fluoresces blue.

Patulin causes chromosomal aberrations in animal and plant cells and is a carcinogen. Originally isolated as an antibiotic, it is effective against many bacterial species.

Sambutoxin causes hemorrhage in the stomach and intestines of rats, and the animals refuse feed and lose weight.

*C. parvum* is an obligate intracellular coccidian parasite that carries out its life cycle in one host.

The oocysts of *C. parvum* are spherical to ovoid and average 4-6 µm in size. The oocysts are highly resistant in the natural environment and may remain viable for several months when kept cold and moist.

Oocysts are very resistant to chlorination but killed by conventional cooking procedures.

Diagnosis of cryptosporidiosis requires the identification of oocysts in stools of victims. Staining methods such as acid-fast, negative staining, and sugar flotation are used.

Another diagnostic method of cryptosporidiosis is direct immunofluorescence test used for the detection of oocysts in feces, which employs a monoclonal antibody against an oocyst wall antigen.

Spiramycin, fluconazole, and amphotericin B show some promise. More recently, the aminoglycoside antibiotics paromomycin and geneticin were found to inhibit the growth of intracellular *C. parvum*. 
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• *Cyclospora cayetanensis*, is a coccidian that is closely related to the cryptosporidia
• Diarrhoea (which may be chronic and relapsing), abdominal cramps, abdominal distention, fatigue, weight loss, dehydration, fever, vomiting, anorexia and nausea.
• Giardiasis is diagnosed by the demonstration of trophozoites in stool specimen by microscopic examinations using either wet mounts or stained specimens.
• *G. lamblia* can be grown in axenic culture, but this does not lend itself to rapid diagnosis.
• Antimicrobial therapy for toxoplasmosis consists of sulfonamides, pyrimethamine, pyrimethamine plus clindamycin, or fluconazole. Pyrimethamine is a folic acid antagonist that inhibits dihydrofolate reductase.
• An allergy is defined as a special reaction of an individual to some ingredient in food. Some people show abnormal sensitivity to foods that are harmless to a non-allergic person.
• The substance that causes the allergy is called an allergen. Allergens may be present in foods such as eggs, wheat, fish, shellfish, chocolate, strawberries, and cow’s milk.
• Food poisonings can be the result of either chemical poisoning or the ingestion of a toxicant.
• The toxicant might be found naturally in certain plants or animals or be a toxic metabolic product excreted by a microorganism.
• Bacterial food intoxication therefore refers to food-borne illnesses caused by the presence of a bacterial toxin formed in the food.
• A bacterial food infection refers to food-borne illnesses caused by the entrance of bacteria into the body through ingestion of contaminated foods and the reaction of the body to their presence or to their metabolites.
• Experiments on dehydrated meat have shown that toxin was produced more slowly when the moisture content was 40 per cent than when it was 60 per cent and that reduction to 30 per cent prevented toxin production.
• A pH near neutrality favours the growth of *C. botulinum*. The minimal pH at which growth and toxin production will take place depends on the kind of food and its temperature.
• Temperature is an important factor in determining whether toxin production will take place and what the rate of production will be.
• Ochratoxin A is toxic to ducklings, rats, chicks, trout, and other animals. It is about one-third as toxic as aflatoxin B, to rats. The other derivatives or analogues are all equally toxic or less toxic than ochratoxin A.
5.5 KEY WORDS

- **Foodborne illness**: Foodborne illness, more commonly referred to as food poisoning, is the result of eating contaminated, spoiled, or toxic food.
- **Botulism**: Botulism is an uncommon type of food poisoning. It is produced by anaerobic spore-forming bacteria found in the soil.
- **Bacillus cereus**: Bacillus cereus is a spore-forming aerobic rod-shaped bacteria which causes a toxic type of food-borne illness.
- **Clostridium perfringens**: Clostridium perfringens is a spore-forming anaerobe found in the human and animal intestinal tract, soil, dust, contaminated raw meat, poultry and some dried foods.
- **Allergy**: An allergy is defined as a special reaction of an individual to some ingredient in food.
- **Allergen**: The substance that causes the allergy is called an allergen.

5.6 SELF ASSESSMENT QUESTIONS AND EXERCISES

**Short Answer Questions**

1. What is food poisoning?
2. Define food borne illness.
3. What is *Bacillus cereus* food poisoning?
4. What is cholera?
5. Write in short about Hepatitis A.
6. What is *Rotavirus*? What are the symptoms of *Rotavirus*?
7. What is mycotoxin and how are they produced?
8. How is *Amebiasis* caused?

**Long Answer Questions**

1. Write a note on food poisoning and food borne infections.
2. Discuss about various bacterial diseases, their symptoms, causative agent, treatment and prevention.
3. What are viral diseases? How are they caused? Write about some of the viral diseases and their symptoms, duration, source and prevention.
4. Write a detailed note on fungal diseases explaining about different types of fungal diseases and their causative agents, symptoms, duration, symptoms and cure.
5. Explain about various types of protozoal diseases and their causative agents, symptoms, duration, symptoms and cure.

6. Write a note on bacterial and fungal toxins.

### 5.7 FURTHER READINGS

### UNIT 6  GENERAL PRINCIPLES OF FOOD PRESERVATION

**Structure**
- 6.0 Introduction
- 6.1 Objectives
- 6.2 General Principles of Food Preservation
  - 6.2.1 History of Food Preservation
  - 6.2.2 Food Preservation and Method
- 6.3 Answers to Check Your Progress Questions
- 6.4 Summary
- 6.5 Key Words
- 6.6 Self Assessment Questions and Exercises
- 6.7 Further Readings

#### 6.0  INTRODUCTION

Food preservation prevents the growth of microorganisms, or other microorganisms, as well as slowing the oxidation of fats that cause rancidity. Food preservation may also include processes that inhibit visual deterioration, such as the enzymatic browning reaction in apples after they are cut during food preparation.

The vast majority of instances of food spoilage can be attributed to one of two major causes: (1) the attack by pathogens (disease-causing microorganisms) such as bacteria and moulds, or (2) oxidation that causes the destruction of essential biochemical compounds and/or the destruction of plant and animal cells. The various methods that have been devised for preserving foods are all designed to reduce or eliminate one or the other (or both) of these causative agents. For example, a simple and common method of preserving food is by heating it to some minimum temperature. This process prevents or retards spoilage because high temperatures kill or inactivate most kinds of pathogens. The addition of compounds known as BHA and BHT to foods also prevents spoilage in another different way. These compounds are known to act as antioxidants, preventing chemical reactions that cause the oxidation of food that results in its spoilage. Almost all techniques of preservation are designed to extend the life of food by acting in one of these two ways.

The search for methods of food preservation probably can be traced to the dawn of human civilization. People who lived through harsh winters found it necessary to find some means of insuring a food supply during seasons when no fresh fruits and vegetables were available. Evidence for the use of dehydration (drying) as a method of food preservation, for example, goes back at least 5,000
years. Among the most primitive forms of food preservation that are still in use today are such methods as smoking, drying, salting, freezing, and fermenting.

Early humans probably discovered by accident that certain foods exposed to smoke seem to last longer than those that are not. Meats, fish, fowl, and cheese were among such foods. It appears that compounds present in wood smoke have antimicrobial actions that prevent the growth of organisms that cause spoilage. Today, the process of smoking has become a sophisticated method of food preservation with both hot and cold forms in use. Hot smoking is used primarily with fresh or frozen foods, while cold smoking is used most often with salted products. The most advantageous conditions for each kind of smoking—air velocity, relative humidity, length of exposure, and salt content, for example—are now generally understood and applied during the smoking process. For example, electrostatic precipitators can be employed to attract smoke particles and improve the penetration of the particles into meat or fish. So many alternative forms of preservation are now available that smoking no longer holds the position of importance it once did with ancient peoples. More frequently, the process is used to add interesting and distinctive flavours to foods.

In this unit, you will study about general principles of food preservation, chemical and physical methods of food preservation in detail.

6.1 OBJECTIVES

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

- Discuss about general principles of food preservation
- Explain chemical and physical methods of food preservation

6.2 GENERAL PRINCIPLES OF FOOD PRESERVATION

The term food preservation refers to any one of a number of techniques used to prevent food from spoiling. It includes methods such as canning, pickling, drying and freeze-drying, irradiation, pasteurization, smoking, and the addition of chemical additives. Food preservation has become an increasingly important component of the food industry as fewer people eat foods produced on their own lands, and as consumers expect to be able to purchase and consume foods that are out of season.

Many processes designed to preserve food involve more than one food preservation method. Preserving fruit by turning it into jam, for example, involves boiling (to reduce the fruit’s moisture content and to kill bacteria, etc.), sugaring (to prevent their re-growth) and sealing within an airtight jar (to prevent recontamination). Some traditional methods of preserving food have been shown
to have a lower energy input and carbon footprint, when compared to modern methods.

Some methods of food preservation are known to create carcinogens. In 2015, the International Agency for Research on Cancer of the World Health Organization classified processed meat, i.e., meat that has undergone salting, curing, fermenting, and smoking, as 'carcinogenic to humans'. Maintaining or creating nutritional value, texture and flavour is an important aspect of food preservation.

6.2.1 History of Food Preservation

Since the beginning of civilization and in different parts of the world, man has been preserving food in one form or the other. In cold regions, he stored meat and fish under ice while in hot areas sun drying of fruits, berries, grains and nuts was practiced. Salting and smoking are other techniques which were invented by the ancient man. Major technological developments in the field of food preservation took place during the First and Second World War which created a huge demand for preserved food for the armed forces. In 1810, Nicolas Appert—a French confectioner and distiller—discovered that heat application to food sealed in glass containers preserved it from spoiling. His subsequent publication 'The Art of Preserving Animal and Vegetable Substances for Many Years' formed the basis for modern canning—a technique also known as 'Appertizing'. Another scientist, Gay-Lussac studied Appert’s process and interpreted that spoilage in food was an oxidative process which could be prevented by exclusion of air from the glass container. It was only in the 1860s that Louis Pasteur discovered microorganisms and proved their role in food spoilage; he developed a heat treatment method, called ‘pasteurization’ (Refer Figure 6.1) to kill harmful microbes and also prevent them from entering the food by sealing the container hermetically.

Fig. 6.1 Louis Pasteur

In 1920s, Clarence Birdseye discovered quick freezing and developed two processes for freezing fish. The first one involved placing the food between two metal plates chilled by a calcium chloride solution, while the other made use of two hollow metal plates cooled by vaporization of ammonia. This technology formed
the basis of multiple plate freezer used in the modern preservation industry.

In addition to the traditional preservation technologies like freezing, heat treatment, control of pH and water activity, newer and more advanced techniques have been developed over the last hundred years (Refer Table 6.1). These include the use of radiation, high intensity light, pulsed electric fields, oscillating magnetic field, high hydrostatic pressure and ultrasound in preservation. Membrane filtration techniques and modified atmosphere packaging are other novel methods in the field of preservation.

### Table 6.1 History of Food Preservation

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1810</td>
<td>Discovery of canning by Nicholas Appert.</td>
</tr>
<tr>
<td>1813</td>
<td>Origin of sulphur dioxide as meat preservative.</td>
</tr>
<tr>
<td>1839</td>
<td>Use of tin cans.</td>
</tr>
<tr>
<td>1840</td>
<td>Canning of fish and fruit.</td>
</tr>
<tr>
<td>1860</td>
<td>Discovery of microorganisms and ‘pasteurization’ by Louis Pasteur.</td>
</tr>
<tr>
<td>1886</td>
<td>Mechanical drying of fruits and vegetables.</td>
</tr>
<tr>
<td>1890</td>
<td>Commercial pasteurization of milk.</td>
</tr>
<tr>
<td>1908</td>
<td>Use of sodium benzoate as preservative.</td>
</tr>
<tr>
<td>1917</td>
<td>Discovery of quick freezing of food by Clarence Birdseye.</td>
</tr>
<tr>
<td>1928</td>
<td>Controlled atmosphere storage of apples.</td>
</tr>
<tr>
<td>1955</td>
<td>Sorbic acid approved as preservative.</td>
</tr>
<tr>
<td>1990</td>
<td>Irradiation of poultry approved in the US.</td>
</tr>
</tbody>
</table>

### Principles of Food Preservation

As mentioned earlier, food preservation is one of the oldest techniques known to man. It is the process which uses appropriate methods to prevent the microbial, chemical and enzymatic changes and thus extend the shelf life of food. The basic principles of food preservation are as follows:

1. **Prevention or delay of microbial changes**
   - By keeping out microorganisms (asepsis)
   - By removing the microorganisms; for example, filtration
   - By hindering the growth and activity of microorganisms; like, use of low temperature, removal of oxygen, drying or adding chemicals
   - By killing the microorganisms; like using high temperature or radiation

2. **Prevention or delay of self-decomposition of food**
   - By destroying or inactivating endogenous enzymes; for example, blanching.
   - By prevention or delaying of purely chemical reactions; for example, use of antioxidants.
• Prevention of damage caused by insects, animals and mechanical causes

On the basis of these principles, several methods of preservation are available, some of which are listed in Table 6.2. Food preservation usually employs a combination of these methods for better effectiveness. Following are the different principles of preservation as well as techniques based on them:

### Table 6.2 Principles and Methods of Preservation

<table>
<thead>
<tr>
<th>Principle of Preservation</th>
<th>Preservation Factor</th>
<th>Method of Preservation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keeping Out Microorganisms</td>
<td>Hygienic/aseptic handling</td>
<td>Aseptic processing, Aseptic packaging</td>
</tr>
<tr>
<td>Hindering the Growth and Activity of Microorganisms</td>
<td>Low temperature, Removal of air, Addition of carbon dioxide, Increasing acidity, Chemical preservatives</td>
<td>Refrigeration, freezing, Drying, addition of salt or sugar, Vacuum packaging, nitrogen packaging, Carbonation, Addition of acid, fermentation, Sulfit, nitrite, benzoate, nisin</td>
</tr>
<tr>
<td>Killing the Microorganisms</td>
<td>Heat, Radiation</td>
<td>Pasteurization, sterilization, canning, use of ionizing radiation (gamma rays)</td>
</tr>
</tbody>
</table>

### Asepsis

The term ‘asepsis’ means absence of infection or keeping out microorganisms; many of its examples can be found in nature. Shells of eggs, peels of fruits and vegetables, skin on meat or fish, as well as the casing on nuts form a protective covering which prevents the entry of microbes in these foods. When—due to some reasons—this protective covering is cut, damaged or removed, the inner tissues are exposed to microorganisms.

In food preservation, the principle of asepsis aims at preventing the entry of microbes especially the ones that cause spoilage and disease. As you know, microorganisms are present in soil, water, air, the equipment used and the personnel handling the food. Therefore, hygienic practices must be followed during harvesting, handling, washing and processing food to keep the microbial load minimum. By keeping the microbial burden low, one can prolong the shelf life of food through minimal use of chemical preservatives and that of high temperature. Appropriate packaging also prevents the entry of microbes in processed food.

One of the recent techniques of preservation is aseptic packaging in which food is placed into an aseptic container and in an aseptic environment. The container is so designed that it maintains aseptic condition until the seal is broken. This technique, which has been used successfully for fruit juices, has many advantages over the conventional thermal techniques. It ensures a high product quality in terms of colour, flavour and nutritive value of food. Moreover, the production costs as well as the energy consumption are also low. The shelf stability of foods packed aseptically is, however, shorter than the commercially sterilized foods and this technique is not suitable for products containing large particles.
Removal of Microorganisms

Several methods can be employed to remove microorganisms from the food; these are filtration, centrifugation, washing and trimming. For filtering clear liquids, a sterilized filter made up of diatomaceous earth, unglazed porcelain or other suitable material is used. Drinking water, fruit juices, wine and beer can be preserved satisfactorily by this method. Membrane filtration is a novel technique for removal of microbes.

Centrifugation at a very high speed removes several spores and sedimentation of water removes some of the impurities yet, this technique does not ensure complete removal of microbes. Centrifugation, therefore, is not a very effective method for preservation. Washing fresh fruits and vegetables with clean water removes soil as well as harmful microorganisms. All the equipment used for processing must be washed thoroughly to remove traces of microbes. Trimming away the spoiled portions of food and removing the decaying samples also help in controlling the spread of spoilage.

Membrane filtration technique is a purely physical operation to separate a single liquid food into two by means of a selective membrane which rejects all microorganisms. So, after permeating through the membrane, the liquid is sterile without any application of heat. This is a novel technique which has three classical membrane processes that are as follows:

- **Reverse Osmosis (RO):** It is used for separating and purifying water. It is also being applied in areas where evaporation is widely used, such as concentration of milk and fruit juices. When a solution is separated from a solvent by a semi-permeable membrane, the solvent flows to the solution; this process is termed as osmosis and the pressure exerted by the flow is known as osmotic pressure. By applying a pressure greater than the osmotic pressure on the solution side of the membrane, the flow of solvent during osmosis is reversed.

- **Ultrafiltration:** It is a recent technique which is pressure-driven and uses a membrane to separate particulate matter from fluids such as water. These membranes have the ability to filter bacteria and most viruses.

- **Microfiltration:** It is a technique similar to ultrafiltration and can filter both the vegetative and spore forms of microbes to produce sterile fluids. Microfiltration membranes have very fine pore size ranging from 0.01 – 0.10 µm, and have been used successfully for clarifying fruit juices and beverages.

Preservation Using High Temperature

Heating can be considered as the foundation of food preservation. Look around and you will find many applications of heat in food processing; for example, heating, blanching, pasteurization and canning. High temperature results in denaturation of proteins and inactivation of enzymes as a result of which microorganisms are killed and the food gets preserved. Selection of heat treatment, however, depends on various factors, such as kind of microorganism to be killed, its state, other methods...
of preservation employed and the effect of heat on quality attributes of food. We will discuss the concept of heat resistance of microorganisms and the factors affecting.

**Methods of Heat Processing**

It is imperative to recognize that there are various degrees of preservation by heat at the commercial level. The purpose is to enhance the shelf life of food and not to sterilize it fully. Various methods of heat processing include blanching, pasteurization and sterilization. All these processes employ varying degree of heats and are as follows:

**Blanching:** It is a heat process which uses a temperature ranging from 75°C – 95°C for 1 – 5 minutes. It is usually carried out in vegetables, prior to dehydration, freezing or canning to achieve the following objectives:

- To inactivate enzymes; particularly, polyphenol oxidase which causes browning.
- To sanitize the vegetables (microbial load may be reduced to some extent and pesticides may be leached away).
- To remove air from the intercellular spaces so as to achieve suitable head space in canned foods.
- To prevent changes in colour and flavour.
- To remove the stringent taste of some plant foods, like amla.
- To pre-heat the food before canning.
- To soften the texture and reduce the volume, so that more can be filled in the can.

Nevertheless, the main function of blanching is to inactivate the enzymes such as catalase, peroxidase, polyphenol oxidase, ascorbic acid oxidase and lipoxygenase. Blanching vegetables prior to drying protects their colour. For example, the carotenoid pigment present in carrots is guarded from the oxidative changes if they are blanched.

Blanching can be carried out using hot water, steam or microwave. In hot water blanching, the prepared vegetables are dipped in boiling water for 1–5 minutes depending on the type of food and its size. During this period, the temperature of food rises to 75 – 95°C. This method has been traditionally used, but it results in loss of water-soluble flavours and nutrients particularly, ascorbic acid through leaching. Steam blanching uses steam at temperature above 100°C instead of boiling water. It prevents loss of water soluble constituents and also saves the effort of changing hot water repeatedly. Microwave blanching is a new technique which further reduces the nutrient loss.

In order to test the efficiency of blanching, peroxidase test is conducted. The rationale behind the selection of peroxidase as an indicator is that peroxidase is a heat resistant enzyme so if it is inactivated, all other enzymes can be assumed to be destroyed.
Pasteurization: It is a mild heat treatment which uses a temperature below 100°C to kill the undesirable microbes. It brings about partial preservation; therefore, it has been followed by other methods of preservation, such as refrigeration of milk and aseptic packaging of fruit juices.

Pasteurization, discovered by Louis Pasteur, is an important process in milk technology. It accomplishes following functions:

- It kills *Mycobacterium tuberculosis* (a pathogen capable of causing tuberculosis) as well as *Brucella abortus* and *Streptococcus pyogenes*.
- It decreases the number of milk-souring bacteria and thereby prolongs the shelf life of milk.
- It destroys the microorganisms which enter milk accidentally through equipment, utensils and personnel.
- If carried out properly, it does not affect chemical composition or organoleptic attributes of milk. The only loss is that of small amount of ascorbic acid naturally present in milk.

The following three processes are used for pasteurization:

- **Low Temperature Long Time (LTLT) Pasteurization**: In this method, milk is held at 63°C for 30 minutes and rapidly cooled to 10°C. Since the temperature is low and the contact time is long, LTLT method affects adversely the flavour and nutritive value of milk.

- **High Temperature Short Time (HTST) Pasteurization**: Here, the milk is heated to a temperature of 72°C and held for only 15 seconds. This method uses a higher temperature and the contact time is very short. As a result, the nutritive value as well as flavour of milk is better preserved.

- **Ultra-Heat Treatment (UHT)**: It is a more recently developed method used for milk (Refer Figure 6.2) and other products like cream, yoghurt, wine and fruit juices. It results in a significant reduction in microbial load and the product has a prolonged shelf-life (four to nine months), without the changes in organoleptic attributes associated with sterilization. The temperature used is 135 °C or more for a short time, that is, 1 – 2 seconds.

![Fig. 6.2 Ultra High Temperature (UHT) Milk](image_url)
For measuring the adequacy of pasteurization, that is, to ensure that the milk is free from *Mycobacterium tuberculosis*, phosphatase test is carried out. Phosphatase is an enzyme which has a slightly higher heat resistance as compared to *Mycobacterium tuberculosis*. Inactivation of this enzyme or a negative phosphatase test ensures that *Mycobacterium tuberculosis* is also destroyed.

**Sterilization:** It is a severe heat treatment carried out at a temperature of 121°C, which results in complete destruction of microorganisms. It implies that each particle of food must receive the extreme heat so that all spore forming bacteria, including *Clostridium botulinum* and putrefactive anaerobe are destroyed. Let us consider the case of a canned food. If we desire to sterilize it, then heat processing at a temperature of 121°C would be required for many hours to transfer the required heat into the can. Such a harsh treatment would no doubt sterilize the canned food, but also alter its organoleptic and nutritive quality in an adverse manner. Therefore, instead of sterilizing the food, it is advisable to commercially sterilize the foods.

**Commercial Sterilization:** It refers to the degree of sterilization which destroys all pathogenic, toxin-forming as well as spoilage-causing organisms. Some heat resistant bacterial spores may be present in commercially sterile foods, but they do not usually multiply; for example, most canned and bottled foods are commercially sterile. The vacuum present in a can, coupled with the high acidity, provides an adverse environment for growth of microbes.

**Canning:** You must have seen many canned foods in the market (Refer Figure 6.3). Canned fruit and vegetables are available as whole (cherries, peas), sliced (pineapple), diced (fruit -tidbits), or pureed (tomatoes). A variety of meat, fish and their products are also available in canned form. Even fruit and vegetable products like jam and pickles are at times canned.
For canning, the food is graded, washed and pre-prepared; and it is then filled in cans along with a medium either sugar syrup for fruits or brine (salt solution) for vegetables. Thereafter, air is exhausted and the can is sealed hermetically before being heat processed at a high temperature for a pre-determined period. The main objective of heat processing is to destroy *Clostridium botulinum* and its spores; but the degree and time of heat treatment depends upon the size of can, nature of food and its pH. Heat penetration is better in a smaller can and at a lower pH. For preservation purposes, acidic fruits are processed at a boiling temperature (100°C) while vegetables being alkaline need a higher temperature (121°C) of processing.

The containers used are usually tin plate steel cans; but glass jars, sterilizable plastic containers or tetra packs may also be used. As mentioned in 1.5.1, aseptic canning is a modern technique in which the food is sterilized separately outside the container; it is placed in previously sterilized package and then sealed in a sterile environment. Aseptic canning employs the High Temperature Short Time (HTST) method for heat transfer, so the quality of food—in terms of colour, flavour and nutritive value—are better preserved.

**Preservation Using Low Temperature**

Use of freezing as a method of preservation dates back in history when Eskimos preserved freshly caught fish in ice. However, commercial refrigeration and freezing came into practice only in the 19th century when mechanical ammonia refrigeration was invented. Keeping food at low temperature slows down the chemical reactions, inhibits the enzymatic action and controls the growth and activity of microorganisms. Consequently, the deterioration of food is delayed or prevented.

Besides preventing food spoilage, there are many other advantages of low temperature preservation.

- It prolongs the shelf life without adversely affecting the nutritive value as well as organoleptic properties of food.
- It retards the chemical as well as enzymatic changes in food.
- It controls the growth and activity of microorganisms.
- It helps in certain unit operations, such as slicing of bread and meat cutting.
- It reduces flavour loss during their juice extraction from citrus fruits.
- It precipitates waxes from edible oils.
- It increases the solubility of carbon dioxide in water—a property used in preparation of soft drinks.

Based on the principle of use of low temperature, following two methods are employed:

- Refrigeration and Cool Storage
- Freezing and Frozen Storage
Refrigeration or cooling is a process of taking heat out of the food. Commercial and household refrigerators maintain a temperature of 4.5°C – 7°C while in cool storage, the temperature ranges from 2°C – 16°C. You may be aware that every microorganism grows best at an optimal temperature and stops multiplying at a minimal temperature. Low temperature of refrigeration does prevent the growth of microbes, but does not usually kill the microorganisms or sterilize the food. As a result, their slow metabolic activity may still continue.

Unlike other methods of preservation; like, canning and dehydration, refrigeration is a gentle method of food preservation. It delays deterioration, but has a mild effect on microbes as well as on the quality attributes of food including its appearance, texture, taste and nutritive value. Ideally, refrigeration of perishable foods like milk, meat, fish poultry, fruits and vegetables should start immediately after slaughter or harvest and maintained throughout transportation and storage. Nonetheless, some foods like bananas, potatoes and bread should not be refrigerated as it can result in adverse change in their quality. At refrigerating temperature, bananas show adverse darkening of skin, potatoes become sweet and rate of staling in bread is hastened.

The major requirements for cool storage are controlled low temperature, air circulation, humidity control and modification of gas atmosphere.

Freezing and Frozen Storage

You must have noticed frozen foods like meat and meat products, vegetables (like peas) and vegetarian snacks being marketed under various brands (Refer Figure 6.4). If kept in frozen storage these foods have a shelf life of many months.
Water is a major constituent of food. While the freezing point of pure water is 0°C, most foods start freezing only when temperature is lowered to -2°C or below. Freezing is a process which converts water into ice and makes it unavailable for microorganisms as well as the enzymes. As a result, it prevents microbial growth entirely and slows down the enzymatic activity.

Moreover, freezing is a major factor in making available a large variety of convenience foods to the present day consumer. If carried out properly, it preserves the foods without causing adverse changes in the quality attributes. This property makes it possible to prepare a food item or a meal entirely prior to freezing. Such frozen foods provide maximum convenience as they can be served just after a single thawing-heating operation. Use of microwaves can ensure faster thawing, which is more beneficial in terms of preserving the quality of food being thawed.

There are three basic methods of commercial freezing—freezing in air, freezing by indirect contact with the refrigerant and freezing by direct immersion in a refrigerating medium.

Frozen storage forms an integral part of the freezing process. It refers to the storage of frozen food at a temperature of -18°C or less, so as to maintain it in a frozen condition. If packaged properly, frozen food in adequate frozen storage can be preserved for many months or few years.

Preservation by Moisture Removal

Drying is possibly the oldest food preservation method practiced by man. Early man used sun and air for drying grains and nuts. Traditional processes based on moisture removal included sun drying, dehydration and concentration. They are discussed in detail and are as follows:

Sun Drying: It is still practiced in many parts of our country, it has many limitations. It is a slow process which is limited to hot and dry climate and is suitable for few fruits (grapes, raw mangoes, figs, dates, etc.), and vegetables (potato, peas, leafy vegetables, onion, garlic, etc.).

Food Dehydration: It refers to nearly complete removal of moisture from food under controlled conditions of temperature, humidity and air velocity, so that the quality of food is best preserved. Following are the benefits of dehydration:

- It prolongs the shelf life of food.
- It decreases the weight and bulk of food and thus lowers the transportation and packaging costs.
- It makes available convenient foods for the consumers. For example instant soup mix, instant coffee, etc.

For this purpose special equipment, known as dehydrator, is used. Based on the type of dehydrator used, drying can be classified as air convection drying,
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Concentration or Evaporation: It is another method based on the principle of moisture removal. It is carried out on liquid foods such as milk, juices and purees to get a more concentrated solution. Fruit juices are concentrated prior to spray drying or drum drying. Though concentration reduces the moisture content up to 20 per cent, it is not a complete method of preservation; it has to be followed by some other preservation technique. For example, evaporated milk is sweetened and canned.

As mentioned earlier in Section 1.4.1, the availability of water in food is expressed in terms of water activity (\(a_w\)). The water activity is defined as the ratio of the vapour pressure of water in the food to the vapour pressure of pure water at a specific temperature. Scientific evidence has revealed that water activity (\(a_w\)), rather than total moisture content, affect the growth, death, survival and spore formation of different microbes.

Based on the water content, foods can be divided into two types, which are as follows:

*Intermediate Moisture (IM) Foods: Primarily, drying is a process of removing moisture from the food, but any other method which reduces the amount of available water in food is also a form of drying. For example, use of high concentration salt or sugar can reduce the amount of available water. Jam is one such example which falls under the category of Intermediate Moisture Foods (IMF). These foods have water activity ranging from 0.60 – 0.90 and the moisture content is 10 – 50 per cent by weight. To ensure safety from microorganisms, other hurdles are also used, such as reduction in pH, addition of preservatives and sometimes use of competitive microorganisms. Many a times, IMF foods receive a thermal treatment during their processing.

In light of better knowledge of water activity, old preservation techniques are being reviewed and rediscovered. Traditional fully dehydrated and
intermediate moisture foods are produced by using older methods, but novel techniques are used for high moisture foods such as minimally processed foods.

- **High Moisture (HM) Foods**: The water activity of high moisture foods is much above 0.90. Therefore, $a_w$ is not the main hurdle. Hurdle technology is carefully applied to increase the shelf life of these foods at ambient temperatures. It uses an intelligent combination of different preservation techniques or factors to achieve multi-target and mild, but reliable preservation effects. Examples of HM foods are minimally processed foods, such as fresh fruits and meat products.

**Preservation by Anaerobic Conditions**

You must be aware that more than 90 per cent of microorganisms are aerobes, that is, they grow only in the presence of oxygen. It is possible to prevent the growth of these microbes just by removing oxygen from their environment. Anaerobic conditions may be created inside a sealed and packaged container to prolong the shelf life of food. For example, exhausting removes air and helps in creating a vacuum in the headspace of a can. Such a condition inhibits the growth of spores of heat resistant aerobes which may have survived the heat processing during canning. Another example is the replacement of air with inert nitrogen gas inside a pack of potato chips; in the absence of oxygen, rancidity of fat used for frying chips is prevented (Refer Figure 6.5).
Preservation by Increasing Acidity

We know that all microorganisms grow best at an optimal pH. If pH is lowered or increased, their growth will be inhibited. Nevertheless, very few food items, like eggs, are edible at a pH exceeding 9. Therefore, acidification or decreasing pH is employed for preserving food, and the role of added organic acids here is very important.

Since, pH alone cannot achieve the desired safety and stability of food, it is necessary to combine it with other methods of preservation. For example, citric acid is added to squashes along with chemical preservatives. Combining low pH with other preservation techniques, such as preservatives, water activity, low temperature and/or oxygen availability is gaining popularity for hurdle technology in minimally processed foods.

Fermentation is a process which increases acidity. Vegetables like cucumbers are non-acidic; they are preserved by partial lactic acid fermentation and along with that vinegar and a high concentration of salt is also added. On the other hand, alcoholic and acetic acid fermentation are used for preparing and preserving alcoholic beverages. Fermentation can be defined as an energy-yielding process in which organic compounds are metabolized, usually under anaerobic or microaerobic conditions to simpler compounds. It commonly refers to processes carried out by microorganisms.

Preservation by Food Additives

Food additives are used to complement a number of processing techniques. Nevertheless, their main function is to prevent the food from microbial spoilage as well as the chemical changes. In addition, they improve the appearance and flavour of the processed food for better consumer acceptance. The most common additives used in processed foods include preservatives, colourants, sweeteners, flavourings, emulsifiers, thickeners and stabilizers. Some important terms related to food additives are as follows:

- **Food Additive:** World Health Organization has defined food additive as 'a substance or a mixture of substances, other than the basic food stuff, which is present in food as a result of any aspect of production, processing, storage or packaging. The term does not include chance contamination'.

  Additives are added to foods to fulfill the following objectives:
  
  - To enhance the sensory qualities such as colour, flavour, consistency and texture.
  - To maintain the nutritional quality.
  - To prevent spoilage and prolong the shelf life.
To make the food attractive and appetizing to the consumer.
To produce preserved foods of uniform quality and composition on a large scale.

However, food additives should not be used in certain conditions, which are as follows:
- In baby foods and foods for invalids.
- To cover the spoilage or inferior quality of food.
- To make the food attractive without considering the nutritive value and safety of food.
- To bring about desirable changes without using better techniques of processing.

- **Acceptable Daily Intake (ADI):** Acceptable daily intake is a measure of the quantity of a particular chemical additive in food, which can be consumed on a daily basis over a lifetime without causing harm to human body.
- **Generally Recognized as Safe (GRAS):** ‘Generally recognized as safe’ is a designation given by Food and Drug Administration of USA to food additives which are generally recognized as safe. For example, benzoic acid and sulfur dioxide have the GRAS designation.
- **Delaney Clause:** Delaney clause is an amendment to the U.S. Food, Drug and Cosmetic Act banning the use of any food substance found to be carcinogenic in humans or experimental animals.
- **‘E’ Number:** Each permitted additive is recognized by an ‘E’ number which provides a short code for some of the lengthier chemical names. More importantly, it approves the safety of the additives used in the food. The additives may be listed on the label of a processed food either by its ‘E’ number or by its generally known name.
- **Chemical Preservatives:** Food additives which are intentionally added to inhibit, retard or arrest the process of decomposition of food are known as food preservatives. They may classified as follows:
  - **Class I Preservatives:** They are safe for human consumption and can be added in any amount to the food; for example salt, sugar, citric acid, vinegar and spices.
Class II Preservatives: They are chemicals such as benzoic acid and benzoates; sulphur dioxide and sulfites; nitrates and nitrites, etc. (Refer Table 6.3). Only restricted use of permitted Class II preservatives is allowed in foods.

Table 6.3 Common Chemical Preservatives Used in Processed Foods

<table>
<thead>
<tr>
<th>Preservative</th>
<th>Organism Affected</th>
<th>Foods to Which Added</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propionates</td>
<td>Molds</td>
<td>Bread, cakes</td>
</tr>
<tr>
<td>Sorbitates</td>
<td>Molds</td>
<td>Cakes, cheese, salad dressing</td>
</tr>
<tr>
<td>Benzoates</td>
<td>Yeast and molds</td>
<td>Tomato ketchup, soft drinks</td>
</tr>
<tr>
<td>Sulphites</td>
<td>All microbes</td>
<td>Squashes, cordial</td>
</tr>
<tr>
<td>Ethylene Oxide</td>
<td>Yeast and molds</td>
<td>Spices, nuts</td>
</tr>
<tr>
<td>Nitrites</td>
<td>Clostridium botulinum</td>
<td>Cured meat products</td>
</tr>
</tbody>
</table>

One of the main reasons to use chemical preservatives is to stop the growth and activity of microorganisms. The preservatives achieve this objective by interfering with the cell membranes, the genetic mechanism or the enzyme activity. An ideal chemical preservative should have the following properties:

- Kill the microorganisms rather than inhibiting their growth.
- Be safe and non-toxic for human consumption.
- Not adversely affect the appearance, flavor, taste or aroma of the food.
- Be economical.
- Not result in development of resistant strains of microbes.
- Not be inactivated by the food or its constituents.

In addition to preservatives, a large variety of food additives are available which can be classified into different groups such as colouring agents, sweeteners, antioxidants, stabilizers, flavouring agents, etc. (Refer Table 6.4).
<table>
<thead>
<tr>
<th>S. No</th>
<th>Type of food additive</th>
<th>Function</th>
<th>Uses</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Preservatives</td>
<td>Prevent food spoilage from micro-organisms</td>
<td>Fruit beverages, baked goods, cured meats</td>
<td>Sodium benzoate, calcium propionate, sodium nitrite, calcium sulfite</td>
</tr>
<tr>
<td>2.</td>
<td>Antioxidants</td>
<td>Prevent changes in colour, flavour or texture and delay rancidity</td>
<td>Oils and margarines, cereals, dressings, snack foods</td>
<td>Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT) tocopherols (Vitamin E)</td>
</tr>
<tr>
<td>3.</td>
<td>Sweeteners</td>
<td>Add sweetness with or without the extra calories</td>
<td>Beverages, baked goods, confectionery, table-top sugar, substitutes, many processed foods</td>
<td>Sucrose (sugar), glucose, fructose, sorbitol, mannitol, corn syrup, aspartame, sucralose, acesulfame potassium</td>
</tr>
<tr>
<td>4.</td>
<td>Colour Additives</td>
<td>Correct natural variations in colour, enhance colours that occur naturally, provide colour to colourless and 'fun' foods</td>
<td>Candies, snack foods margarine, cheese, soft drinks, jams/jellies, gelatins, pudding and pie fillings</td>
<td>FD&amp;C Blue, FD&amp;C Green, FD&amp;C Red, FD&amp;C Yellow, Orange B, Citrus Red No. 3, annatto extract, beta-carotene, caramel, fruit and vegetable juices, vitamin D</td>
</tr>
<tr>
<td>5.</td>
<td>Flavour Enhancers</td>
<td>Enhance flavours already present in foods</td>
<td>Many processed foods</td>
<td>Monosodium glutamate, hydrolyzed soy protein</td>
</tr>
<tr>
<td>6.</td>
<td>Fat Replacers</td>
<td>Provide expected texture and a creamy &quot;mouth-feel&quot; in reduced-fat foods</td>
<td>Baked goods, dressings, frozen desserts, confections, cake mixes, dairy products</td>
<td>Olestra, cellulose gel, carrageenan, modified food starch, guar gum, xanthan gum, whey protein concentrate</td>
</tr>
<tr>
<td>7.</td>
<td>Nutrients</td>
<td>Replace micronutrients lost in processing (enrichment), add nutrients lacking in the diet (fortification)</td>
<td>Flour, breads, cereals, rice, margarine, salt, milk, fruit beverages, energy bars, instant breakfast drinks</td>
<td>Trimine, riboflavin, niacin, folic, beta carotene, potassium iodide, iron or ferrous sulfate, tocopherols, ascorbic acid, pantothenic acid, Vitamin D</td>
</tr>
<tr>
<td>8.</td>
<td>Emulsifiers</td>
<td>Prevent separation and keep emulsified products stable</td>
<td>Salad dressings, peanut butter, chocolate, margarine, frozen desserts</td>
<td>Soy lecithin, mono- and di-glycerides, egg yolks, vegetable monoesterate</td>
</tr>
<tr>
<td>9.</td>
<td>Stabilizers, Thickeners</td>
<td>Produce uniform texture, improve &quot;mouth-feel&quot;</td>
<td>Frozen desserts, dairy products, cakes, dressings, sauces</td>
<td>Gelatin, pectin, guar gum, carrageenan, xanthan gum, whey</td>
</tr>
<tr>
<td>10.</td>
<td>Leavening Agents</td>
<td>Promote rising of baked goods</td>
<td>Breads and other baked goods</td>
<td>Baking soda, monocalcium phosphate, calcium carbonate</td>
</tr>
<tr>
<td>11.</td>
<td>Anti-caking Agents</td>
<td>Keep powdered foods from flowing, prevent moisture absorption</td>
<td>Salt, baking powder, confectioners sugar, sprayable starches</td>
<td>Calcium silicate, iron ammonium citrate, silicon dioxide</td>
</tr>
<tr>
<td>12.</td>
<td>Humectants</td>
<td>Retain moistness</td>
<td>Soft candies</td>
<td>Glycerin, sorbitol</td>
</tr>
<tr>
<td>13.</td>
<td>Dough Conditioners</td>
<td>Produce more stable dough</td>
<td>Breads and other baked goods</td>
<td>Ammonium sulfate, L-cysteine</td>
</tr>
<tr>
<td>14.</td>
<td>Firming Agents</td>
<td>Maintain crispness and firmness</td>
<td>Processed fruits and vegetables</td>
<td>Calcium chloride, calcium lactate</td>
</tr>
</tbody>
</table>
Preservation by Radiation

Food radiation is a preservation technique in which the food is exposed either pre-packaged or in bulk to ionizing rays, such as gamma rays to kill bacteria, insects and, in some cases, to prevent sprouting. Though the food absorbs radiation, it does not become radioactive itself. The radiation absorbed by food may result in some chemical and physical changes. Therefore, the radiation dose is controlled in a manner that the objective of preservation is achieved without harming the quality of food.

Most often, gamma radiation from the isotopes of Cobalt-60 or Cesium-137 or electrons produced from machines are used for treating the food. Irradiation may be equated with pasteurization which decreases the microbial load, but does not kill the microbes completely. As a result, refrigerated storage life of irradiated foods; like meat, fish and poultry is prolonged, but it does not replace the need for cold storage. In other words, radiation complements other preservation techniques, but does not replace them completely.

Another benefit of radiation is that it can be used as an alternative for chemical preservatives in grains, herbs, spices to control insect infestation and also in onions and potatoes to inhibit sprouting. It must, however, be reiterated that no residue is left after irradiation therefore, it does not provide any protection against re-infestation.

Earlier, the term used for unit of radiation was ‘rad’ or ‘radiation absorbed dose’ which represents the absorption of 100 erg per gram of the irradiation. It has now been replaced by Gray (Gy) which is equal to 100 rads. The Codex Alimentarius Commission has recommended an upper limit of 10 K Gy as dose for food irradiation. The three different types of doses are as follows:

- **Low-dose** (less than 1 kGy) can be used effectively for controlling insect infestation in stored grains, pulses and food products, as well as for killing parasites in meat and meat products.
- **Medium dose** (1 – 10 kGy) can destroy microbes in fresh fruits, meat, fish and poultry as well as spices and herbs.
- **High dose** (above 10 kGy) results in sterilization of food for special requirements; these foods are shelf-stable foods and do not require any refrigeration. High doses of radiation may result in adverse changes in nutritive value, colour, flavor, taste and physical properties of food.

The commonly employed methods of radiation are the following:

- **Radappertization**: It achieves destruction of practically all microbes by very high dose of radiation (25 – 45 kGy). It is equivalent to commercial sterilization and results in adverse changes in the quality attributes of the radiated food.
- **Radicidation**: It destroys non-spore forming bacteria particularly *Salmonella*, but spores of *Clostridium botulinum* are not eliminated. It
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uses a low dose of radiation (2 – 8 kGy) so the colour, flavor and texture of food are not affected adversely. This process is equivalent to pasteurization of milk.

- **Radurization**: It makes use of a low dose of radiation (0.4 – 10 kGy) to kill most of the harmful microorganisms. It prolongs the shelf life of food and is equivalent to pasteurization.

**Preservation by Carbonation**

Carbonation is the process of dissolving carbon dioxide in a liquid under pressure. As a result, the air present in the liquid is displaced. In the absence of oxygen, aerobes are not able to grow and the liquid is preserved. This method is used for carbonating water as well as soft drinks. As mentioned earlier, the solubility of carbon dioxide increases when the temperature is lowered. That is the reason why carbonated drinks are served chilled.

**Preservation by Combination of Procedures**

Despite recent advancements in the field of food preservation, no single procedure alone can be considered satisfactory in delaying food spoilage. Most often a combination of preservation procedures is used to prevent deterioration of food effectively. For example, cold storage of fresh fruits and vegetables is combined with modified gas atmosphere where the concentration of oxygen is lowered and that of carbon dioxide is increased. Similarly, preservation of fruit beverages with chemical preservatives is combined with addition of acid to lower pH. When foods are preserved by a combination of procedures, the process is referred to as hurdle technology.

By now, you must have got a good understanding of the various methods of preservation. All these methods aim at preventing food spoilage. Next, we will discuss about food-borne diseases which may or may not alter the organoleptic properties of food.

**Low Temperature Preservation**

Low temperature preserves food by decreasing the growth and activity of microorganisms and by retarding the chemical, as well as the enzymatic reactions. In other words, lower the degree of temperature, slower are the chemical reactions, enzymatic action and microbial growth. When the temperature is lowered below a limit, microbial growth is almost halted.

Raw foods from animal or plant sources contain a wide variety of microorganisms such as yeast, bacteria and molds that have a potential to grow, provided they get optimal environment for multiplying. Every microorganism needs an optimal temperature for its growth and it can tolerate a maximum and minimum temperature. In low temperature preservation, the temperature is lowered from optimal to minimal. This results in a gradual decline in the rate of microbial growth, which becomes negligible at the minimal temperature. Though the growth of
microorganisms is inhibited at lowered temperature, their metabolic activity may continue. Low temperature also lowers the action of enzymes which are necessary for the growth and metabolic activity of microbes.

Refrigeration and freezing are the techniques of low temperature preservation, but they differ from each other in terms of the degree of temperature used, the effect on microbial activity as well as the shelf life and safety of the food (Refer Table 6.5). These differences can be summarized as following:

- The temperature for refrigeration varies from 4.5 – 7.0°C and that of cold storage is -2 – 16°C; while for freezing and frozen storage it is below 0 and -18°C, respectively.
- Most of the microorganisms stop growing at refrigerating temperatures; nearly all microbes cease to grow and some are killed at a freezing temperature which is below -9.5°C. Freezing, however, does not sterilize the food or inactivate the enzymes.
- Refrigeration or cold storage extend the shelf life of food to few days or weeks while frozen storage prolongs it to months or even years.
- The tissue structure of refrigerated food is moderately affected while it may be severely affected in case of frozen foods.
- The water activity of refrigerated foods is high but it is low in frozen foods. Reduced water activity and the resulting concentration of solutes contribute in extending the shelf life of frozen foods.

Table 6.5 Difference Between Refrigeration and Freezing

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Characteristics</th>
<th>Refrigeration</th>
<th>Freezing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Temperature</td>
<td>4.5 – 7.0°C for refrigeration; -2 – 18°C for cold storage</td>
<td>&lt; 0°C for freezing; &lt;-18°C for frozen storage</td>
</tr>
<tr>
<td>1.</td>
<td>Effect on Micro-Organisms</td>
<td>Most stop growing on refrigeration</td>
<td>Nearly all cease to grow and some are killed on freezing</td>
</tr>
<tr>
<td>2.</td>
<td>Shelf Life</td>
<td>Few days or weeks</td>
<td>Months or even years</td>
</tr>
<tr>
<td>3.</td>
<td>Effect on Tissue Structure</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td>4.</td>
<td>Water Activity (a_w)</td>
<td>High</td>
<td>Low</td>
</tr>
</tbody>
</table>

Refrigeration and Cold Storage

Refrigeration or chilling can be described as a process where the temperature of a food is lowered so that it is between -1°C and 7°C. As a result, the rate of biochemical and microbiological changes is reduced and the shelf life of fresh and processed foods is increased yet, the alterations in sensory quality as well as nutritional attributes are minimal. The consumers perceive chilled foods as high quality, natural, healthy and fresh foods.
Compared to other methods of preservation such as freezing, dehydration and canning, refrigeration and cold storage are mild methods of preservation. The slowed growth of microbes at refrigerating temperatures extends the shelf life to few days or weeks. Since refrigeration offers short-term preservation, the period of refrigerated storage should not be too long.

Besides the preserving action, low temperature has many other benefits. It retains the nutritive value as well as organoleptic properties of food. Cooling helps in certain unit operations, such as while slicing bread and cutting meat. It also reduces flavour loss during the extraction of juice from the citrus fruits. Low temperature can, further, help in precipitating waxes from edible oils and in increasing the solubility of carbon dioxide in soft drinks.

Perishable foods like fruits and vegetables, milk and meat should ideally be refrigerated right from the time they are harvested, slaughtered or produced. Delay in refrigeration can substantially increase the deterioration rate among these products. Besides, they should continue to be under refrigeration throughout their transportation, storing, marketing as well as storage prior to consumption. Many perishable food products are also chilled; for example, prepared salads, ready meals and sandwiches.

Refrigerated storage generally refers to the storage of fruits, vegetables and other foods at refrigerating temperature in a large room with circulating air and humidity control.

Requirements of Refrigerated Storage

There are three primary requirements of refrigerated storage and these are controlled low temperature, air circulation and humidity control as well as modification of gas atmosphere. The warehouse must be insulated and airtight.

Controlled Low Temperature

Maintaining a low temperature is the most important pre-requisite for refrigerated storage. Temperature fluctuations must be minimized. There are many factors which may lead to a temperature rise in the storage room; these include electric lights and workers working inside; the type of food as well as the frequency and length of opening the doors. Control over these factors must be maintained to reduce the subsequent loss in quality of the food.

Fruits and vegetables are kept in cold storage so that they are available throughout the year. They are living things that respire and produce heat that must be dissipated. The rate of respiration as well as the specific heat of these foods is kept in mind while calculating their refrigerating temperature. Some foods are sensitive to low temperatures, such as bananas, which are best stored at a temperature ranging from 13.3 to 16.7°C.

Air Circulation and Humidity Control

It is necessary to have adequate circulation of air in the cold storage so as to keep heat away from food surface. The circulating air should not be too moist or too...
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High moisture in air can condense on the food surface making it prone to mold growth while dry air can result in dehydration of the product. Optimal humidity desired for different foods varies; green leafy vegetables require a high relative humidity while nuts need only 70 per cent humidity in the circulating air. Generally, a relative humidity of 80 – 95 per cent is maintained to reduce transpiration losses in fruits and vegetables.

Before keeping foods in refrigerated storage, packaging them is a good option to prevent mold growth as well as dehydration of the surface. Accordingly, larger cuts of meat are packaged and sealed in plastic bags, cheese is wrapped in plastic films and eggs are coated with a thin layer of edible oil to seal the pores.

**Modification of Gas Atmosphere**

As mentioned earlier, harvested fruits and vegetables respire during storage. They take in oxygen and produce heat and carbon dioxide. The rate of respiration can be significantly lowered by use of low temperature. Besides, modification of gas atmosphere, such as decreasing (but not eliminating) oxygen and increasing carbon dioxide can also help in achieving the purpose. The optimal temperature, relative humidity and the concentration of gases required vary from one fruit to another.

There are two main methods of storage for fruits and vegetables. They are the Controlled Atmosphere Storage (CAS) and Modified Atmosphere Storage (MAS). They are described as follows:

- **Controlled Atmosphere Storage (CAS)** is a process used extensively for apples. In this case, an insulated and gas-tight warehouse is brought to a desired low temperature and stuffed with fruits. Selected gases are filled in the required proportion and some water vapour is added for achieving the optimal humidity. The warehouse is then sealed till the time it has to be emptied. Apples can be kept fresh in such atmosphere for up to six months. Besides the gases, some other substances may be introduced in CAS, such as antimicrobial fumigants or vapours for controlling mold growth and ethylene gas for accelerating the ripening process. In CAS, the composition of gas atmosphere is continuously monitored and controlled. The concept of controlled atmosphere storage is also being applied for individually packed foods like fruits and vegetables, meat, pasta and fish.

- **Modified Atmosphere Storage (MAS)** does not maintain a strict control over the concentration of gases. Instead, respiration is allowed to proceed so as to lower the concentration of oxygen and increase that of carbon dioxide in the sealed warehouse. When combined with low temperature, MAS can significantly slow down the physiological activity of fruits and vegetables as well as the growth of microorganisms and thus prolong the storage life.

CAS and MAS are suitable for fruits and vegetables that are perishable and that ripen after harvest. However, they have some limitations, which are as follows:
They are not economical for most crops which do not have enough sales to justify the investment. Besides, different crops have different requirements for the optimal atmosphere, so they cannot be stored together.

Low concentration of oxygen and high concentration of carbon dioxide needed for CAS and MAS may be detrimental to many foods.

CAS may increase the concentration of ethylene and thereby hasten the ripening process.

Inappropriate gas composition may lead to development of off-flavour and loss of characteristic flavour.

Besides CAS and MAS, there are some terms which are used for modified atmosphere in packaged foods. These are as follows:

- **Modified Atmosphere Packaging (MAP)** is a term which is used for replacing the air in a package with a single or mixture of gases to prevent deteriorative changes. The gases used for the purpose are carbon dioxide, nitrogen and oxygen.
- **Vacuum Packaging (VP)** involves simple removal of air from a package prior to sealing it hermetically.
- **Active Packaging (AP)** refers to the incorporation of certain additives in packaging with an objective of maintaining and extending the shelf life of food product.

**Equipment for Refrigeration**

The equipment used for refrigeration may work either in batch or in continuous operation. Irrespective of their mode of operation, they lower the temperature of food quickly from the danger zone of 10 – 50°C. The refrigerators use different methods for heat removal and on that basis they can be grouped into two classes—mechanical refrigeration systems and cryogenic systems.

**Mechanical Refrigeration Systems**

Copper is primarily used in construction of refrigerating equipments since its low heat conductivity increases the rate of heat transfer. Mechanical refrigeration systems comprise of four elements; they are evaporator, compressor, condenser and expansion valve (Refer Figure 6.6). A refrigerant circulates between the elements and changes its state from liquid to gas and back to liquid. Following are the functions of the four elements:

- Evaporator is the most important component of a refrigerator. When the liquid refrigerant evaporates in the evaporator under lowered pressure it absorbs the latent heat of vaporization from the surroundings and cools the freezing medium.
- The vaporized refrigerant passes to the compressor where pressure is raised.
It then passes to condenser where high pressure is maintained and the vapour is condensed to liquid state.

Liquid refrigerant passes through the expansion valve where the pressure is lowered to start the refrigeration cycle all over again.

**Fig. 6.6 Components of Mechanical Refrigeration System**

**Requirements of a Refrigerant**

The refrigerant used should fulfill certain requirements. It should not only have a low boiling point and a high latent heat of vaporization, but also be non-toxic, non-inflammable and inexpensive. Its miscibility with oil should be low. Available refrigerants are ammonia, carbon dioxide, Chloro Fluoro-Carbons (CFCs) and freon 22, and each has its advantages and limitations. Ammonia is immiscible with oil and has high heat transfer properties, but it is toxic, inflammable and corrosive to the carbon pipes. On the other hand, carbon dioxide is non-inflammable and non-toxic, but it needs higher operating pressures. Chlorofluoro-Carbons (CFCs) have the advantage of being non-inflammable and non-toxic. In addition, they have good heat transfer properties and are low cost; but they have been proven to be harmful to the environment. On the contrary, partially hydrogenated CFCs (also known as HCFCs) are less detrimental to the environment. In recent years ozone-friendly HCFCs have been developed.

The refrigeration systems make use of moving air or direct contact with a metallic surface for the purpose of chilling. Retail chill cabinets use chilled air which is circulated by natural convection to keep the food chilled.

There are many methods of cooling the freshly harvested fruits and vegetables. For example, leafy vegetables having a large surface area are vacuum cooled; fruits and vegetables are immersed in chilled water (hydrocooling), or cooled by forced air.

**Cryogenic Refrigeration Systems**

Cryogenic systems use a cryogen, which is a refrigerant that changes its phase by absorbing latent heat to lower the temperature of food. Examples of cryogens are solid or liquid carbon dioxide and liquid nitrogen.
Carbon dioxide has a higher boiling and sublimation point than nitrogen; so, it does not affect the food adversely, however, its major limitation is that it can cause asphyxia. Carbon dioxide can be used in different forms such as ‘dry ice’, pellets or ‘snow’. When liquid carbon dioxide is injected in the air to convert it into a fine mist of solid particles, it is referred to as ‘snow’. In its snow form, the sublimation to vapour form is very rapid.

Liquid nitrogen can also be used for refrigeration purposes. It may be injected in a batch type chilling cabinet where it results in immediate vaporization to produce uniform reduction in the temperature.

**Foods Commonly Refrigerated**

Refrigeration is very often combined with other preservation techniques, such as pasteurization. For example, pasteurized milk is refrigerated for prolonging its shelf life. On the basis of temperature range, refrigerated foods can be grouped as following:

- **Fresh**: It comprises of meat, fish, sausages and ground meat that are chilled at -1°C ±1°C.
- **Pasteurized**: It comprises of milk, meat, cream, yoghurt, prepared salads, sandwiches, baked goods, pizza, pastries that are chilled at 0°C ±5°C.
- **Fully cooked**: It comprises of meats, cured meats, cheese, butter, margarine, fruit juices, soft drinks that are refrigerated at 0°C ±8°C.

Refrigeration is employed for fresh foods, processed foods as well as cook-chill foods. The quality of chilled foods depends on low temperature distribution system, including chill stores, refrigerated transport, retail chill display units as well as home refrigerators. This chain of refrigeration must be controlled especially for low acid foods like milk, milk products, meat and meat products to prevent them from spoilage.

**Refrigeration of Fresh Foods**

We know that refrigeration decreases the rate of enzymatic and microbial changes. In case of fresh fruits and vegetables, it also reduces rate of respiration. When temperature is lowered below the optimum level for a particular fruit, certain undesirable changes termed as ‘chilling injury’ may become evident. These include failure to ripen, browning of pulp and skin blemishes. Though reasons for these changes are not clearly understood, it is assumed that the balance of metabolic activity is disturbed at low temperature, which results in over-production of metabolites that harm the tissues. For example, chilling injury is seen in bananas at a temperature lower than 12°C and in mangoes at a temperature less than 10°C.

In case of animals, aerobic respiration slows down when they are slaughtered. Anaerobic respiration of glucose is then initiated, which decreases the pH of meat and rigor mortis sets in. You must be aware that rigor mortis is the process where the muscle of a dead animal become firm and inextensible. It is
necessary to cool the meat during anaerobic respiration to reduce bacterial spoilage and to produce the desired texture and colour of meat.

**Refrigeration of Processed Foods**

The refrigerating temperatures prevent the growth of thermophils and mesophils. However, during extended refrigeration, some pathogens can grow and cause food poisoning. Examples of such pathogens are *Listeria, Yersinia enterocolitica, Bacillus cereus, Vibrio parahaemolyticus* and enteropathic *Escherichia coli.* We learnt in the previous unit that these organisms can result in food infections. It is, therefore, necessary to follow Good Manufacturing Practices (GMPs), such as safe water supply, cleanliness of contact surfaces, prevention of cross contamination, personal hygiene, pest control and waste management.

The shelf life of refrigerated processed foods is decided by:

- The type of food,
- Extent of microbial destruction and enzyme activity resulting from the processing,
- Hygienic practices followed during processing and packaging,
- The impermeability of the package and
- Temperature during processing, distribution and storage.

**Refrigeration of Cook-Chill Foods**

A large variety of cook-chill foods are available for the convenience of consumers. Some are prepared from a mixture of cooked and raw ingredients while other are cooked completely and packaged. There are other examples where the food is first packaged and then cooked. It is necessary to follow hygienic practices while handling these foods to prevent the microbial contamination.

**Effects of Refrigeration on Food Quality**

Refrigerating food to an appropriate temperature does not harm its quality adversely. The most apparent alteration in sensory quality is hardening, which is the result of solidification of fats and oils. In addition, certain chemical, biochemical and physical changes take place that lead to loss in quality. These changes include enzymatic browning, lipolysis, colour and flavour changes, and retrogradation of starch. Lipid oxidation in cook-chilled products leads to major quality loss. For example, cooked meat develops ‘warmed over flavour’ on chilling.

Physico-chemical changes include syneresis in sauces and gravies, drying of unpacked cheese or meat, dripping from fish, faster staling of bread and cakes as well as moisture migration from filling in sandwiches.

When stored at a very low temperature, fruits and vegetables lose their firmness and crispness. Some of them may even get ‘chilling injury’. For example, internal browning and a soggy texture in certain varieties of apples, water-soaked areas in cucumbers and lady’s finger, pitting and discoloration near calyx of capsicum and rind disorders in oranges. Refrigeration may also result in exchange.
of flavour among different foods during refrigeration which can be prevented by adequate and effective packaging.

Generally, the nutrients are retained at low temperature; there are negligible losses of thiamin, riboflavin and vitamin A; however, vitamin C is lost to some extent.

6.2.2 Food Preservation and Method

You will be able to understand the process of preserving food better with the help of an example. If you keep fruits, vegetables and cereals in the refrigerator, it is not food preservation as the items are not able to retain the colour and taste of food, which is necessary in case of preservation. To quote another example, grapes and raisins are preserved by sun-drying. During the process of drying, the colour of the grapes changes from green to brown and it also becomes a little sweeter in taste. Although, once they have undergone these changes, their colour and taste does not change any further. Also, the texture of food must not change. For example, in the case of mango murabba, it should remain firm and not lose its texture after some time.

Importance of Preserving Food

There are several reasons to preserve food, some of which have been mentioned as follows:

- The major reason behind food preservation is to take care of that amount of food that has been produced in excess.
- Preserving food also adds variety to our meals, thereby, making them interesting. In this way, we get the opportunity to eat vegetables that are not in season, at that point of time. Preservation of vegetables when they are in season makes this situation possible.
- Another reason to preserve food is that it makes the reach of things possible in those regions, where the food item is not grown otherwise. For example, in the desert areas of Rajasthan, many vegetables and fruits cannot be grown. Availability of some food items that are preserved adds variety to the meal. Examples of such foods can be dehydrated peas, green leafy vegetables, and so on.
- Another prominent reason for food preservation is that it makes transportation and storage of food easier. It reduces the bulk thereby making their transportation and storage simpler and easier, since it requires very little space in comparison to fresh foods.

Methods of Food Preservation

You have already read about the process of treating and handling food so as to stop or slow down the process of food spoilage caused or accelerated by microbes, which is known as food preservation. The spoilage can be in the form of quality
loss, edibility loss or nutritive value loss. Some of the methods of preserving food use benign bacteria, yeast and fungi to add a specific quality to the food product and also to preserve them. Examples of such usage are wine or any other alcoholic beverage and cheeses. In preserving the value of the food, it is important to maintain or create nutritional value, texture and flavor. This is dependent on the cultures of various places and what qualifies as fit for human consumption in one culture may not be fit for food in another culture.

Common methods of food preservation are drying, canning, freezing, spray drying, freeze drying, vacuum packing, food irradiation, pickling, salting, smoking, sugar crystallization and curing (Refer Figure 6.8).

Preservation processes basically include the following:

- Heating to kill or denature micro-organisms (like boiling).
- Oxidation (such as the use of sulphur dioxide).
- Toxic inhibition (like smoking, use of carbon dioxide, vinegar, alcohol).
- Dehydration (by drying).
- Osmotic inhibition (like the use of syrups).
- Low temperature inactivation (that is, freezing).
- Ultra-high water pressure (like fresherization, a kind of cold pasteurization where the pressure kills naturally occurring pathogens).
- Chelation.

Some of the most important ways of preserving foods have been discussed as follows:

- **Dry Canning**: This method of food preservation is used to preserve raisins, grains, beans, coconut, pasta, rice, legumes or any kind of dried fruits or vegetables. Anything that is dry can be preserved by using this method of food preservation of dry canning. A canner machine, cans and seals are required for the process (Refer Figure 6.9).
Bottling: This is a popular method of food preservation and has been used for centuries. This method is very useful as it helps in retaining the flavour of the fruit, vegetable or meat which has been preserved (Refer Figure 6.10). The only drawback of this method is that many nutrients are lost from the food during this process. It should be done very carefully because if not done in a proper way, botulism (a deadly poison) might occur.

Dehydration: For most fruits and vegetables, drying is an excellent way of preservation. Drying means taking the moisture out from the food, thereby preventing spoilage. The advantage of this method is that the weight of the dry products is very little and its size also reduces making it easier to store. Another important advantage is that the food retains its nutrients. Several ways are used to dry the foods. They can be dried in the sun, oven or a dehydrator. Since, the first two methods are a bit difficult, the dehydrator is
considered to be the best, most effective and easiest way to dry food which is later stored in air-tight containers.

- **Smoking**: This method of food preservation is basically needed for preserving meat. The smokers are quite costly (Refer Figure 6.11). Smoked meat retains most of the nutrients and is very delicious as well.

![Fig. 6.11 Smoker for Preserving Meat](image)

- **Freezing**: Another method of food preservation, which is very effective, is done by the help of a freezer and food items are stored after being frozen (Refer Figure 6.12). The drawback of this method is that in case of power failures, a generator would be required to run the freezer or the food may get spoilt.

![Fig. 6.12 Freezing](image)
General Principles of Food Preservation

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- **Vacuum Packing**: In this method, food is stored in a vacuum environment in an air-tight bag or bottle. The vacuum environment helps in preserving food by stripping bacteria of oxygen which is necessary for survival. This method is often used to preserve nuts.

- **Salt**: Salting is the method that saps moisture from the meat using the principle of osmosis. Meat is either cured with salt or sugar or a combination of both. Nitrates and nitrites are also used to cure meat and also impart a characteristic pink colour and inhibit clostridium botulinum.

- **Sugar**: It is used to preserve fruits either in a syrup or in the crystallized form wherein the preserved substance is cooked in sugar until the crystallization and the resultant product is stored dry. This method is generally used to preserve the skins of citrus fruits, angelica and ginger. A modification of this process is glazed fruit where the fruit is preserved in sugar but is then extracted from the syrup and sold with a coating of sugar on them.

- **Pickling**: It is a method of preserving food in an edible anti-microbial liquid. This process can be broadly categorized into chemical pickling and fermentation pickling. Examples of the former method are by the use of brine, vinegar, alcohol, etc. In chemical pickling, the food is placed in an edible liquid that kills bacteria and other microbes. These processes also involve heating or boiling so that the food that is preserved is saturated with the pickling agent. The foods that are chemically pickled are cucumber, beef, herring, eggs, apples or other fruits (Refer Figure 6.13). In the case of fermentation pickling, the food itself produces the preservation agent typically by a process that produces lactic acid. These types of pickles are sauerkraut, kimchi, curtido, etc. In the case of commercial pickles, a preservative (sodium benzoate or EDTA) is added to enhance their shelf life.

Fig. 6.13 Pickling of Fruits
• **Lye:** Sodium hydroxide or lye is used to make food too alkaline for the growth of bacteria. It acts by saponifying fats in the food that change their flavor and texture.

• **Jellying:** Food is also preserved by cooking in a substance that forms a gel upon solidification. Such substances are gelatin, agar, arrowroot flour and maize flour. Some foods on cooking automatically form a protein gel such as eels and elvers. Jellied eels (eaten along with mashed potatoes) are a delicacy in the East end of London. The foods that are preserved using the process of jellying are known as jams, marmalades or fruit preserves. In this case, the jellying agent that is used is pectin, which is added either during cooking or arising naturally from the fruit. Most preserved fruit is also sugared in jars (Refer Figure 6.14). Heating and packaging acid and sugar provide the preservation.

• **Potting:** This method of food preservation is a traditional British way of preserving meat and shrimps by setting them in a pot and sealing it with a layer of fat.

• **Jugging:** Meat is also preserved by using the method of jugging wherein the stewed meat is kept in a covered earthenware jug or a casserole. The animal that has to be juggled is generally cut into pieces and placed in a tightly sealed jug that is filled with brine or gravy and stewed. Jugging was a very popular method of preserving food until the mid-twentieth century.

• **Irradiation:** Food irradiation basically refers to the exposure of food to ionizing radiation, that is, either high energy electrons or X-rays from accelerators or gamma rays that are emitted from radioactive sources such as Cobalt 60 or Caesium 137. The process has a wide range of effects such as the killing of bacteria, moulds, insects, pests, etc. along with the reduction of the ripening and spoiling of fruits. If rendered in a higher intensity, it results in sterility. This process is similar to pasteurization and is also known
as cold pasteurization. However, this method of irradiation is not effective against viruses and cannot eliminate toxins that are already formed by microbes and are only useful for food of high initial quality (Refer Figure 6.15).

- **Modified Atmosphere**: Modifications in the atmosphere is another important way of preserving food. For example, salad crops which are usually very difficult to preserve are now being packaged in sealed bags with a controlled atmosphere to reduce the concentration of oxygen and increase that of carbon dioxide.

- **Burial in the Ground**: This is a unique way of preserving food that protects the food from a variety of factors such as lack of light and oxygen, harsh temperatures, pH level, etc. The burial method can also accompany other methods such as salting or fermentation. Many root vegetables are prone to spoilage and need to be stored in cool and dark conditions such as being buried in the ground.

- **High Pressure Food Preservation**: It refers to high pressure for the preservation of food. Pressed inside a vessel exerting immense force, food
can be processed so that it retains its fresh appearance, flavour, texture and nutrients while disabling harmful microorganisms and slowing spoilage.

Table 6.6 portrays all the major forms of preserving food along with their effect on the growth of microbes.

<table>
<thead>
<tr>
<th>Method</th>
<th>Effect on Microbial Growth or Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refrigeration</td>
<td>Low temperature to retard growth</td>
</tr>
<tr>
<td>Freezing</td>
<td>Low temperature and reduction of water activity to prevent microbial growth, slowing of oxidation reactions</td>
</tr>
<tr>
<td>Drying, Curing and Conserving</td>
<td>Reduction in water activity sufficient to delay or prevent microbial growth</td>
</tr>
<tr>
<td>Vacuum and Oxygen-Free Packaging</td>
<td>Low oxygen tension inhibits strict aerobes and delays growth of facultative anaerobes</td>
</tr>
<tr>
<td>Carbon Dioxide–Enriched Packaging</td>
<td>Specific inhibition of some microorganisms</td>
</tr>
<tr>
<td>Addition of Weak Acids</td>
<td>Reduction of the intracellular pH of microorganisms</td>
</tr>
<tr>
<td>Lactic Fermentation</td>
<td>Reduction of pH value by microbial action and sometimes additional inhibition by the lactic and acetic acids formed by other microbial products</td>
</tr>
<tr>
<td>Sugar Preservation</td>
<td>Cooking in high sucrose concentration creating a high osmotic pressure for most microbial survival</td>
</tr>
<tr>
<td>Ethanol Preservation</td>
<td>Steeping or cooking in ethanol produces toxic inhibition of microbes and can be combined with sugar preservation</td>
</tr>
<tr>
<td>Emulsification</td>
<td>Compartmentalization and nutrient limitation within the aqueous droplets in water-in-oil emulsion foods</td>
</tr>
<tr>
<td>Addition of Preservatives such as Nitrite Ions</td>
<td>Inhibition of specific groups of microorganisms</td>
</tr>
<tr>
<td>Pasteurization and Appertization</td>
<td>Delivery of heat sufficient to inactivate target microorganisms</td>
</tr>
<tr>
<td>Food Irradiation</td>
<td>Delivery of ionizing radiation to disrupt cellular RNA</td>
</tr>
<tr>
<td>Application of High Hydrostatic Pressure (Pasculation)</td>
<td>Pressure-inactivation of vegetative bacteria, yeasts and moulds</td>
</tr>
<tr>
<td>Pulsed Electric Field Processing</td>
<td>Short bursts of electricity for microbial inactivation</td>
</tr>
</tbody>
</table>

Chelating Agents

Chelating agents have gained immense popularity in past few years in food processing. These are basically chemicals that integrate with metal ions and remove the ions from their sphere of action. Chelating agents are also known as sequestrants. These agent are used to remove traces of metal ions which might cause food to deteriorate and reduce absorption of a mineral or increase its excretion in food manufacturing process. Citrates, tartrates, phosphates and EDTA are chelating agents.
agents. Many natural constituents of food can also act as chelating agents, such as carboxylic acids, hydroxyl acids, polyphosphoric acids, amino acids, peptides, proteins and porphyrins.

Many essential biological chemicals are chelates. Chelates play an important role in the transport of oxygen and in photosynthesis. Furthermore, many biological catalysts (enzymes) are chelates. In addition to their significance in living organisms, chelates are also economically important, both as products in themselves and as agents in the production of other chemicals.

The formation or the presence of two or more separate bindings between a single central atom and polydentate ligand is termed as chelation (Refer Figure 6.16). These ligands are basically organic compounds and are called chelants, chelators, chelating agents or sequestering agents.

![Fig. 6.16 Chelation](image)

Ligand forms a chelate complex with the substrate. They are then contrasted with coordination complexes with monodentate ligands. Monodentate ligands are the ligands that form only one bond with the central atom.

For fat or oil oxidation, grains of heavy metal ions act as catalysts. Their binding with chelating agents increases antioxidant efficiency and inhibits oxidation of ascorbic acid and fat soluble vitamins. Owing to antioxidants, the aroma and colour of canned vegetables improves a lot. The combination of a chelating agent and an antioxidant provides an improved extract quality in the production of herb and spices extracts. These are also used in dairy products.

Prior to blanching, chelating agents are added to the vegetables thereby inhibiting discoloration induced by metals and also calcium is removed from pectin in cell walls, thereby promoting tenderness. To prevent metal-induced oxidation...
of flavour compounds such as terpenes, citric acid and phosphoric acid are added to the drinks. In fermented malt beverages sequestrants complex copper is added and thus, polyphenol oxidation prevents the formation of haze.

**Check Your Progress**

1. What are the basic principles of food preservation?
2. Distinguish between ultrafiltration and microfiltration.
3. What is blanching and why is it done?
4. Give the functions of pasteurization.
5. What are the processes used for pasteurization?
6. What are the advantages of low temperature preservation?
7. Write a brief note on concentration or evaporation.
8. Why additives are added to foods?
9. What are the conditions in which food additives should not be used?

**6.3 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS**

1. The basic principles of food preservation are as follows:
   - Prevention or delay of microbial changes
     - By keeping out microorganisms (asepsis)
     - By removing the microorganisms; for example, filtration
     - By hindering the growth and activity of microorganisms; like, use of low temperature, removal of oxygen, drying or adding chemicals
     - By killing the microorganisms; like using high temperature or radiation
   - Prevention or delay of self-decomposition of food
     - By destroying or inactivating endogenous enzymes; for example, blanching.
     - By prevention or delaying of purely chemical reactions; for example, use of antioxidants.
   - Prevention of damage caused by insects, animals and mechanical causes

2. Ultrafiltration is a recent technique which is pressure-driven and uses a membrane to separate particulate matter from fluids such as water. These membranes have the ability to filter bacteria and most viruses. Microfiltration is a technique similar to ultrafiltration and can filter both the vegetative and spore forms of microbes to produce sterile fluids. Microfiltration membranes have very fine pore size ranging from 0.01 –
0.10 µm, and have been used successfully for clarifying fruit juices and beverages.

3. Blanching is a heat process which uses a temperature ranging from 75°C – 95°C for 1 – 5 minutes. It is usually carried out in vegetables, prior to dehydration, freezing or canning to achieve the following objectives:

- To inactivate enzymes; particularly, polyphenol oxidase which causes browning.
- To sanitize the vegetables (microbial load may be reduced to some extent and pesticides may be leached away).
- To remove air from the intercellular spaces so as to achieve suitable head space in canned foods.
- To prevent changes in colour and flavour.
- To remove the stringent taste of some plant foods, like amla.
- To pre-heat the food before canning.
- To soften the texture and reduce the volume, so that more can be filled in the can.

4. Pasteurization accomplishes following functions:

- It kills *Mycobacterium tuberculosis* (a pathogen capable of causing tuberculosis) as well as *Brucella abortus* and *Streptococcus pyogenes*.
- It decreases the number of milk-souring bacteria and thereby prolongs the shelf life of milk.
- It destroys the microorganisms which enter milk accidentally through equipment, utensils and personnel.
- If carried out properly, it does not affect chemical composition or organoleptic attributes of milk. The only loss is that of small amount of ascorbic acid naturally present in milk.

5. The following three processes are used for pasteurization:

- Low Temperature Long Time (LTLT) Pasteurization: In this method, milk is held at 63°C for 30 minutes and rapidly cooled to 10°C. Since the temperature is low and the contact time is long, LTLT method affects adversely the flavour and nutritive value of milk.
- High Temperature Short Time (HTST) Pasteurization: Here, the milk is heated to a temperature of 72°C and held for only 15 seconds. This method uses a higher temperature and the contact time is very short. As a result, the nutritive value as well as flavour of milk is better preserved.
- Ultra-Heat Treatment (UHT): It is a more recently developed method used for milk and other products like cream, yoghurt, wine and fruit juices. It results in a significant reduction in microbial load and the
product has a prolonged shelf-life (four to nine months), without the changes in organoleptic attributes associated with sterilization. The temperature used is 135 °C or more for a short time, that is, 1 – 2 seconds.

6. Besides preventing food spoilage, there are many other advantages of low temperature preservation that are as follows:
   - It prolongs the shelf life without adversely affecting the nutritive value as well as organoleptic properties of food.
   - It retards the chemical as well as enzymatic changes in food.
   - It controls the growth and activity of microorganisms.
   - It helps in certain unit operations, such as slicing of bread and meat cutting.
   - It reduces flavour loss during their juice extraction from citrus fruits.
   - It precipitates waxes from edible oils.
   - It increases the solubility of carbon dioxide in water—a property used in preparation of soft drinks.

7. Concentration or evaporation is another method based on the principle of moisture removal. It is carried out on liquid foods such as milk, juices and purées to get a more concentrated solution. Fruit juices are concentrated prior to spray drying or drum drying. Though concentration reduces the moisture content up to 20 per cent, it is not a complete method of preservation; it has to be followed by some other preservation technique. For example, evaporated milk is sweetened and canned.

8. Additives are added to foods to fulfill the following objectives:
   - To enhance the sensory qualities such as colour, flavour, consistency and texture.
   - To maintain the nutritional quality.
   - To prevent spoilage and prolong the shelf life.
   - To make the food attractive and appetizing to the consumer.
   - To produce preserved foods of uniform quality and composition on a large scale.

9. Food additives should not be used in certain conditions, which are as follows:
   - In baby foods and foods for invalids.
   - To cover the spoilage or inferior quality of food.
   - To make the food attractive without considering the nutritive value and safety of food.
   - To bring about desirable changes without using better techniques of processing.
Since the beginning of civilization and in different parts of the world, man has been preserving food in one form or the other.

Major technological developments in the field of food preservation took place during the First and Second World War which created a huge demand for preserved food for the armed forces. In 1810, Nicolas Appert—a French confectioner and distiller—discovered that heat application to food sealed in glass containers preserved it from spoiling.

In 1920s, Clarence Birdseye discovered quick freezing and developed two processes for freezing fish.

The term ‘asepsis’ means absence of infection or keeping out microorganisms; many of its examples can be found in nature. Shells of eggs, peels of fruits and vegetables, skin on meat or fish, as well as the casing on nuts form a protective covering which prevents the entry of microbes in these foods.

In food preservation, the principle of asepsis aims at preventing the entry of microbes especially the ones that cause spoilage and disease.

Centrifugation at a very high speed removes several spores and sedimentation of water removes some of the impurities yet, this technique does not ensure complete removal of microbes.

Reverse Osmosis (RO) is used for separating and purifying water. It is also being applied in areas where evaporation is widely used, such as concentration of milk and fruit juices.

Ultrafiltration is a recent technique which is pressure-driven and uses a membrane to separate particulate matter from fluids such as water.

Microfiltration is a technique similar to ultrafiltration and can filter both the vegetative and spore forms of microbes to produce sterile fluids.

Microfiltration membranes have very fine pore size ranging from 0.01 – 0.10 µm, and have been used successfully for clarifying fruit juices and beverages.

Heating can be considered as the foundation of food preservation. Look around and you will find many applications of heat in food processing; for example, heating, blanching, pasteurization and canning.

Blanching is a heat process which uses a temperature ranging from 75ºC – 95ºC for 1 – 5 minutes.

Blanching can be carried out using hot water, steam or microwave. In hot water blanching, the prepared vegetables are dipped in boiling water for 1–5 minutes depending on the type of food and its size.

Pasteurization is a mild heat treatment which uses a temperature below 100ºC to kill the undesirable microbes. It brings about partial preservation;
therefore, it has been followed by other methods of preservation, such as refrigeration of milk and aseptic packaging of fruit juices.

- In Low Temperature Long Time (LTLT) pasteurization method, milk is held at 63°C for 30 minutes and rapidly cooled to 10°C. Since the temperature is low and the contact time is long, LTLT method affects adversely the flavour and nutritive value of milk.

- In High Temperature Short Time (HTST) Pasteurization the milk is heated to a temperature of 72°C and held for only 15 seconds. This method uses a higher temperature and the contact time is very short. As a result, the nutritive value as well as flavour of milk is better preserved.

- In Ultra-Heat Treatment (UHT) a more recently developed method used for milk and other products like cream, yoghurt, wine and fruit juices. It results in a significant reduction in microbial load and the product has a prolonged shelf-life (four to nine months), without the changes in organoleptic attributes associated with sterilization. The temperature used is 135°C or more for a short time, that is, 1 – 2 seconds.

- Sterilization is a severe heat treatment carried out at a temperature of 121°C, which results in complete destruction of microorganisms. It implies that each particle of food must receive the extreme heat so that all spore forming bacteria, including Clostridium botulinum and putrefactive anaerobe are destroyed.

- Commercial sterilization refers to the degree of sterilization which destroys all pathogenic, toxin-forming as well as spoilage-causing organisms. Some heat resistant bacterial spores may be present in commercially sterile foods, but they do not usually multiply.

- Canned fruit and vegetables are available as whole (cherries, peas), sliced (pineapple), diced (fruit tidbits), or pureed (tomatoes).

- A variety of meat, fish and their products are also available in canned form. Even fruit and vegetable products like jam and pickles are at times canned.

- Refrigeration or cooling is a process of taking heat out of the food. Commercial and household refrigerators maintain a temperature of 4.5°C – 7°C while in cool storage, the temperature ranges from 2°C – 16°C.

- Water is a major constituent of food. While the freezing point of pure water is 0°C, most foods start freezing only when temperature is lowered to -2°C or below.

- Freezing is a process which converts water into ice and makes it unavailable for microorganisms as well as the enzymes. As a result, it prevents microbial growth entirely and slows down the enzymatic activity.

- Acceptable Daily Intake (ADI) is a measure of the quantity of a particular chemical additive in food, which can be consumed on a daily basis over a lifetime without causing harm to human body.
• Generally Recognized As Safe (GRAS) is a designation given by Food and Drug Administration of USA to food additives which are generally recognized as safe. For example, benzoic acid and sulfur dioxide have the GRAS designation.

• Delaney clause is an amendment to the U S Food, Drug and Cosmetic Act banning the use of any food substance found to be carcinogenic in humans or experimental animals.

• Each permitted additive is recognized by an ‘E’ number which provides a short code for some of the lengthier chemical names. More importantly, it approves the safety of the additives used in the food.

• Food additives which are intentionally added to inhibit, retard or arrest the process of decomposition of food are known as food preservatives.

• Food radiation is a preservation technique in which the food is exposed either pre-packaged or in bulk to ionizing rays, such as gamma rays to kill bacteria, insects and, in some cases, to prevent sprouting.

• Refrigeration or chilling can be described as a process where the temperature of a food is lowered so that it is between -1°C and 7°C.

• Perishable foods like fruits and vegetables, milk and meat should ideally be refrigerated right from the time they are harvested, slaughtered or produced. Delay in refrigeration can substantially increase the deterioration rate among these products.

• Refrigerated storage generally refers to the storage of fruits, vegetables and other foods at refrigerating temperature in a large room with circulating air and humidity control.

• Cryogenic systems use a cryogen, which is a refrigerant that changes its phase by absorbing latent heat to lower the temperature of food.

• Carbon dioxide has a higher boiling and sublimation point than nitrogen; so, it does not affect the food adversely, however, its major limitation is that it can cause asphyxia.

• Carbon dioxide can be used in different forms such as ‘dry ice’, pellets or ‘snow’. When liquid carbon dioxide is injected in the air to convert it into a fine mist of solid particles, it is referred to as ‘snow’. In its snow form, the sublimation to vapour form is very rapid.

• Liquid nitrogen can also be used for refrigeration purposes. It may be injected in a batch type chilling cabinet where it results in immediate vaporization to produce uniform reduction in the temperature.

• Physico-chemical changes include syneresis in sauces and gravies, drying of unpacked cheese or meat, dripping from fish, faster staling of bread and cakes as well as moisture migration from filling in sandwiches.

• The major reason behind food preservation is to take care of that amount of food that has been produced in excess.
Preserving food also adds variety to our meals, thereby, making them interesting. In this way, we get the opportunity to eat vegetables that are not in season, at that point of time.

Chelating agents have gained immense popularity in past few years in food processing. These are basically chemicals that integrate with metal ions and remove the ions from their sphere of action.

Many essential biological chemicals are chelates. Chelates play an important role in the transport of oxygen and in photosynthesis.

6.5 KEY WORDS

- **Food preservation**: It is the process which uses appropriate methods to prevent the microbial, chemical and enzymatic changes and thus extend the shelf life of food.
- **Asepsis**: The term ‘asepsis’ means absence of infection or keeping out microorganisms;
- **Ultrafiltration**: A recent pressure-driven technique that uses a membrane to separate particulate matter from fluids such as water.
- **Microfiltration**: It is a technique similar to ultrafiltration and can filter both the vegetative and spore forms of microbes to produce sterile fluids.
- **Pasteurization**: It is a mild heat treatment which uses a temperature below 100°C to kill the undesirine microbes.
- **Sterilization**: It is a severe heat treatment carried out at a temperature of 121°C, which results in complete destruction of microorganisms.
- **Commercial sterilization**: It refers to the degree of sterilization which destroys all pathogenic, toxin-forming as well as spoilage-causing organisms.
- **Freezing**: Freezing is a process which converts water into ice and makes it unavailable for microorganisms as well as the enzymes.

6.6 SELF ASSESSMENT QUESTIONS AND EXERCISES

**Short Answer Questions**

1. What is asepsis?
2. Define the term Reverse Osmosis (RO).
3. What is ultrafiltration?
4. Give the methods of heat processing.
5. What is pasteurization?

7. How is preservation done by increasing acidity?

8. What is radappertization?

9. Write a short note on refrigeration and cold storage.

10. Distinguish between dry canning and dehydration.

**Long Answer Questions**

1. Write a note on history of food preservation.

2. What are the principles of food preservation? Principles of Food Preservation

3. How is removal of microorganisms done?

4. Explain how preservation is done using high temperature and low temperature.

5. How preservation is done by anaerobic conditions.

6. Discuss about preservation by food additives.

7. Elaborate a note on chelating agents.

### 6.7 FURTHER READINGS


UNIT 7 DAIRY MICROBIOLOGY

7.0 INTRODUCTION

The area of dairy microbiology is large and diverse. The bacteria present in dairy products may cause disease or spoilage. Some bacteria may be specifically added to milk for fermentation to produce products like yogurt and cheese. A detailed discussion of fermentation bacteria is outside the scope of this website, although these organisms are discussed briefly in the section on yogurt production and cheese production. This section of the website is dedicated to the discussion of pathogens because of their importance in human health.

Milk is virtually sterile when it is synthesized in a healthy cow’s udder (mammary gland). Cows, like humans, are natural reservoirs of bacteria. Many of these bacteria are not harmful to humans, but some may be harmful to humans even though the cows are not affected and appear healthy.

Milk may become contaminated with bacteria during or after milking. The mammary glands of cows can become inflamed due to a bacterial infection called mastitis. During a mastitis infection, very high numbers of bacteria present can be in the udder and in the milk. Some disease-causing organisms can be shed through cow feces and may contaminate the outside of the udder and teats, the farm environment and the milking equipment. Although optimal growth conditions for bacteria are different for different organisms, milk contains important nutritional components for mammal growth, and, therefore, it is also an ideal medium for the growth of many different bacteria. Temperature plays an important role in bacterial growth. Many bacteria prefer to grow at body temperature (86-98°F; 30-37°C), but will grow at lower temperatures at slower rates.

In this unit, you will study about dairy microbiology, normal flora of milk and milk products, spoilage of milk and milk products in detail.
7.1 OBJECTIVES

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

- Understand what dairy microbiology is
- Discuss normal flora of milk and milk products
- Explain the spoilage of milk and milk products

7.2 DAIRY MICROBIOLOGY: NORMAL FLORA OF MILK AND MILK PRODUCTS

Milk is used throughout the world as a human food. The milk of goats and sheep is similar in composition to that of cows (Refer Table 7.1). Overall, the chemical composition of whole cow’s milk makes it an ideal growth medium for heterotrophic microorganisms, including the nutritionally fastidious Gram-Positive lactic acid bacteria.

Nutritional Value of Milk

Milk is the excellent medium for the growth of microorganisms as it is a low acid (6.3 to 6.5) and a high moisture food (more than 85%). It is Rich in nutrient such as carbohydrates, protein, vitamin, etc. (Refer Table 7.1).

Table 7.1 Composition of Milk

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>87.0%</td>
<td>Most of the part is water.</td>
</tr>
<tr>
<td>Protein</td>
<td>3.5%</td>
<td>Protein content of milk is considerably lower (3.5%). It consists mainly of casein, imparts color to the milk. If milk pH falls below 4.6, the casein precipitates. When casein precipitates, rest of the liquid portion (15%) is referred to as whey. The remaining proteins are found in whey and they include serum albumin, immunoglobulins, α-lactalbumin, etc.</td>
</tr>
<tr>
<td>Fat</td>
<td>3.8%</td>
<td>Fat consists mainly of triglycerides of C14, C16, C18, and C18:1 fatty acid. The fat content of different pasteurized milk is: full cream milk 6%, double toned milk 1.5%, skimmed milk 0.5%, single toned milk 3%. Smaller quantities of diglycerides and phospholipids occur. Milk lipids exist largely in the form of fat globules that are surrounded by a phospholipid layer.</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>4.9%</td>
<td>Carbohydrate content is considerably higher (14.9%). Although lactose is the main sugar, smaller quantities of glucose and citric acid exist.</td>
</tr>
<tr>
<td>Ash</td>
<td>0.7%</td>
<td>Consists of a relatively high level of Ca²⁺ and a lower level of Fe²⁺.</td>
</tr>
<tr>
<td>pH</td>
<td>6.6%</td>
<td>The pH of fresh whole milk is around 6.6 but it may reach ca. 6.8 from a cow that has mastitis. Mastitis is an infection of the udder that is most often caused by Streptococcus agalactiae and S. uberis but sometimes by Staphylococcus aureus or Streptococcus dysgalactiae. Fresh milk from a mastitic cow typically contains leukocytes (white blood cells) &gt;10⁷/ml in contrast to non-mastitic milk that contains leukocytes around 70,000/ml.</td>
</tr>
</tbody>
</table>
Dairy Microbiology

Vitamins
Milk contains a very adequate supply of B vitamins with pantothenic acid and riboflavin being the two most abundant. Vitamins A and D are added for human consumption, and their presence has no known effect on the activity of microorganisms.

Milk also contain enzymes, such as phosphates, protease.

Minerals, such as calcium, phosphorus, iron, zinc, potassium constitute the milk.

Normal Flora of Milk and Milk Products
Theoretically, milk that is secreted to the udder of a cow should be free of microorganisms. However, during the milking operation, microorganism can enter the milk from the hands of person drawing, from hides of the animal, from atmosphere, utensils, dust particles in air or from water that is added to dilute the milk and constitute normal microflora of the milk.

The normal microflora of milk is very rich in bacteria (Refer Table 7.2). The growth of these microorganisms has to be controlled because if milk is allowed to stand at room temperature, normal microflora will grow and cause spoilage. Spoilage includes curdling of milk, change in colour of milk, milk becoming slimy, orropy in nature. The growth of normal microflora should be inhibited to prevent spoilage and transmission of disease.

Table 7.2 Microbial Groups Associated with Raw Milk

<table>
<thead>
<tr>
<th>Microbial Type</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-Negative Bacteria</td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>Escherichia, Enterobacter, Citrobacter and Klebsiella.</td>
</tr>
<tr>
<td>Non-Enterobacteriaceae</td>
<td>Citrobacter, Aeromonas, Flavobacterium, Moraxella, and Pseudomonas.</td>
</tr>
<tr>
<td>Spore-Forming Bacteria</td>
<td></td>
</tr>
<tr>
<td>Acrobic</td>
<td>Gram-Positive Bacteria belonging to the genera Bacillus (aerobic or facultative anaerobic). B. sporothermodurans produce highly heat-resistant endospores, which can survive even UHT processing of milk. B. cereus known to cause food spoilage via the production of extracellular enzymes (proteases, lipases) and/or food poisoning through the production of toxins (food intoxications). Other aerobic, spore formers isolated from raw milk belong to the genera Paenibacillus, Oceanobacillus, Brevibacillus, Lysinibacillus, Ureibacillus, Ornithinibacillus and Sporosarcina.</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>Clostridium species like C. perfringens, C. botulinum.</td>
</tr>
<tr>
<td>Psychrotrophic Bacteria</td>
<td>Members of the genera Saprospiraceae spp., Pseudomonaceae spp., and Moraxellaceae spp. have been isolated from raw milk samples after heat treatment at 80°C for 10 min, i.e., a heat treatment more intense than laboratory pasteurization.</td>
</tr>
<tr>
<td>Mesophilic bacteria that are capable of proliferation at refrigeration temperatures (0-7°C).</td>
<td>Members of the genera Saprospiraceae spp., Pseudomonaceae spp., and Moraxellaceae spp. have been isolated from raw milk samples after heat treatment at 80°C for 10 min, i.e., a heat treatment more intense than laboratory pasteurization.</td>
</tr>
<tr>
<td>Thermoduric Bacteria (usually isolated from Raw Milk after Pasteurization)</td>
<td>Members of the genera Saprospiraceae spp., Pseudomonaceae spp., and Moraxellaceae spp. have been isolated from raw milk samples after heat treatment at 80°C for 10 min, i.e., a heat treatment more intense than laboratory pasteurization.</td>
</tr>
<tr>
<td>Lactic Acid Bacteria (LAB)</td>
<td>Raw-milk LAB belong to all major LAB genera (Lactococcus, Lactobacillus, Streptococcus, Leuconostoc, Pediococcus, Enterococcus.</td>
</tr>
</tbody>
</table>
Mastitis-Causing Organisms (Intra-Mammary Infection)
Contagious mastitis is usually caused by *Staphylococcus aureus*, *Streptococcus agalactiae* and *Mycoplasma* spp. Environmental mastitis is usually the result of infection with coliform bacteria (*Escherichia coli*, *Klebsiella* spp., *Enterobacteriaceae*), non-coliform Gram-Negative Bacteria, such as *Serratia* spp., *Pseudomonas* spp., *Proteus* spp., Gram-Positive Bacteria, such as *Enterococcus faecalis* and *E. faecium*, some *Bacillus* spp. and *Streptococci* other than *Strep. Agalactiae*.

Human Pathogenic Bacteria
14 pathogenic bacterial families, genera or species commonly associated with raw milk (*Bacillus cereus*, *Brucella* spp., *Clostridium* spp., *Coxiella burnetii*, *Escherichia coli*, *Enterobacteriaceae*, *Enterococcus* spp., *Listeria monocytogenes*, *Mycobacterium* spp., *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus* and *Yersinia enterocolitica*).

*Mycobacterium Avium* sub sp. *Paratuberculosis* (MAP) is the causative agent of paratuberculosis (Johne’s disease), a chronic debilitating disease affecting ruminants and other animals.

*Mycobacterium bovis* and *Coxiella burnetti*, the causative agents of tuberculosis and Q-fever in human.

*Helicobacter pylori* is the causative agent of gastric ulcers and other pathogenic conditions in humans.

Fungi (Yeasts and Molds)
Mold include *Penicillium* spp., *Aspergillus* spp., and *Eurotium* spp., *Chrysosporium*, *Cladosporium*, *Engyodontium*, *Fusarium*, *Torrubiella*.

Sources of Contamination

Below are discussed some sources of contamination in raw milk:

**Contamination on the Farm**
Utensils and milk contact surfaces including the milk pail and milking machines, as the case may be, strainers, milk cans or pipelines and the bulk milk cooler. If dairy utensils or the milk contact surfaces are adequately cleaned, sanitized and dried, they add few bacteria per millimeter of the milk but under very poor condition these sources may increase the bacterial content of the milk by millions per milliliter.

Undesirable bacteria from these sources include lactic streptococci, coliform bacteria, psychrotrophic Gram-Negative rods, and thermodurics, those which survive pasteurization, for example micrococci, enterococci, bacilli, and *Brevibacteria*, in general these bacteria grow well in milk and hence endanger its keeping quality.

Application of quaternary ammonium compounds as sanitizing agents tend to increase the percentage of Gram Negative rods on the utensils (psychrotropics, coliforms), whereas hypochlorides favor Gram-Positive Bacteria (*micrococci, bacilli*).

Modern dairy utensils and milk contact surfaces particularly milk machines, pipelines, and bulk milk coolers are designed to provide easy access for cleaning, sanitizing and drying. Farm bulk milk coolers are also equipped with excellent refrigeration capacity and agitation to ensure proper cooling if the milk. Hands and arms of the milker or dairy workers, the air of the barn or milking.

**Parlor and Flies**
When milking a cow by hand where the milk is collected in an open pail, an infected person (or carrier) may contaminate the milk by coughing or by hand...
Contact. It was largely the connection between raw milk and cases of human scarlet fever, diphtheria, and typhoid fever. The quality of farm water supply used in the milking parlor for cleaning and rinsing, etc. will have some effect on the quality of the milk.

The numbers of bacteria per milliliters of milk added from the various sources depends on the care taken to avoid contamination. For example, the exterior of the cow contributes comparatively few organisms if precautions are taken and a milking machine is used, but under very poor conditions thousands per milliliters could enter the milk.

**Contamination in Transit and at the Manufacturing Level**

Other sources of contamination after the milk leaves the farm include the tanker, truck, transfer pipes, sampling utensils and equipment at the market milk plant, cheese factory, condenser or other processing plant. Again the most significant sources of contamination are the milk contact surfaces. Pipelines, vat, tanks, pumps, valves, separators, clarifiers, homogenizers, coolers, strainers, stirrers and fillers may serve as possible source of bacteria. The amount or level of contamination from each of these sources depends and cleaning and sanitizing methods. In addition the employees, particularly their hands and arms are possible sources of contamination and pathogens. The paper stock used for packaging fluid milk is also an important source of contamination.

In should be kept in mind that the number and types of organisms in milk or other dairy products may be increased either by contamination or by growth of the organisms already present. Methods of production, handling, storage and manufacture are designed to prevent both.

**Spoilage of Milk and Milk Products**

**Milk and Cream**

Spoilage of milk at different temperature are as follows:

- At refrigeration temperatures, proteolysis may be initiated by psychrotropic bacteria, such as *Pseudomonas*. During cold storage of pasteurized milk, psychrotrophic *Bacillus weihenstephanensis* causes ‘sweet curdling’ due to its production of proteases and peptidases.
- At room temperature an acid fermentation is most probable, first by *Streptococcus lactis* and then by coliform bacteria, enterococci, acid tolerant lactobacilli. Then mold or film yeasts on the surface lower the acidity, permitting the formation of more acid. Eventually when most of the acid has been destroyed, proteolytic or putrefactive bacteria complete the decomposition.
- Pasteurization as applied commercially in HTST systems kills yeast, molds, most psychotropic bacteria, the coliforms, and rapid acid producers, such as *Streptococcus lactis*. 

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*NOTES*

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In spite of the widespread use of pasteurization, milk continues to be a vehicle for some diseases.

- For the years 1973–1992, 46 outbreaks (in United States) and 1,733 cases of human gastroenteritis were traced to raw milk. Of the 1,733 cases, 57% were caused by *Campylobacter*, 26% by *Salmonella*, and 2% by *E. coli* 0157:H7. In the case of campylobacteriosis, it is not surprising that its etiologic agent can be found in milk since the organism (*C. jejuni*) exists in cow feces.

- Of 219 raw milk samples tested in Brazil, 37 (16.9%) contained *Listeria* spp. and 32.4% were *Y. enterocolitica*. Of 280 pasteurized milk samples, 13.7% were positive for *Yersinia* spp. with 41.5% being *Y. enterocolitica*. The latter species was the most common in raw milk while *Y. frederiksenii* at 56.1% was the most prevalent in pasteurized milk.

- The two largest human outbreaks of salmonellosis to occur in the United States involved milk and ice cream.

- In regard to aflatoxin M1 in milk, a study of 2902-liter samples of pasteurized and ultrapasteurized milk was carried out in Mexico on the seven most widely used brands with varying fat content. Forty percent contained AFM1 at levels ≥0.05 ppm and 9.7% contained ≥ 0.5 ppm, and the range was 0–8.35 ppm in 40% of those with the lower concentration and in 10% of those with the higher concentration. Milk with the highest fat content had a slightly higher probability of containing AFM1.

- An outbreak of *E. coli* 0157:H7 was traced in fecal samples and raw milk in east Tennessee, where in study found 8 of 415 (2%) fecal samples and 2 of 268 (0.7%) milk samples positive.

- The coliform bacteria are the most conspicuous utilizers of lactose among Gram-Negative Bacteria. Thus, the bacterial spoilage of either raw or pasteurized milk is conspicuous by the production of lactic acid by lactose users, with the normal pH of around 6.6 being reduced to 4.5 or so that leads to the precipitation of casein (curdling). The thermoduric *Streptococcus salivarius* subsp. *thermophilus* strains preferentially use the glucose moiety of lactose and excrete galactose, which is a ready substrate for non-lactose users.

- A bacterium of continuing concern in milk is the etiologic agent of Johne’s disease of cattle, which appears to play some role in Crohn’s disease of humans. *M. avium* subsp. *paratuberculosis* is an obligate pathogen of ruminants was found in cow’s milk because of its possible role in Crohn’s disease.

The spoilage of pasteurized milk then depends on:

- The spoilage of UHT-treated milk can result from the actions of heat-resistant proteases and lipases that are produced by thermodurics and spore former
bacteria, such as *B. cereus*, *B. licheniformis*, *B. badius*, and *B. sporothermodurans* that survives pasteurization.

- The bacteria that enter the milk following pasteurization, post pasteurization contamination from equipment, filling operation and the package itself.
- The possible presence of heat resistant residual microbial enzymes.
- The temperature of storage.

**Gas Production**
Gas production by bacteria usually is accompanied by acid formation. The chief gas formers are:

- *Coliform bacteria*, *Clostridium* spp., and gas forming *Bacillus* species that yield both hydrogen and carbon dioxide.
- The yeasts, propionic and heterofermentative lactis that produce only carbon dioxide.

The production of gas in milks is evidenced by foam at the top if milk is liquid and is supersaturated with the gas, by gas bubble caught in the curd or furrowing it, by floating curd containing gas bubble, or by a ripping apart of the curd by rapid gas production, causing the so called stormy fermentation of milk. In raw milk the coliform bacteria are the main gas formers. In milk heated at pasteurizing temperature or above, the chief acid formers are killed, and gas formation by the spore formers (clostridium and bacillus) may take place.

**Proteolysis**
The hydrolysis of milk proteins by microorganisms is accompanied by the production of a bitter flavour caused by some of the peptides released. Proteolysis is favoured by:

- Storage at a low temperature.
- By the destruction of lactis and other acid formers by heat.
- By the destruction of formed acid in the milk by molds and film yeasts.
- The neutralization of acids by products of the organisms.

The types of change produced by proteolytic organisms include:

- Acid proteolysis, in which acid production and proteolysis occur together.
- Proteolysis with little acidity or even with alkalinity.
- Sweet cursing, which is caused by rennin like enzymes of the bacteria at an early stage of proteolysis.
- Slow proteolysis by intracellular enzymes of bacteria after their autolysis.
- Residual proteolytic activity of heat stable protease. For example, *Pseudomonads florescense* produces a protease that will survive pasteurization even though the bacterium does not.
Acid Proteolysis causes the production of a shrunken curd and the expression of much whey. This is followed by a slow digestion of the curd, which changes in appearance from opaqueness to translucency and may be completely dissolved by some kind of bacteria. Acid proteolysis may be caused by several species of Micrococcus, some of which grow in the udder of the cow and cause acid proteolysis of aseptically drawn milk. One of the intestinal Streptococci or Enterococci, Streptococcus faecalis var. liquefaciens, is a lactic acid organism that also is actively proteolytic. It is thermodurics like the other enterococci, and may cause acid proteolysis in pasteurized milk. Spores of lactose fermenting, proteolytic strains of some species Bacillus can survive pasteurization or a more vigorous heat treatment of milk and cause acid proteolysis.

Proteolysis by bacteria unable to ferment lactose varies from digestion of the Protein Casein to slight proteolysis. Little acidity is produced by most of these bacteria, in fact, the milk usually become alkaline from products of protein decomposition. Actively proteolytic bacteria are from genera of non-spor forming bacteria as Micrococcus, Alcaligenes, Pseudomonads, Proteus, Flavobacterium and Serratia, and spore formers Bacillus and Clostridium. Some species of genera Micrococcus, Pseudomonas, Alcaligenes, Flavobacterium and Bacillus can grow at low temperature and hence are likely to cause some proteolysis and/or bitterness of milk held at chilling temperatures. Bacillus cereus has been implicated in sweet curdling of the pasteurized milk.

Slow proteolysis by intracellular enzymes of bacteria is significant when a long time is allowed for their action, as in curing cheese or in products with a long time shelf life.

Ropiness

Ropiness is a form of bacterial spoilage in milk that makes the milk highly viscous or sticky. Ropy milk has characteristic silk-like threads that may vary in length from a few inches to several feet.

Non-bacterial ropiness or sliminess in milk and cream may be due to the following reasons:

- Stringiness caused by mastitis and in particular by fibrin and leukocytes from the cow’s blood (in contrast to ropiness produced by bacteria it is present when the milk is drawn, not developed during holding of the milk).
- Sliminess resulting from the thickness of cream, for example at the top of a bottle, iii) stringiness due to thin film of casein or lactalbumin during cooling.

Bacterial Ropiness: It is caused by slimy capsular material from the cells, usually gums or mucins, ordinarily develops best at low storage temperatures. The ropiness usually decreases as the acidity of the milk or cream increases. There are two main types of bacterial ropiness,

- Surface Ropiness
- Throughout Ropiness
Surface Ropiness: In which the milk is most ropy at the top. Surface ropiness is caused most often by *Alcaligenes vissolactis*. Its growth is favored by low-temperature maintenance of raw milk for several days. Some of the thermoduric micrococci, for example *Micrococcus freudenreichii* can cause surface ropiness.

Throughout Ropiness: In which milk becomes ropy throughout. Ropiness throughout the milk may be caused by any of a number of kinds of bacteria:

- *Enteribacter aerogenes*, *E. cloacae*, *Klebsiella oxytoca* and rarely *Escherichia coli*. Ropiness caused by *Enteribacter* usually is worse near the top of the milk.
- Certain strains of some of the common species of lactic acid bacteria. *Streptococcus lactis var. hollandicus* causes ropiness in milk and is used in making Scandinavian fermented milk. *Lactobacillus casei*, *L. bulgaricus* and *L. plantarum* occasionally produce ropiness, as do strains of *Streptococcus cremoris*.
- Miscellaneous other bacteria among the alkali formers micrococci, streptococci, and bacilli.

Since the sources of bacteria causing ropiness are water, manure, utensils, and feed, the reduction or elimination of contamination from these sources helps prevent ropiness. Adequate pasteurization of milk readily destroys most of these kinds of bacteria.

Changes in Milk Fat

The following changes in milk fat takes place:

- Oxidation of the unsaturated fatty acids, which coupled with other decomposition, yields aldehyde, acids and ketones and results in animal fat odors and tastes. The reaction is favored by metals, sunlight and oxidizing microorganisms.
- Hydrolysis of butter fat to fatty acids and glycerol by the enzymes lipases. The lipase may have been in the original milk or may be microbial.
- Combined Oxidation and Hydrolysis to produce rancidity. Species of lipase forming bacteria are found in many of the bacterial genera, for example *Pseudomonas*, *Proteus*, *Alcaligenes*, *Bacillus*, *Micrococcus*, *Clostridium*. *Pseudomonas fragi* and *Staphylococcus aureus* produce fairly heat resistant lipase which may survive pasteurization if present in the raw milk. Many of the molds and some species of the yeasts are lipolytic.

Alkali Production

The group of alkali formers includes bacteria which causes an alkaline reaction in milk without any evidence of proteolysis. The alkaline reaction may result from the formation of ammonia, as from urea, or of carbonates, as from organic acids such as...
as citric acid. Most of these bacteria grow from moderate to low temperatures and many can survive pasteurization. Examples of alkali formers are *Pseudomonas fluorescense* and *A. vascolactis*.

**Flavor Changes**

Some of the off-flavours caused by microorganisms are described as follows:

**Sour or Acid Flavour**

The acidity may be described as clean as produced by *Streptococcus lactis* and other lactis; as aromatic, when lactic streptococci and aroma forming *Leuconostoc* species are growing together and sharp when appreciable amounts of volatile fatty acids (formic, acetic or butyric) are produced from coliform bacteria, clostridium and, other organism. Clean and aromatic flavours are desired in fermented milk products but sharp flavours are undesirable.

**Bitter Flavours**

Bitterness usually results from proteolysis but may follow lipolysis or even fermentation of lactose. Milk from cows late in their lactation period sometimes is slightly bitter. Other organisms causing bitterness are certain strains of coliform bacteria and of asporogenous yeasts. Some cocci causes very bitter milk and actinomycetes sometimes gives bitter musty flavours.

**Burnt or Caramel Flavour**

Certain strains of *Streptococcus lactis* var. *maltigenes* produce this flavour, which resembles the cooked flavour of overheated milk.

**Colour Changes**

The colour of the milk or cream is affected by its physical and chemical composition, for example by the amount and yellowness of the butterfat and thinness of the milk, the content of the blood and puss and the feed of the animal.

- **Blue Milk**: *Pseudomonas synchyanea* produces a blue grey to brownish colour in milk in pure culture but when growing with an acid former like *Streptococcus lactis* a deep blue colour.

- **Yellow Milk**: *Pseudomonas synxantha* may cause a glow colour in the cream layer of milk, coincidence to lipolysis and proteolysis. Species of *Flavobacterium* can also give yellowness.

- **Red Milk**: Red milk is usually caused by *Serratia marcescens*, but is rare because other bacteria ordinarily outgrow the red pigmented species. *Brevibacterium erythrogenes* produces a red layer at the top of the milk, followed by proteolysis. *Micrococcus roseus* may grow and produce red sediment, and yeast may produce pink or red colonies on the surface of sour milk or cream.
- **Brown Milk**: A brown colour may result from *Pseudomonas putrefaciens* or by the enzymic oxidation of tyrosine by *P. fluorescens*.

**Fig. 7.1 Spoolage of a Dairy Product: Fresh (left) and Curdled (right) Milk**

**Condensed and Dry Milk Products**

Condensed and dry milk products include evaporated milk (unsweetened), bulk condensed milk, frozen milk, sweetened condensed milk, condensed whey or butter milk and condensed sour skim milk and dry milk. The quality of all of these products depends on the quality of starting material dried and condensed. The entire condensed products have a fairly high concentration of solutes that inhibits the growth of some bacteria. Dry milk is so low in moisture that offers no microbial storage problems when properly handled. The only spoilage of condensed butter milk and sour skim milk is by molds when the surface is exposed to air. The high concentration of acid and solutes prevents the growth of bacteria or yeasts.

**Bulk Condensed Milk**

Although refrigerated, this product has only a short storage life and is subject to spoilage by thermoduric bacteria that tolerate the increased concentration of solutes in the condensed product. In superheated condensed milk, the temperature of the milk is raised to 65.6 to 76.7°C during the introduction of steam, a process that probably destroys most of the vegetative cells of bacteria but not the spores. This product too has a short storage life.
Evaporated Milk (Unsweetened)

Unsweetened evaporated milk is canned and heat processed under stem pressure in an attempt to destroy all the microorganisms present. Spoilage can take place only when the heat process is inadequate or defects in the can permit the entrance of organisms. Bacterial spores that survive the heat process may be the cause of can swelling, milk coagulation or development of a bitter flavour.

- Swelling of the can is primarily caused by gas forming anaerobic spore formers (*clostridium*), although overfilling of the can with cold milk may cause swelling. Acid constituent of the milk acting on the iron of the can may produce hydrogen gas and cause bulging on long storage.
- Coagulation of the milk in the can may vary from a few flakes to a solid curd. Species of *Bacillus* either mesophiles, such as *B. cereus*, *B. subtilis*, and *B. megatarium*; a facultative thermophile, such as *B. coagulans*, or an obligate thermophile, *B. calidolactis*.
- Bitterness usually results from proteolysis by species of *Bacillus* and less commonly by the species of *Clostridium*.

Sweetened Condensed Milk: Sweetened condensed milk has been subjected to a fairly high temperature (71.1 to 100 degree C) during forewarming and to a milder heat treatment (48.9 to 54.4 degree C) during condensing so that the yeasts molds and most of the vegetative cells of bacteria are destroyed. In addition there is a high concentration of sugars about 55 to 60% of total sugar (lactose plus added sugar). Also, the can is evacuated and sealed. Spoilage is then primarily due to organisms that have entered after the heat treatment, especially if air is present.

The chief types of spoilage are:

- Gas formation by sucrose fermenting yeasts or more rarely by coliform bacteria,
- Thickening caused by micrococci which probably produce rennin like enzymes,
- Buttons, which are mold colonies growing in the milk surface. Species of *Aspergillus*, for example *A. repens* and of *Penicillium* have been incriminated.

Frozen Desserts

Frozen dessert including, ice cream, ice milk, frozen custard, sherbets and ices. The ingredients may be various combinations of milk, cream, evaporated milk, condensed milk, dried milk, coloring material, flavors, fruits, and nuts, sweetening agents, egg products and stabilizers. Any of these may contribute microorganisms...
to the product and affect the quality of desserts. The desserts are not ordinarily subject to spoilage however as long as they are kept frozen.

**Butter**

Many of the defects if the butter originate in the cream from which is made especially when the cream has been held for several days on the farm before collection by the creamery. During this time lactic acid bacteria, gas formers and other spoilage organism may grow and be followed by molds *Geotrichium candidum*. Lactose fermenting yeasts which are present only occasionally may develop high gas pressure in the can of cream.

The probability and type of spoilage will depend on the kind of butter and the environment in which it is kept. Because of the high salt concentration in the small amount of moisture present, salted butter is less likely to support microbial growth than is unsalted butter. In general, sweet cream butter keeps better than sour cream butter. Today most butter in this country is made from pasteurized cream, in which most of the spoilage microorganisms have been destroyed. Also butter commonly is kept refrigerated and during the commercial storage is kept at about -17.8°C where no microbial growth can take place, for these reasons bacteria usually do not grow in butter. However, relatively small amounts of growth may cause appreciable damage to the flavour.

**Flavour Defects**

Undesirable flavour may come from the cream which may receive such flavours from the feed of the cow, absorb them from the atmosphere or develop them during microbial growth. Feeds, such as onion, garlic, French weed, peppergrass and poor silage contributes off-flavors to the cream. Volatile products that may be absorbed from the air are odors from the barn and from the chemicals used their, for example kerosine, gasoline, fly sprays, disinfectant, etc.

**Microbially Produced Defects**

**Defects in the Cream:** Growth of microorganisms in the cream and in the milk from which it is separated may result in any of the following bad flavours:

- Cheesiness, caused by *Lactobacilli*.
- Rancidity; this condition is caused by the hydrolysis of butterfat with the liberation of free fatty acids. The causative organisms are lipolytic bacteria, such as *Pseudomonas fragi*.
- Barny flavours, produced by species of *Enterobacter*.
- Malty flavour; produced by *Streptococcus lactis var maltigenes*.
- Yeast flavour; produced by yeasts.
● Musty flavours; caused by molds and actinomyces.
● Metallic flavours, caused by dissolved metals in highly acidic cream.
● Flat flavour; resulting from destruction of diacetyl by bacteria like some of the *Pseudomonas* species.
● Highly acid flavour, when the cream has excessive acidity.

**Defects in Butter:** Growth Like cream, butter readily absorbs volatile materials from the air, microorganisms in the butter can cause following defects:

- Surface taint also called rabbito and putridity. This condition is caused by *Pseudomonas putrefaciens* as a result of its growth on the surface of finished butter. It develops at temperatures within the range 4–7°C and may become apparent within 7–10 days. The odour of this condition is apparently due to certain organic acids, especially isovaleric acid. Surface taint along with an apple odour is caused also by *Chryseobacterium joosteii*.
- Fishiness; caused by *Aeromonas hydrophilia*.
- Ester like flavours, resulting from the action of *P. fragi*.
- Skunk like flavours; caused by *Pseudomonas mephitica*.
- Roquefort like flavours, produced by molds.

Chemically produced flavours include:

- Rancidity produced by lipase in their cream.
- Tallowiness from oxidations if unsaturated fats catalyzed by copper or bacterial enzymes and favoured by a low pH, low temperature pasteurization, salt, air, ozone.
- Fishiness, where trimethylamine is produced from lecithin. This defect is favoured by high acidity, salt, overworking of the butter, and the presence of the copper.

**Colour Defects**

**Microbially Produced Defects:** Discolouration; chiefly at the surface, may be caused by molds, yeasts or bacteria that come from churns, wrappers, liners, circles, tubs, the air or the cream if it is unpasteurized.

- Coloured growth of molds results in the smudged or *Alternaria* type of discolouration, with dark smoky or rarely greenish areas where *Alternaria* or *Cladosporium* species have grown or small black spots of *Stemphyllium*.
- *Penicillium* produces green colouration.
- *Phoma* or *Alternaria* molds brown areas.
- *Geotrichium* (syn *Oospora*) species produces orange or yellow spots.
• *Fusarium culmorum* can cause bright reddish link areas.
• Yeasts sometimes grow in pink colonies.
• *Pseudomonas nigrifaciens* causes the black discolorations of butter.

Some of the colour defects not caused by microorganisms are:
• Mottling because of improper working.
• A pink colour caused by the sulfur dioxide refrigerant on the butter colour.
• Surface darkening resulting from the loss of water from surface layers.
• Bleaching that accompanies tallowiness.

### Check Your Progress

1. What is the nutritional value of milk?
2. How much fat is present in milk?
3. How does contamination occur in the farm?
4. What are the chief formers of gas production?
5. What is proteolysis and what favours it?

### 7.3 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Milk is the excellent medium for the growth of microorganisms as it is a low acid (6.3 to 6.5) and a high moisture food (more than 85%). It is rich in nutrient, such as carbohydrates, protein, vitamin, etc.

2. Fat composition in the milk is 3.9%. Fat consists mainly of triglycerides of C14, C16, C18, and C18:1 fatty acid. The fat content of different pasteurized milk is: full cream milk 6%, double toned milk 1.5%, skimmed milk 0.5%, single toned milk 3%. Smaller quantities of diglycerides and phospholipids occur. Milk lipids exist largely in the form of fat globules that are surrounded by a phospholipid layer.

3. Utensils and milk contact surfaces including the milk pail and milking machines, as the case may be, strainers, milk cans or pipelines and the bulk milk cooler. If dairy utensils or the milk contact surfaces are adequately cleaned, sanitized and dried, they add few bacteria per milliliter of the milk but under very poor condition these sources may increase the bacterial content of the milk by millions per milliliter.

4. Gas production by bacteria usually is accompanied by acid formation. The chief gas formers are:
   • Coliform bacteria, *Clostridium* spp., and gas forming *Bacillus* species that yield both hydrogen and carbon dioxide.
• The yeasts, propionic and heterofermentative lactis that produce only carbon dioxide.

5. The hydrolysis of milk proteins by microorganisms is accompanied by the production of a bitter flavour caused by some of the peptides released. Proteolysis is favoured by:
   • Storage at a low temperature.
   • By the destruction of lactis and other acid formers by heat.
   • By the destruction of formed acid in the milk by molds and film yeasts.
   • The neutralization of acids by products of the organisms.

7.4 SUMMARY

• Milk is used throughout the world as a human food. The milk of goats and sheep is similar in composition to that of cows.
• Overall, the chemical composition of whole cow’s milk makes it an ideal growth medium for heterotrophic microorganisms, including the nutritionally fastidious Gram-Positive Lactic Acid Bacteria.
• Milk is the excellent medium for the growth of microorganisms as it is a low acid (6.3 to 6.5) and a high moisture food (more than 85%).
• Theoretically, milk that is secreted to the udder of a cow should be free of microorganisms.
• During the milking operation, microorganism can enter the milk from the hands of person drawing, from hides of the animal, from atmosphere, utensils, dust particles in air or from water that is added to dilute the milk and constitute normal microflora of the milk.
• The normal microflora of milk is very rich in bacteria. The growth of these microorganisms has to be controlled because if milk is allowed to stand at room temperature, normal microflora will grow and cause spoilage.
• Spoilage includes curdling of milk, change in color of milk, milk becoming slimy, or ropy in nature. The growth of normal microflora should be inhibited to prevent spoilage and transmission of disease.
• Gram-Positive Bacteria belonging to the genera *Bacillus* (aerobic or facultative anaerobic).
• *B. sporothermodurans* produce highly heat-resistant endospores, which can survive even UHT processing of milk.
• *B. cereus* known to cause food spoilage via the production of extracellular enzymes (proteases, lipases) and/or food poisoning though the production of toxins (food intoxications).
Other aerobic spore formers isolated from raw milk belong to the genera *Paenibacillus*, *Oceanobacillus*, *Brevibacillus*, *Lysinibacillus*, *Ureibacillus*, *Ornithinibacillus* and *Sporosarcina*.

‘Lactic Bacteriophage’ (phage attacking and inactivating LAB) are often present in raw milk. The presence of lactic bacteriophage in the milk used for milk fermentations leads to loss of fermentative capacity associated with starter culture lysis, which can significantly retard or even halt the fermentation process.

Utensils and milk contact surfaces including the milk pail and milking machines, as the case may be, strainers, milk cans or pipelines and the bulk milk cooler.

If dairy utensils or the milk contact surfaces are adequately cleaned, sanitized and dried, they add few bacteria per millimeter of the milk but under very poor condition these sources may increase the bacterial content of the milk by millions per milliliter.

Undesirable bacteria from these sources include lactic *Streptococci*, coliform bacteria, psychrotrophic Gram-Negative rods, and thermodurics, those which survive pasteurization, for example micrococci, enterococci, bacilli, and *Brevibacteria*. In general these bacteria grow well in milk and hence endanger its keeping quality.

Application of quaternary ammonium compounds as sanitizing agents tend to increase the percentage of Gram-Negative rods on the utensils (psychrotropics, coliforms), whereas hypochlorides favor Gram-Positive Bacteria (*micrococci*, *bacilli*).

Modern dairy utensils and milk contact surfaces particularly milk machines, pipelines, and bulk milk coolers are designed to provide easy access for cleaning, sanitizing and drying.

Farm bulk milk coolers are also equipped with excellent refrigeration capacity and agitation to ensure proper cooling if the milk.

When milking a cow by hand where the milk is collected in an open pail, an infected person (or carrier) may contaminate the milk by coughing or by hand contact.

The quality of farm water supply used in the milking parlor for cleaning and rinsing, etc., will have some effect on the quality of the milk.

The numbers of bacteria per milliliters of milk added from the various sources depends on the care taken to avoid contamination.

Other sources of contamination after the milk leaves the farm include the tanker, truck, transfer pipes, sampling utensils and equipment at the market milk plant, cheese factory, condenser or other processing plant.
• The most significant sources of contamination are the milk contact surfaces.
• Pipelines, vat, tanks, pumps, valves, separators, clarifiers, homogenizers, coolers, strainers, stirrers and fillers may serve as possible source of bacteria.
• The amount or level of contamination from each of these sources depends and cleaning and sanitizing methods.
• The paper stock used for packaging fluid milk is also an important source of contamination.
• The number and types of organisms in milk or other dairy products may be increased either by contamination or by growth of the organisms already present.
• Methods of production, handling, storage and manufacture are designed to prevent both.
• The spoilage of UHT-treated milk can result from the actions of heat-resistant proteases and lipases that are produced by thermodurics and spore former bacteria, such as *B. cereus*, *B. licheniformis*, *B. badius*, and *B. sporothermodurans* that survives pasteurization.
• The bacteria that enter the milk following pasteurization, post pasteurization contamination from equipment, filling operation and the package itself.
• The production of gas in milks is evidenced by foam at the top if milk is liquid and is supersaturated with the gas, by gas bubble caught in the curd or furrowing it, by floating curd containing gas bubble, or by a ripping apart of the curd by rapid gas production, causing the so called stormy fermentation of milk.
• In raw milk the coliform bacteria are the main gas formers. In milk heated at pasteurizing temperature or above, the chief acid formers are killed, and gas formation by the spore formers (clostridium and bacillus) may take place.
• Acid proteolysis causes the production of a shrunken curd and the expression of much whey. This is followed by a slow digestion of the curd which changes in appearance from opaqueness to translucency and may be completely dissolved by some kind of bacteria.
• Acid proteolysis may be caused by several species of *Micrococcus*, some of which grow in the udder of the cow and cause acid proteolysis of aseptically drawn milk.
• One of the intestinal *Streptococci* or *Enterococci*, *Streptococcus faecalis var. liquefaciens*, is a lactic acid organism that also is actively proteolytic.
• Spores of lactose fermenting, proteolytic strains of some species Bacillus can survive pasteurization or a more vigorous heat treatment of milk and cause acid proteolysis.
• Proteolysis by bacteria unable to ferment lactose varies from digestion of the casein to slight proteolysis.
• Little acidity is produced by most of these bacteria, in fact, the milk usually become alkaline from products of protein decomposition.
• Actively proteolytic bacteria are from genera of non-spore forming bacteria as *Micrococcus*, *Alcaligenes*, *Pseudomonas*, *Proteus*, *Flavobacterium* and *Serratia*, and spore formers *Bacillus* and *Clostridium*.
• Bacterial ropiness is caused by slimy capsular material from the cells, usually gums or mucins, ordinarily develops best at low storage temperatures.
• The group of alkali formers includes bacteria which causes an alkaline reaction in milk without any evidence of proteolysis.
• The alkaline reaction may result from the formation of ammonia, as from urea, or of carbonates, as from organic acids, such as citric acid.
• Bitterness usually results from proteolysis but may follow lipolysis or even fermentation of lactose.
• Other organisms causing bitterness are certain strains of coliform bacteria and of asporogenous yeasts.
• Some cocci causes’ very bitter milk and actinomycetes sometimes gives bitter musty flavours.
• Condensed and dry milk products include evaporated milk (unsweetened), bulk condensed milk, frozen milk, sweetened condensed milk, condensed whey or butter milk and condensed sour skim milk and dry milk.
• The entire condensed products have a fairly high concentration of solutes that inhibits the growth of some bacteria.
• Dry milk is so low in moisture that offers no microbial storage problems when properly handled.
• The only spoilage of condensed butter milk and sour skim milk is by molds when the surface is exposed to airs.
• The high concentration of acid and solutes prevents the growth of bacteria or yeasts.
• The desserts are not ordinarily subject to spoilage however as long as they are kept frozen.
• Undesirable flavour may come from the cream which may receive such flavors from the feed of the cow, absorb them from the atmosphere or develop them during microbial growth.
• Feeds such as onion, garlic, French weed, peppergrass and poor silage contributes off-flavours to the cream.
Volatile products that may be absorbed from the air are odours from the barn and from the chemicals used there, for example kerosine, gasoline, fly sprays, disinfectant, etc.

7.5 KEY WORDS

- **Lactic bacteriophage**: These are often present in raw milk and is used for milk fermentations, for example, cheese making.
- **Ropiness**: Ropiness is a form of bacterial spoilage in milk that makes the milk highly viscous or sticky.
- **Surface ropiness**: In surface ropiness the milk is most ropy at the top.
- **Throughout ropiness**: In throughout ropiness milk becomes ropy throughout.
- **Gas production**: It is done by bacteria and is usually accompanied by acid formation.
- **Proteolysis**: The hydrolysis of milk proteins by microorganisms is accompanied by the production of a bitter flavour caused by some of the peptides released.
- **Acid proteolysis**: In this acid production and proteolysis occur together, which causes the production of a shrunken curd and the expression of much whey.

7.6 SELF ASSESSMENT QUESTIONS AND EXERCISES

**Short Answer Questions**

1. What is the amount of protein composition in milk?
2. Write a note on spore-forming bacteria.
3. How does contamination occur with parlor and flies?
4. Briefly describe the contamination in transit and at the manufacturing level.
5. Give short note on milk and cream spoilage.
6. What are the factors on which the spoilage of pasteurized milk depends?
7. What is acid proteolysis?
8. Define about the changes in milk fat.
9. List the chemically produced flavours.
Long Answer Questions

1. Briefly discuss about the nutritional value of milk.
2. Discuss about the composition of milk.
3. Explain about the normal flora of milk and milk products giving examples.
4. What are the microbial groups associated with raw milk?
5. Explain the different sources of contamination.
6. Discuss about spoilage of milk and milk products.
7. Elaborate a note on ropiness and its types.
8. What is alkali production? Explain giving examples.
9. Explain colour defects with the help of examples.

7.7 FURTHER READINGS


UNIT 8 FERMENTED MILK PRODUCTS

8.0 INTRODUCTION

Fermented milk products, also known as cultured dairy foods, cultured dairy products, or cultured milk products, are dairy foods that have been fermented with Lactic Acid Bacteria such as *Lactobacillus*, *Lactococcus*, and *Leuconostoc*. The fermentation process increases the shelf life of the product, while enhancing the taste and improving the digestibility of milk. There is evidence that fermented milk products have been produced since around 10,000 BC. A range of different Lactobacilli strains has been grown in laboratories allowing for a wide range of cultured milk products with different tastes.

The primary function of fermenting milk was, originally, to extend its shelf life. With this came numerous advantages, such as an improved taste and enhanced digestibility of the milk, as well as the manufacture of a wide variety of products. Historically the fermentation of milk can be traced back to around 10,000 B.C. It is likely that fermentation initially arose spontaneously from indigenous microflora found in milk. Fortunately, the bacteria were *lactococci* and *lactobacilli* which typically suppress spoilage and pathogenic organisms effectively. The evolution of these products likely came as a result of the climate of the region in which they were produced: thermophilic lactic acid fermentation favours the heat of the subtropics; mesophilic lactic acid fermentation occurs at cooler temperatures. Today the fermentations are controlled with specific starter cultures and conditions. Some of the many fermented milk products are: acidophilus milk, cultured buttermilk, kefir, koumiss, sour cream, and viili. Yogurt and cheese are also fermented milk products.

Qualities of fermented dairy product are influenced by the composition and quality of milk. Milk contains protein, lactose, lipids, minerals, traces of vitamins and water. Microorganisms use the nutrients in milk in their metabolism for growth.
They are present in the fermented milk products, not only as viable cells, but also as autolyzed cells that they release cell components, metabolites and enzymes. Fermentation increases digestibility of nutrients and nutritive value and makes nutrients more available for intestinal absorption. The primary microflora used in the production of fermented milk products are homofermentative Lactic Acid Bacteria (LAB). Other types of secondary microflora of dairy products are mixtures of yeasts, molds and bacteria. These microorganisms can be added directly to the milk or are smeared, sprayed, or rubbed onto the cheese surface.

In this unit, you will study about fermented products, acidophilus and bifidus milk, yoghurt, manufacture of cheese and evolution of quality milk in detail.

8.1 OBJECTIVES

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

- Understand what fermented products are
- Discuss about acidophilus and bifidus milk
- Explain yoghurt manufacture of cheese
- Analyse evolution of quality milk

8.2 FERMENTED MILK PRODUCTS

Milk is a highly unstable food as it is a very good medium for the growth of many microorganisms. Fermentation of milk primarily aims at preserving the same by converting into more stable, nutritious and desirable products such as cheese, yoghurt, butter milk and butter. Nearly every civilization has developed fermented milk products of some type exhibiting a wide variety of textures ranging from liquid drinks such as buttermilk, kefir, koumiss, and acidophilus milk to semisolid or firm products including yogurt and dahi. There are other various types of traditional fermented milk produced in the name of Ymer in Denmark, lactafil and filmjolk in Sweden and Nordic ropy milk made in Norway.

In today's world, consumers demand products of high quality in terms of nutritional, biological and dietetic value where these fermented products find a good place. So, over the past several decades the consumption of fermented milk products has greatly increased in part due to increased knowledge regarding the health benefits of fermented milk products.

Milk from cows, sheep, goats and other mammals may be used directly for fermentation or after preliminary treatment such as pasteurization, ultrafiltration or lactose hydrolysis. The fermentation may be initiated spontaneously by native microflora or by addition of specific starter cultures or material from previous fermentation. The fermented milk may undergo further processing to yield a more...
concentrated product or may be blended with other ingredients such as salts, fruits, herbs, spices, sugars and natural colourants to change flavour, appearance and texture of such products.

The microorganisms used in the industrial scale of fermentation of cow’s milk include mainly lactic acid producing bacteria such as the mesophilic *Lactococcus* and *Leuconostoc* species with optimum growth temperatures in the range of 20 to 30°C and thermophilic *Lactobacillus* and *Streptococcus* species used at temperatures up to 45°C. Other bacteria such as *Corynebacterium*, *Propionibacterium*, yeasts such as *Saccharomyces* and moulds such as *Penicillium camemberti* and *Penicillium candidum* are also used. Table 8.1 lists some fermented dairy products and Table 8.2 lists some organisms involved in making fermented milk.

<table>
<thead>
<tr>
<th>Table 8.1 Some Fermented Dairy Products</th>
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<tr>
<td><strong>Foodstuff</strong></td>
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<tr>
<td>Acido phosphus milk</td>
</tr>
<tr>
<td>Chal</td>
</tr>
<tr>
<td>Cultured buttermilk</td>
</tr>
<tr>
<td>Filmjolk</td>
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<tr>
<td>Kefir</td>
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<tr>
<td>Kumiss</td>
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<tr>
<td>Lass</td>
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<tr>
<td>Quark</td>
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<tr>
<td>Ricotta</td>
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</tbody>
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Over the last several thousand years, fermentation has been a major way of preserving food. Microbial growth, either of natural or inoculated populations, causes chemical and/or textural changes to form a product that can be stored for extended periods. The fermentation process also is used to create new, pleasing food flavors and odors.

Throughout the world, at least 400 different fermented milks are produced. These fermentations are carried out by mesophilic, thermophilic, and probiotic lactic acid bacteria, as well as by yeasts and molds (Refer Table 8.3). The fermented milk products are of two main types: natural and artificial. Natural products are made with milk from animals that have not been treated with antibiotics, and artificial products are made with milk that has been treated with antibiotics. The table below lists the organisms involved in making fermented milks:

<table>
<thead>
<tr>
<th>Foodstuff</th>
<th>Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidophilus milk</td>
<td><em>Lactobacillus acidophilus</em></td>
</tr>
<tr>
<td>Cultured buttermilk</td>
<td><em>Lactococcus lactis ssp. cremoris</em></td>
</tr>
<tr>
<td></td>
<td><em>Lactococcus lactis ssp. lactis</em></td>
</tr>
<tr>
<td></td>
<td><em>Lactococcus lactis ssp. lactis biovar diacetylactis</em></td>
</tr>
<tr>
<td>Kefir</td>
<td><em>Lactococcus lactis ssp. cremoris</em></td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus delbrueckii ssp. bulgaricus</em></td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus helveticus</em></td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus delbrueckii ssp. lactis</em></td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus casei</em></td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus brevis</em></td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus kefir</em></td>
</tr>
<tr>
<td></td>
<td><em>Leuconostoc mesenteroides</em></td>
</tr>
<tr>
<td></td>
<td><em>Leuconostoc dextranicum</em></td>
</tr>
<tr>
<td></td>
<td><em>Acetobacter acetii</em></td>
</tr>
<tr>
<td></td>
<td><em>Candida kefir</em></td>
</tr>
<tr>
<td></td>
<td><em>Kluyveromyces marxianus ssp. marxianus</em></td>
</tr>
<tr>
<td></td>
<td><em>Saccharomyces cerevisiae</em></td>
</tr>
<tr>
<td></td>
<td><em>Torulaspora delbrueckii</em></td>
</tr>
<tr>
<td>Kumiss</td>
<td><em>Lactobacillus delbrueckii ssp. bulgaricus</em></td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus kefir</em></td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus lactis</em></td>
</tr>
<tr>
<td></td>
<td><em>Acetobacter acetii</em></td>
</tr>
<tr>
<td></td>
<td><em>Mycoderma sp.</em></td>
</tr>
<tr>
<td></td>
<td><em>Saccharomyces carillaginosus</em></td>
</tr>
<tr>
<td></td>
<td><em>Saccharomyces lactis</em></td>
</tr>
<tr>
<td>Yoghurt</td>
<td><em>Lactobacillus delbrueckii ssp. bulgaricus</em></td>
</tr>
<tr>
<td></td>
<td><em>Streptococcus salivarius ssp. thermophilus</em></td>
</tr>
</tbody>
</table>
Fermented Milk Products

milks are the products obtained by souring of milk by use of starter cultures (Refer Table 8.4). The majority of fermented milk products rely on Lactic Acid Bacteria (LAB). The art of fermentation developed long before the science, and fermented milks were produced for thousands of years before Louis Pasteur discovered lactic acid fermentation.

Pasteur’s work enabled the development of pure LAB starter cultures and the industrialization of milk fermentation. LAB includes species belonging to the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Streptococcus*. These bacteria are low G+C Gram-Positives that tolerate acidic conditions, are non-sporing, and are aerotolerant with a strictly fermentative metabolism.

**Table 8.3 Different Fermentation Methods Involved in Making Fermented Milk Products**

<table>
<thead>
<tr>
<th>Category</th>
<th>Typical Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Lactic Fermentations</td>
<td></td>
</tr>
<tr>
<td>Mesophilic</td>
<td>Buttermilk, Cultured buttermilk, Langofil, Tetmjoék, Yrner.</td>
</tr>
<tr>
<td>Thermophilic</td>
<td>Yogurt, laban, zabadi, labneh, skyr, Bulgarian buttermilk.</td>
</tr>
<tr>
<td>Probiotic</td>
<td>Biogarde, Bifighurt, Acidophilus milk, yakult, Cultura-AB.</td>
</tr>
<tr>
<td>II. Yeast-Lactic Fermentations</td>
<td>Kefir, koumiss, acidophilus yeast milk.</td>
</tr>
<tr>
<td>III. Mold-Lactic Fermentations</td>
<td>Viili.</td>
</tr>
</tbody>
</table>

The fermented milks are popular because of following reasons:

- **Preservation**: Fermented milk has higher shelf-life than normal raw milk. This is because fermentation of milk results in a product that is hostile to undesirable bacteria.
- **Organoleptic Properties**: A variety of flavours in fermented milk, refreshing taste, and specific consistency and viscosity favourably influence the consumption of these products.
- **Production of Varieties**: It is possible to make several modifications in fermented milks to have varieties like sweet or sour, salted or spiced, beverage or gel, etc.
- **Ideal for Probiotics**: Fermented milk is an ideal medium for carrying probiotic microorganisms and also makes probiotic and symbiotic foods.
**Table 8.4 A Summary of Fermented Milk with their Starter Fermenting Organisms/Starter Cultures**

<table>
<thead>
<tr>
<th>Fermented Milk Products</th>
<th>Raw Ingredients</th>
<th>Fermenting Organisms/Starter Cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yogurt</td>
<td>Milk, Milk Solids</td>
<td><em>L. delbrueckii subsp. bulgaricus</em>, <em>S. salivarius subsp. Thermophiles</em></td>
</tr>
<tr>
<td>Curd</td>
<td>Milk</td>
<td><em>Lactococcus lactis subsp. lactis, cremoris and diacetylactis</em>, <em>Leuconostocs</em>, <em>Lactobacilli</em>, and <em>Streptococcus thermophilus</em>.</td>
</tr>
<tr>
<td>Kefir</td>
<td>Milk</td>
<td>Two bacteria (<em>Lactobacillus helveticus</em> and <em>Lactococcus lactis</em>) and one yeast (<em>S. cerevisiae</em>) isolated from kefir grains and combined with two yoghurt strains (<em>Lactobacillus delbrueckii subsp. bulgaricus</em>, and <em>Streptococcus thermophilus</em>).</td>
</tr>
<tr>
<td>Koumiss</td>
<td>Raw Mare's Milk</td>
<td><em>L. bulgaricus</em> and <em>Saccharomyces boulardii</em>.</td>
</tr>
<tr>
<td>Cultured Buttermilk</td>
<td>Skim Milk</td>
<td>Normal mesophilic lactic acid bacteria.</td>
</tr>
<tr>
<td>Cheeses (Ripened)</td>
<td>Milk Curd</td>
<td><em>S. salivarius subsp. thermophilus</em> is employed for acid production in cooked curds (up to 60 degree C) because it is more heat tolerant than either of the other more commonly used lactic starters; or a combination of <em>S. salivarius subsp. thermophilus</em> and <em>L. lactis subsp. lactis</em> is employed for curds that receive an intermediate cook.</td>
</tr>
<tr>
<td>Acidophilus Milk</td>
<td>Milk</td>
<td><em>Lactobacillus acidophilus</em>.</td>
</tr>
<tr>
<td>Bifidus Milk</td>
<td>Milk</td>
<td><em>Bifidobacterium bifidum</em>.</td>
</tr>
<tr>
<td>Bulgarian buttermilk</td>
<td>Milk</td>
<td><em>L. delbrueckii subsp. bulgaricus</em>.</td>
</tr>
</tbody>
</table>

**Yogurt (Yoghurt)**

Yoghurt is the most popular and widespread fermented milk product in the United States and is produced commercially and at home with yogurt-making kits.

**Nutritional Value of Yoghurt**

Milk contains well-balanced macronutrients including carbohydrate, fat, and protein and micronutrients including calcium, phosphorus, magnesium, and zinc. Milk proteins have high nutritive value due to the favourable balance of essential amino acids. While fermentation of this milk for making yoghurt, most of these nutrients remain and hence yoghurt has excellent nutritive value. However quality and quantity of nutrients can be affected during fermentation process depending upon fermentation conditions and the type of cultures used. Some of the changes due to fermentation are:

- During fermentation some nutrients like lactose, protein, fat and some vitamins decrease while nutrients like lactic acid, peptides, amino acids, volatile flavor compounds, vitamins, enzymes and bacterial proteins increase.
- Fermentation improves the digestibility of the proteins. It helps in formation of softer curd and its digestibility is facilitated by presence of lactic acid.
- Availability of some minerals like calcium and phosphorous increases in yoghurt mainly because of the lactic acid and their more soluble forms.
• Vitamin content may increase or decrease depends upon the strain of the culture. Some yoghurt cultures are known to synthesize vitamin B2, B12, Folic acid, Niacin, etc. and hence they can improve its nutritive value.

Commercial Production of Yogurt
In commercial production, non-fat or low-fat milk is pasteurized, cooled to 43°C or lower, and inoculated with a 1:1 ratio of Streptococcus salivarius subsp. thermophilus and Lactobacillus delbrueckii subspecies bulgaricus (L. bulgaricus). S. thermophilus grows more rapidly at first and renders the milk anaerobic and weakly acidic L. bulgaricus then acidifies the milk even more. Acting together, the two species ferment almost all of the lactose to lactic acid and flavor the yogurt with diacetyl (S. thermophilus) and acetaldehyde (L. bulgaricus). Freshly prepared yogurt contains about 10⁹ bacteria per gram. Fruits or fruit flavors to be added are pasteurized separately and then combined with the yogurt. It may also contain sugar, corn syrup or glucose syrup in sweetened yoghurt and fruits in fruits yoghurt. It shall be free from vegetable oil/fat, animal body fat, mineral oil and any other substance foreign to milk.

Quality Testing of Yoghurt
Yoghurt is tested in laboratory for various quality aspects. Various set of tests applied to judge the quality are given below:

• Physical Tests: Physical appearance, free whey, presence of gas slits, package conditions, etc.
• Organoleptic Tests: Colour and appearance, body and texture, flavour.
• Chemical Tests: Titratable acidity, pH, presence of flavour compounds, flavour, etc.
• Microbiological Tests: Microscopic examination, starter bacterial count, Coli form count, Yeast-mold count, etc.
• Tests for Specific Properties: If any specific property or function is claimed from the product, a test to justify is required to be done.

Shelf-Life
Shelf-life of a good quality yogurt is 2-3 weeks at refrigeration temperature. The produce is required to be stored in cold conditions and transported and distributed in cold chain, otherwise several defects develop and the shelf-life is reduced.

Defects in Yoghurt
Defects in yoghurt can be classified as:

• Appearance Defects.
• Body and Texture Defects.
• Flavor Defects.
Therapeutic Value of Yoghurt

Yoghurt and related products have been used for health properties since very long time. The normal yoghurt cultures do not survive passage through digestive system and do not implant in intestine and hence it do not give the benefits of ingestion of live probiotics. However, yoghurt cultures do confer several health benefits. Some of the applications of yoghurt for therapeutic benefits are listed below.

- **Acidification Defects.**
- **Storage Related Defects.**

**Used as Therapeutic Agents in Gastro-Intestinal Disorders:** Yoghurt flora is known to inhibit several food spoilage organisms and intestinal pathogens due to presence of several inhibitory substance and low pH. This helps in prevention of infections and control of several gastro-intestinal tract illness including diarrhea and constipation.

**Can be Digested by Lactose Intolerant People:** Yoghurt has lower lactose due to fermentation and it also provides lactase from autolysed bacteria in gut and hence it helps in digestion of lactose.

**May have Hypocholesterolemic Activity:** There are some reports that yoghurt flora can reduce cholesterol in the body by different mechanisms. This can help in reducing the risk of heart diseases.

**Anticarcinogenic Activities:** Yoghurt, especially having probiotic cultures are known to have some tumor inhibiting properties. They also reduce the activity of enzymes that convert pro-carcinogen to carcinogen in gut which reduces the possibility of colon cancer.

**Immunostimulatory Properties:** Increase in specific and non-specific immune functions have been reported by consumption of fermented milks. Some reports on anti-mutagenic activity are also published.

**Other Applications:** Yoghurt and other fermented milk products have been found to be useful in several other health conditions also. However, the therapeutic effect is dependent on the strain of the culture used for the preparation of the product.

**Curd**

Curd is considered the oldest Indian fermented milk product and is equivalent to Western Yogurt. It is believed that curd has valuable therapeutic properties and helps curing gastrointestinal disorders. In the Indian system of medicine curd has been recommended for curing dyspepsia, dysentery, and other intestinal disorders. Curd is prepared and consumed in the household on a day to day. Recently several commercial dairies have come up with production of curd on large scale and are having good market.
Desirable Properties of Curd
A good quality curd made from whole milk has a creamy layer on the top, the rest being made up of a homogenous body of curd and the surface being smooth and glossy, while the cut surface should be firm and free from cracks of gas bubbles. It should have firm, solid body and texture and be uniform with negligible whey separation. Curd should have a pleasing flavour and a clean acid taste, devoid of undesirable flavor.

Nutritional Value of Curd
- Fat ranging from 5 to 8 per cent.
- Protein 3.3 to 3.4 per cent.
- Ash 0.75 to 0.79 per cent.
- Lactic acid .5 to 1.1 per cent.
- Diacetyl is the major flavor compound in curd.

Curd has acidity value between 1-2 % w/v and the pH value lies between 4.4-4.7. The pH of decreases as the microorganisms in starter culture ferment lactose into lactic acid. This results in the coagulation of milk protein casein. The coagulated milk protein is called curd. Curd has soft custard like consistency. Curd consists of two components- curd and whey. Whey is a pale yellow, straw colored liquid which oozes out from the coagulated protein. Whey contains other soluble protein, unfermented lactose. It is also rich in minerals. The other products contribute to flavor and aroma of curd. As the acidity value of curd decreases, its sourness increases. The shelf life of curd is 10-15 days.

Methods of Production of Curd
In the household, milk is first boiled to reduce the normal microflora. This leads to less competition when lactic acid starter culture is added. Cooled to ambient temperature and inoculated with 0.5 – 1 per cent of starter (previous day’s curd or butter milk) and allowed to set overnight. Fermentation is carried out at temperatures of 42 degree C (optimum). Usually the temperature ranges from 40 to 45 degree C. Further fermentation is stopped by cooling to a temperature of 5-7 degree C. It is then stored under refrigeration and consumed. In cooler weather the curd setting vessel is usually wrapped in a woolen cloth to maintain warmth. In shops, the method is more or less the same except that milk is concentrated somewhat before inoculation and the curd is usually set in a shallow circular earthen pot, which helps in the absorption of any whey that may ooze out. Restaurants and sweetmeat shops make curd by the short set method (curd within 4-6 hours). They use inoculum at the rate of 2-4% followed by incubation at 42-45°C till setting of the curd.

Starter Cultures for Curd
The inoculum used to initiate fermentation in milk is called starter. Traditionally, the previous day curd or buttermilk (chhash), containing an unknown mixture of lactic
acid bacteria was used as starter culture. However, to manufacture curd on large scale with predictable uniform quality, it is desirable to use known mixtures of starters.

Curd contains following Lactic Acid Bacteria:

- **Homofermentative**: Produce only lactic acid, for example *Streptococcus thermophilus*, *Enterococcus*, *Pediococcus*, some species of *Lactobacillus* (e.g., *L. lactis*, *L. cremoris*, *L. delbrueckii*, *L. plantarum*).
- **Heterofermentative**: In addition to lactic acid, these bacteria also produce ethanol and carbon dioxide, for example some species of *Lactobacillus* (e.g., *L. bulgaricus*), all species of *Leuconostoc*.

Lactobacilli dominate in sour curd due to their higher acid resistance, while streptococci dominate in sweet curd.

**Quality of Milk as a Raw Material for Curd Making**

The type and quality of milk used for curd making has great influence on the quality of curd as it affects the starter activity as well as sensory characteristics of the curd. The milk should be fresh, clean and free from developed acidity and any off flavours. It should be free from antibiotic residues and inhibitory substances which can adversely affect the starter activity. The chemical composition, status of fat, protein, minerals, enzymes and vitamins, etc. of curd can vary with the type of milk used in its manufacture. Milks of different species of mammals have been used for the production of curd depending on the availability. Milk with abnormal composition like colostrum, late lactation milk and mastitic milk should not be used as it adversely affects the growth of starter culture which subsequently results into poor quality product.

**Modified Curd - Mishti Curd**

Mishti curd is an indigenous sweetened fermented milk product popular in the eastern parts of the India. Mishti curd has creamish to light brown color, firm consistency, smooth texture, and pleasant aroma. Lactobacilli dominate in sour curd due to their higher acid resistance, while streptococci dominate in sweet curd.

**Kefir**

Kefir is a viscous, acidic, and mildly alcoholic (2%) milk beverage produced by Yeast-lactic fermentation of milk. This unique fermented milk originated in the Caucasus Mountains and it is produced east into Mongolia. The Codex Alimentarius description of kefir state it as Starter culture prepared from kefir grains, *Lactobacillus kefir*, and species of the genera *Leuconostoc*, *Lactococcus* and *Acetobacter* growing in a strong specific relationship. Kefir grains constitute both lactose-fermenting yeasts (*Kluyveromyces marxianus*) and non-lactose-fermenting yeasts (*Saccharomyces unisporus*, *Saccharomyces cerevisiae* and *Saccharomyces exiguus*).
Nutritional Value of Kefir as Prescribed by Codex

<table>
<thead>
<tr>
<th>Nutritional Value</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk protein (% w/w)</td>
<td>min. 2.8</td>
</tr>
<tr>
<td>Milk fat (% m/m)</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Titratable acidity, expressed as % of lactic acid</td>
<td>min. 0.6</td>
</tr>
<tr>
<td>Ethanol (% vol. /w)</td>
<td>not stated</td>
</tr>
<tr>
<td>Sum of specific microorganisms constituting the starter culture (CFU/g, in total)</td>
<td>$10^{7}$ (minimum)</td>
</tr>
<tr>
<td>Yeasts (CFU/g)</td>
<td>$10^{4}$ (minimum)</td>
</tr>
</tbody>
</table>

Kefir products tend to be foamy and frothy, due to active CO$_2$ production. The amino acids valine, leucine, lysine and serine are formed during fermentation, while the quantities of alanine and aspartic acid increase as compared to raw milk. Appreciable amounts of pyridoxine, Vitamin B12, folic acid and biotin were synthesized during kefir production, depending on the source of kefir grains used.

Kefir Manufacture

In this fermentation, the kefir grains are used to inoculate the fresh milk and then recovered at the end of the fermentation. Fermentation of the milk proceeds for approximately 24 hours, during which time homofermentative lactic acid streptococci grow rapidly, initially causing a drop in pH. This low pH favours the growth of lactobacilli, but causes the streptococci numbers to decline. The presence of yeasts in the mixture, together with fermentation temperature (21-23°C), encourages the growth of aroma producing heterofermentative streptococci. As fermentation proceeds, growth of lactic acid bacteria is favoured over growth of yeasts and acetic acid bacteria. Originally, kefir was produced in leather sacks hung by the front door during the day, and passersby were expected to push and knead the sack to mix and stimulate the fermentation. Fresh milk could be added occasionally to maintain activity.

Starter consisting of two bacteria (Lactobacillus helveticus and Lactococcus lactis) and one yeasts (S. cerevisiae) isolated from kefir grain and combined with two yoghurt strains (Lactobacillus delbrueckii subsp bulgaricus, and Streptococcus thermophilus).

A commercial kefir is being produced in the United States using a mixture of defined microorganisms rather than kefir grains. This starter culture mixture has been reported to contain Streptococcus lactis, L. plantarum, Streptococcus cremoris, L. casei, Lactococcus lactis subsp Lactis bio var diacetylactis, Leuconostoc cremoris and Saccharomyces florentinus.

Kefir Grains

Kefir grains are coagulated lumps of casein that contain yeasts, lactic acid bacteria, and acetic acid bacteria. Kefir grains resemble small cauliflower florets: they measure 1-3 cm in length, are lobed, irregularly shaped, white to yellow-white in colour,
and have a slimy but firm texture. Grains are kept viable by transferring them daily into fresh milk and allowing them to grow for approximately 20 hours (Refer Table 8.5).

| Table 8.5 Bacteria Found in Kefir Grains and Kefir |
|-----------------|----------------------------------|
| **Lactobacilli** | L. kefir, L. delbrueckii, L. casei, L. parakefir, L. paracasei, L. brevis, L. plantarum, L. fermentum, L. acidophilus, L. kefiranofaciens subsp. kefiranofaciens, L. kefiranofaciens subsp. Kefirgranum. The last two are responsible for the production of kefiran (a water-soluble polysaccharide), which accounts for about 24% of kefir grains. |
| **Lactococci** | L. lactis subsp. lactis, L. lactis subsp. cremoris. |
| **Enterococci** | Enterococcus durans. |
| **Streptococci** | Streptococcus thermophilus. |
| **Leuconostocs** | Leuconostoc mesenteroides. |
| **Acetic Acid Bacteria** | Acetobacter pasteurianus, Acetobacter aceti. |
| **Yeast Species of the Genera** | Candida, Kluyveromyces, Saccharomyces |

**Therapeutic Significance of Kefir**
- It has been proposed that stimulation of the immune system may be one mechanism whereby probiotic bacteria may exert many of their beneficial effects.
- Anti-tumour effects of a water-soluble polysaccharide isolated from kefir grains is reported.
- Some kefir grains have been shown to possess β-galactosidase activity which remains active when consumed and thus can be beneficial for lactose intolerant people.
- Many lactobacilli are capable of producing a wide range of antimicrobial compounds, including organic acids (lactic and acetic acids), carbon dioxide, hydrogen peroxide, ethanol, diacetyl and peptides (bacteriocins) that may be beneficial not only in the reduction of food borne pathogens and spoilage bacteria during food production and storage, but also in the treatment and prevention of gastrointestinal disorders.

**Koumiss**
It is a traditional drink of nomards-cattle-breeders and remains important to the people of the Central Asia. It is also known as ‘Milk Champagne’. The name Koumiss was derived from a tribe called Kumanes, who lived along the river.
Kumane in the Asiatic Steppes. In earlier days, to accelerate the fermentation of Koumiss, pieces of horse flesh or tendon or some vegetable matter were added to the mare’s milk placed into bags made from the skin of lamb presumably to provide microflora needed for fermentation.

Koumiss is similar to kefir except that mare’s milk is used, the culture organisms do not form grains, and the alcohol may reach 2%. Mare’s milk does not coagulate at the isoelectric point of casein and hence koumiss is not considered as a curdled product.

**Nutritive Value of Koumiss**

Koumiss has almost a similar nutritive value as the milk from which it is prepared except that during the making of koumiss, the sugar has been to a large extent replaced by lactic acid, alcohol and carbon dioxide gas. The casein has been partly precipitated in a state of very fine division and partly predigested and dissolved. There was not much change in fat and salts.

**Type of Koumiss**

Based on different concentration of lactic acid and alcohol, three types of koumiss can be prepared.

- **Weak Koumiss**: (0.7% lactic acid, 1.0 % alcohol).
- **Ordinary Koumiss**: (1.1% lactic acid, 1.8% alcohol).
- **Strong Koumiss**: (1.8 % lactic acid, 2.5% alcohol).

In addition to acid and alcohol, carbon dioxide is also produced to impart fizziness to the final product.

**Production of Koumiss**

Koumiss is made by fermenting mare’s milk. Industrial-scale production of koumiss generally uses cow’s milk because of the limited availability of mare’s milk even in the areas of the world where koumiss is popular today. Cow milk is richer in fat and protein but lower in lactose than the milk from a horse. Therefore, the cow’s milk is fortified in one of the several ways. Sucrose may be added, to allow a comparable fermentation. Another technique adds modified whey in order to better approximate the composition of mare’s milk. During fermentation, the lactose in mare’s milk is converted into lactic acid, ethanol, and carbon dioxide, and the milk becomes an accessible source of nutrition for people who are lactose intolerant. One of the essential points in the making of koumiss is that during the whole process of fermentation milk should be kept constantly agitated by stirring. This helps in incorporation of oxygen to the fermenting fluid. It also causes breaking up of the precipitated casein into very fine particles. This contributes to ease of digestibility of koumiss.

**Starter Cultures**

It mainly comprised of *L. bulgaricus* and *Saccharomyces lactis*. Various strains of lactic acid bacteria and yeasts have been isolated from commercial koumiss.
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viz., *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei* subsp. *tolerans*, *Candida kefir* and *Kluyveromyces marxianus* subsp. *lactis*. It is also possible to find lactic streptococci, coliforms and some spore forming bacilli in koumiss.

**Therapeutic Advantages of Koumiss**

The acid and alcohol produced help in the process of digestion and ease of absorption of fat and minerals. Nutritive and stimulating qualities of Koumiss render it very valuable for nursing females. Koumiss is considered to be a refreshing therapeutic drink. In USSR, this preparation is used for the treatment of pulmonary tuberculosis. Koumiss possesses diuretic properties and helps in restraining intestinal putrefaction. Koumiss has been and is employed with success in diabetes.

**Butter**

In the case of butter, where cream is inoculated, the acidified cream is then churned to yield butter, which is washed, salted, and packaged.

**Nutritional Value of Butter**

Butter contains around 15% water, 81% fat, and generally less than 0.5% carbohydrate and protein.

**Spoilage of Butter**

Although it is not a highly perishable product, it does undergo spoilage by bacteria and molds. The main source of microorganisms for butter is cream, whether sweet or sour, pasteurized or non-pasteurized. The biota of whole milk may be expected to be found in cream because as the fat droplets rise to the surface of milk, they carry up microorganisms. The processing of both raw and pasteurized creams to yield butter brings about a reduction in the numbers of all microorganisms.

**Bacterial Spoilage**

- **Surface Taint or Putridity:** This condition is caused by *Pseudomonas putrefaciens* as a result of its growth on the surface of finished butter. It develops at temperatures within the range 4–7 degree C and may become apparent within 7–10 days. The odor of this condition is apparently due to certain organic acids, especially isovaleric acid. Surface taint along with an apple odor is caused also by *Chryseobacterium joostei*.

- **Rancidity:** This condition is caused by the hydrolysis of butterfat with the liberation of free fatty acids. The causative organism is *Pseudomonas fragi*, although *P. fluorescens* is sometimes found.

- **Other Less Common Spoilage Conditions in Butter:**
  - **Malty Flavor** is reported to be due to the growth of *Lactococcus lactis* var. *maltigenes*.
  - **Skunk like Odor** is reported to be caused by *Pseudomonas mephitica*.
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- **Black Discolorations** of butter have been reported to be caused by *Pseudomonas nigrifaciens*.

**Fungal Spoilage of Butter**

The generally high lipid content and low water content make butter more susceptible to spoilage by molds than by bacteria.

- Butter undergoes fungal spoilage rather commonly by species of *Cladosporium, Alternaria, Aspergillus, Mucor, Rhizopus, Penicillium*, and *Geotrichum*, especially *G. candidum* (*Oospora lactis*). These organisms can be seen growing on the surface of butter, where they produce colorations referable to their particular spore colors.
- Black yeasts of the genus *Torula* also have been reported to cause discolorations on butter.

**Butter Milk/ Cultured Butter Milk**

Buttermilk, as the name suggests, is the milk that remains after cream is churned for the production of butter. True buttermilk is the fluid remaining after cream is churned into butter. If butter is made from sweet cream, its buttermilk has approximately the same composition as skim milk.

**Production of Cultured Butter Milk**

Production of cultured butter milk on industrial scale involves selection of good quality raw milk, starter cultures and optimized process of fermentation, packaging and storage.

- **Starter Culture:** Cultured butter milk is prepared with the help of normal mesophilic lactic acid bacteria. There should be satisfactory balance between acid and flavor producers in the starter.
- **Selection of Milk:** The quality of raw material decides the quality of final product. The raw milk selected for Cultured Butter Milk manufacture should have normal composition, be free from off flavor and odours and free from inhibitory substances. It should have lower microbial count.

The commercial product is usually prepared by inoculating skim milk with a lactic or buttermilk starter culture and holding until souring occurs. The resulting curd is broken up into fine particles by agitation, and this product is termed cultured buttermilk.

This product has most of the fermented milk solids except fat which goes in butter. It also has mixed lactic acid bacteria, especially *Lactococci* and *Leuconostoc*, which gives it a desirable flavor and aroma, typical diacetyl flavour.

**Characteristics of Good Quality Cultured Butter Milk**

A good quality butter milk, after packaging has a pH 4.5 and possess smooth viscous body giving a slow even flow when poured. The flavor should be clean acid with an integrated aromatic diacetyl and free volatile acid background. It
Fermented Milk Products

exhibits no free whey or whey separation. The keeping quality of good buttermilk at 5°C is approximately 2 weeks.

Cheese

About 2,000 distinct varieties of cheese are produced throughout the world, representing approximately 20 general types.

Classification of Cheese

Often cheeses are classified based on texture or hardness as soft cheeses (cottage, cream, Brie), semisoft cheeses (Muenster, Limburger, blue), hard cheeses (cheddar, Colby, Swiss), or very hard cheeses (Parmesan). The final hardness of the cheese is partially a function of the length of ripening. Soft cheeses are ripened for only about 1 to 5 months, whereas hard cheeses need 3 to 12 months, and very hard cheeses like Parmesan require 12 to 16 months ripening.

**Table 8.6 Classification of Cheese Based on Texture or Hardness**

<table>
<thead>
<tr>
<th>Cheese (Country of Origin)</th>
<th>Contributing Microorganism</th>
<th>Earlier Stages of Production</th>
<th>Later Stages of Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft, Unripened</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cottage Cheese</td>
<td>Lactococcus lactis</td>
<td>Lactococcus cremoris</td>
<td></td>
</tr>
<tr>
<td>Cream</td>
<td>L. cremoris,</td>
<td>L. diacetylactis,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Streptococcus thermophilus,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mozzarella (Italy)</td>
<td>L. thermophilus,</td>
<td>L. diacetylactis,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L. bulgaricus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semi-Hard</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese (Country of Origin)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soft, Ripened</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limburger (France)</td>
<td>Bacterial ripened (1-5 months)</td>
<td>Lactococcus lactis,</td>
<td>Penicillium camemberti,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. cremoris</td>
<td>Brevibacterium linens</td>
</tr>
<tr>
<td>Camembert (France)</td>
<td>Mold-ripened (1-3 months)</td>
<td>L. lactis, L. cremoris</td>
<td>Penicillium camemberti,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Brevibacterium linens</td>
</tr>
<tr>
<td>Semi-Hard</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue (France)</td>
<td>Mold-ripened (1-2 months)</td>
<td>Lactococcus lactis,</td>
<td>Penicillium camemberti,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. cremoris</td>
<td>Brevibacterium linens</td>
</tr>
<tr>
<td>Brick, Muenster (United States)</td>
<td>Mold-ripened (2-12 months)</td>
<td>L. lactis, L. cremoris</td>
<td>B. linens</td>
</tr>
<tr>
<td>Limburger (Belgium)</td>
<td>Bacterial ripened (1-7 months)</td>
<td>L. lactis, L. cremoris</td>
<td>Penicillium camemberti,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. linens</td>
<td>Brevibacterium linens</td>
</tr>
<tr>
<td>Gouda</td>
<td>Bacterial ripened (1-2 months)</td>
<td>L. lactis, L. cremoris</td>
<td>Penicillium camemberti,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. linens</td>
<td>Brevibacterium linens</td>
</tr>
<tr>
<td>Hard, Ripened</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheddar, Colby (Britain)</td>
<td>Bacterial ripened (2-16 months)</td>
<td>Lactococcus lactis,</td>
<td>Lactobacillus casei,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. cremoris</td>
<td>L. plantarum</td>
</tr>
<tr>
<td>Swiss Provolone (Switzerland)</td>
<td>Bacterial ripened (2-16 months)</td>
<td>L. lactis, L. helveticus,</td>
<td>Propionibacterium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. thermophilus</td>
<td>thermophilus,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P. freudenreichii</td>
</tr>
<tr>
<td>Very Hard, Ripened</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parmesan (Italy)</td>
<td>Bacterial ripened (12-16 months)</td>
<td>Lactococcus lactis,</td>
<td>Lactobacillus bulgaricus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. cremoris</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. thermophilus</td>
<td></td>
</tr>
</tbody>
</table>
Peculiar Characteristics of some Cheeses

Although most ripened cheeses are the product of metabolic activities of the lactic acid bacteria, several well-known cheeses owe their particular character to other related organisms (Refer Figure 8.1):

- In the case of Swiss cheese, a mixed culture of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* is usually employed along with a culture of *Propionibacterium shermanii* or *P. freudenreichii* added to function during the ripening process in flavor development and eye formation.

- In some cases, molds are used to further enhance the cheese. Obvious examples are Roquefort and blue cheese. For these cheeses, *Penicillium roqueforti* spores are added to the curds just before the final cheese processing, which effect ripening and impart the blue-veined appearance characteristic of this type of cheese.

- Sometimes the surface of an already formed cheese is inoculated at the start of ripening; for example, Camembert cheese is inoculated with spores of *Penicillium camemberti*.

- Similarly, two Coryneform bacteria of the genu *Brachybacterium* have been recovered from the surfaces of French Gruyère and Beaufort cheeses.

- In a study of *L. monocytogenes* in European red smear cheese (soft, semisoft, and hard), 5.8% of 329 test samples contained *Listeria sp.* with 6.4% being *L. monocytogenes* and 10.6% *L. innocua.*

(a)  (b)  
(c)  (d)
In the above Figure 8.1 different types of cheese are shown in which, (a) Gouda (top left) and cheddar cheese (lower right). Note the typical indentations on the surface of Gouda caused by the cheese cloth and red wax covering; (b) Brie (left) and Limburger (right) cheeses are soft, ripened cheeses. Ripening results from the surface growth of microorganisms like Penicillium camemberti (Brie) and Brevibacterium linens (Limburger); (c) Roquefort cheese crumbled for use in salad dressing. The dark areas are the result of extensive Penicillium growth; (d) Cottage cheese and cream cheese (spread on crackers) are soft, unripened cheeses. They are sold immediately after production; (e) Swiss cheese, a hard, ripened cheese, contains holes formed by carbon dioxide from a Propionibacterium fermentation.

Production of Cheese

All cheeses result from a lactic fermentation of milk, which results in coagulation of milk proteins and formation of a curd. Rennin, an enzyme from calf stomachs, or by genetically engineered microorganisms, can also be used to promote curd formation. After the curd is formed, it is heated and pressed to remove the watery part of the milk (called the whey), salted, and then usually ripened. The cheese curd can be packaged for ripening with or without additional microorganisms.

Starter: The starter for cheese production may differ depending on the amount of heat applied to the curds. *S. salivarius* subsp. *thermophilus* is employed for acid production in cooked curds (up to 60 degree C) because it is more heat tolerant than either of the other more commonly used lactic starters; or a combination of *S. salivarius* subsp. *thermophilus* and *L. lactis* subsp. *lactis* is employed for curds that receive an intermediate cook.

*Lactococcus lactis* is used as a starter culture for a number of cheeses including Gouda and chedda. Starter culture density is often over $10^9$ Colony Forming Units (CFUs) per gram of cheese before ripening. However, the high salt, low pH, and the temperatures that characterize the cheese microenvironment reduce these numbers rather quickly. This enables other bacteria, sometimes called Nonstarter Lactic Acid Bacteria (NSLAB) to grow; their numbers can reach $10^7$ to $10^9$ CFUs/g after several months of aging. Thus both starter and nonstarter LAB contribute to the final taste, texture, odor, and appearance of the cheese.
Spoilage of Cheese

The low moisture content of hard and semi-hard ripened cheeses makes them insusceptible to spoilage by most organisms, although molds can grow on these products. Some ripened cheeses have sufficiently low oxidation-reduction potentials to support the growth of anaerobes. It is not surprising to find that anaerobic bacteria sometimes cause the spoilage of these products when a\textsubscript{w} (water activity) permits growth to occur. *Clostridium pasteurianum*, *C. butyricum*, *C. sporogenes*, and *C. tyrobutyricum*, have been reported to cause late gassiness of cheeses. One of these (*C. tyrobutyricum*) is well established as the cause of butyric acid fermentation or the late-blowing defect in cheeses, such as Gouda and Emmentaler.

Acidophilus Milk

Acidophilus milk is a sour product produced by the inoculation of an intestinal implantable strain of *Lactobacillus acidophilus* into sterile skim milk. The inoculum of 1–2% is added, followed by holding the product at 37 degree C until a smooth curd develops. The numbers of *L. acidophilus* should be in the $10^7$–$10^8$/ml range.

This acidophilus milk is considered as a probiotic. After taking a treatment based on antibiotics, the intestinal bacteria is damaged and in order to restore the normal balance, eating acidophilus-rich foods or taking acidophilus milk will help repopulate the intestines with the right kind of microorganisms. *L. acidophilus* may modify the microbial flora in the lower intestine, thus improving general health. The benefits of consuming fermented milks may involve minimizing lactose intolerance, lowering serum cholesterol, and possibly exhibiting anticancer activity. Several lactobacilli have antitumor compounds in their cell walls. Such findings suggest that diets including lactic acid bacteria, especially *L. acidophilus*, may contribute to the prevention of colon cancer. Acidophilus milk differs from Indian curd or curd in body, texture, consistency, flavour, chemical composition and in antibacterial activities.

Sweet Acidophilus Milk

As natural fermented acidophilus milk was sour and having medicinal type of flavor, this gave birth to sweet acidophilus milk. It is probiotic dairy product based on unfermented milk. It is produced by adding concentrated probiotic bacteria to intensively heat treated and chilled milk. Heat treatment is necessary to achieve sufficient microbiological stability during storage of the final product. In some cases it is also prepared by adding large numbers of viable *L. acidophilus* cells as frozen concentrates in chilled pasteurized milk. As long as the milk remains under refrigeration, the organisms do not grow, but when it is drunk, the consumer gets the benefit of viable *L. acidophilus* cells. It is ‘sweet’ because it lacks the tartness of traditional acidophilus milk.

It has been reported to prevent symptoms of lactose intolerance (lactose malabsorption, intestinal hypolactemia). When lactose malabsorbers consume
certain quantities of milk they immediately experience flatulence and diarrhea. The condition is due to the absence or reduced amounts of intestinal lactase, and this allows the bacteria in the colon to utilize lactose with the production of gases.

**Bifidus Milk**

The product is named accordingly to the bacteria used in fermentation, i.e., *bifidobacteria*. The genus *Bifidobacterium* contains irregular, non-sporing, Gram-Positive rods that may be club-shaped or forked at the end (Refer Figure 8.2). *Bifidobacteria* are non-motile, anaerobic, and ferment lactose and other sugars to acetic and lactic acids. *Bifidobacteria* sp. are the predominant intestinal flora and the major components of large intestine of human adults and it is considered to be a probiotic strain with beneficial effects. The inflammation in the colon can be reduced by taking milk supplemented with *Bifidobacterium bifidum*.

*Fig. 8.2 Bifidobacteria*

*Fig. 8.3 Some Bifid-Amended Dairy Products*
**Health Benefits of Bifidobacterium**

- **Act as Probiotic:** *Bifidobacteria* are thought to help maintain the normal intestinal balance, while improving lactose tolerance; The inflammation in the colon can be reduced by taking milk supplemented with *Bifidobacterium bifidum*, decreases the development of harmful bacteria, and encourage the production of organic composites, such as lactic acid, acetic acid and hydrogen peroxide leading to a healthy function of the lower intestine.

- **It has also been suggested that bifidobacteria reduce or prevent the excretion of rotaviruses, a cause of diarrhea among children.**

- **Intake of Calcium:** *Bifidobacterium* promote calcium absorption and the synthesis of B-complex vitamins.

- **Increases Immunity:** Taking *Bifidobacterium bifidum* in regular diet can boost immunity. Histamine discharge is prevented by *Bifidobacterium* as it encourages a proper digestion. Additionally, the organism’s immune response is strengthened by *Bifidobacterium*. These reasons should make you think of taking *Bifidobacterium* supplements in order to prevent intestinal pathogens, digestive disorders and histamine production.

- **Prevents Cancer:** Scientists believe that *Bifidobacterium* may stop the multiplication of cancerous cells in the body as harmful bacteria found in the gut can produce tumors. Bifidum may stop normal cells to become cancerous and protecting the colon. Moreover, *bifidobacterium* can also be used in abdominal cramps and diarrhea treatments.

**Milk Based Acidophilus/Bifidus Products**

Milk based acidophilus and bifidus products are listed in Table 8.7.

<table>
<thead>
<tr>
<th>Name</th>
<th>Physical Type</th>
<th>Country of Origin</th>
<th>Microflora</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermented Acidophilus Gel</td>
<td>Most countries</td>
<td>Lactobacillus acidophilus</td>
<td></td>
</tr>
<tr>
<td>Sweet Acidophilus Liquid</td>
<td>Denmark</td>
<td>Lactobacillus acidophilus</td>
<td></td>
</tr>
<tr>
<td>Culture Liquid</td>
<td>Denmark</td>
<td>L. acidophilus + B. bifidum</td>
<td></td>
</tr>
<tr>
<td>AB – Yoghurt Gel</td>
<td>---</td>
<td>L. acidophilus + B. bifidum + Yoghurt culture</td>
<td></td>
</tr>
<tr>
<td>Biograde Liquid</td>
<td>---</td>
<td>L. acidophilus + B. bifidum + S. thermophilus</td>
<td></td>
</tr>
<tr>
<td>Big M (Cheese Whey) Liquid FRG</td>
<td>---</td>
<td>L. acidophilus + S. thermophilus + B. bifidum</td>
<td></td>
</tr>
<tr>
<td>Mil – Mil E Frozen</td>
<td>---</td>
<td>L. acidophilus + B. bifidum + Yoghurt culture</td>
<td></td>
</tr>
</tbody>
</table>

**Therapeutic Acidophilus/Bifidus Products used as Probiotics in Market**

- **L. casei Shirota-Yakult.**
- **L. acidophilus La 5-Chr. Hansen.**
- L. acidophilus NCFM-Rhodia.
- L. acidophilus Johnsonii Lai-Nestle.

**Bulgarian Buttermilk**

Bulgarian buttermilk is produced in a similar manner by the use of *L. bulgaricus* as the inoculum or starter, but unlike *L. acidophilus*, *L. bulgaricus* is not implantable in the human intestines.

**Kishk**

Kishk is a typical wheat milk mixture fermented food popular in Egypt and most of the Arab World. It consists of small, round or irregular pieces, yellowish brown in colour, which have a rough surface and hard texture. Kishk is rich in nutritive constituents, and is a possible source of many vitamins and growth factors associated with the microbial fermentative processes. It is of good keeping quality and consumed throughout the year.

**Micro Flora in Kishk**

The most predominant microflora, which are responsible for fermentation are lactic acid bacteria, homo and hetero fermentative lactobacilli, mainly *L. plantarum*, *L. casei* and *L. brevis*.

**Manufacture of Kishk**

Kishk preparation involves three main stages, i.e., as follows:

**Preparation of Par-Boiled Wheat**: Wheat grains, are placed in large cooking pan, covered with water and heated slowly to boiling and simmered until soft. The cooked wheat or *helila* is then washed with cold water, spread on straw mats and left to dry in air. The dry material which is hard in texture is coarsely ground in stone mills, then sieved and seed coats are removed.

**Preparation of Laban Zeer**: Laban-zeer is sour coagulated milk. Traditionally, the milk was churned in skin bag and left out fluid is called as sour milk. In hot weather, milk coagulates spontaneously and is then often considered unsuitable for cheese making. Such sour milk is stored in earthenware containers known as *zeer*. A suitable quantity of salt judged by taste is added and the contents of the *zeer* are mixed. The whey oozes through the walls and thus consistency of sour milk becomes considerably thicker. Accumulated laban zeer is especially used in summer, when wheat is plentiful in the farmers store for making kishk.

**Preparation of Hamma (Kishk Mix)**: The coarser power of the par boiled wheat is placed in large pots and moistened with slightly salted boiling water. Then raw milk or laban zeer diluted with water or milk with syrupy consistency, is added in portions

After 24h, the *hamma*, which has meanwhile increased in volume as a result of fermentation, is kneaded by hand. Later on Laban zeer, twice the volume added before is diluted with water or milk with syrupy consistency, is added in portions
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to the hamma and left for a further 24h. Subsequently, the fermented mix is thoroughly remixed, cut in small balls and placed on straw mats to dry in the open. The dried product is Kishk. Sometimes spices like cumin or pepper are added for enhancing taste. Complete drying of product takes 5-7 days and during this period also fermentation goes on gradually at reducing rates.

Advantages of Kishk Fermentation

- Higher protein content. Net protein utilization is comparable with casein (60 for casein, 59 for Kishk).
- Contains fair amounts of iron, phosphorous, calcium.
- Contains higher amounts amino acid, such as phenylalanine, threonine, isoleucine, leucine, arginine, valine, tyrosine, lysine; while low amount of tryptophane and sulphur containing amino acids.
- Has higher digestibility.
- Wheat is relatively good source of iron while milk is deficient in iron. The complementary effect makes the product nutritionally sound.
- Excellent shelf-life of more than one year at room temperature.
- Convenient to use. It can be taken as breakfast food along with milk or used for preparation of vegetable curries for lunch or dinner.

Yakult

Yakult is a probiotic fermented milk product which originated from Japan almost 70 years before. It is made with the help of a culture of *Lactobacillus casei* strain Shirota, which is named after the inventor Prof. Minoru Shirota. The product is most widely studied for clinical benefits and it is claimed that the product aids digestion, modulates immunity and prevents infection. Yakult is originally made from skim milk and sweetener agents like sucrose, glucose, liquid sugar and starch syrup. It may contain fruit juices and flavouring essences.

The Average Composition of Yakult

- Fat: 1.1%
- Protein: 1.2%
- Lactose: 1.1%
- Other Sugar: 14.1%
- Ash: 0.34%

It has a junket like consistency. Colour is slightly brown, which indicates that the milk/sugar base may be highly heat treated. Now it is available in different flavours blended with different fruits juices. The product is proprietary item of M/s Yakult Hansa Ltd. in Japan and is being consumed now by 25 million people.
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**Villi**

Mold-lactic fermentation results in a unique Finnish fermented milk product made from pasteurized non-homogenized milk called villi. The milk is placed in a cup and inoculated with a mixture of the fungus *Geotrichum candidum* and lactic acid bacteria. The cream rises to the surface and after incubation at 18 to 20 degree C for 24 hours, lactic acid reaches a concentration of 0.9%. The fungus forms a velvety layer across the top of the final product, which also can be made with a bottom fruit layer.

**Starters for Villi**

*Lactic streptococci, Lactococcus lactis subsp. lactis, L. lactis subsp. cremoris, Mold Geotrichium candidum.* *Geotrichum candidum* has different metabolic activity and is symbiotic with lactic streptococci. It assimilates glucose and galactose but not lactose and sucrose, while assimilating sugars in villi, the mold consumes oxygen from air space of air tight cup and produce CO₂. When all O₂ is consumed its growth is restricted. So much CO₂ is produced by mold that it is partially dissolved in the milk to form carbonic acid and carbonates, and as a consequence of it, slight under pressure is generated in the cup. The mold also shows lipolytic activity, while growing on the surface layer, the concentration of monoglycerides and free fatty acids increases and gives typical flavor to the product.

**Evolution of Quality Milk**

Milk quality plays a key role not only for its wide consumption but also because milk is involved in a large series of derived products, such as yogurt, butter, cheese, and ice cream. Milk is the excellent medium for the growth of microorganisms. The growth of these microorganisms has to be controlled because if milk is allowed to stand at room temperature, normal microflora will grow and cause spoilage. Spoilage includes curdling of milk, change in color of milk, milk becoming slimy, or ropy in nature. The growth of normal microflora should be therefore, inhibited to prevent spoilage and transmission of disease.

**Some of the Preventive Measures Include**

- High temperature measures, such as boiling, pasteurization leads to the destruction of disease causing microorganism and also prevent spoilage.
- Low temporary measures, such as a refrigeration do not allow normal microflora to flourish rapidly.
- Aseptic packaging keeps out undesirable microorganisms.

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[564x283]NOTES

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Pasteurization

Pasteurization was given by Louis Pasteur in 1864. It is used to kill harmful pathogenic bacteria resent in milk. Pasteurization reduces the natural microflora of milk. It helps to kill some pathogens in milk, for example *Mycobacterium tuberculosis*, the Q fever agent, *Coxiella burnetii*.

There are Different Types of Pasteurization

- **Low Temperature Long Time Method (LTLT):** This is referred to as the normal holding method. In this milk is pasteurized at 62.8 °C for 30 minutes.
- **High Temperature Short Time Method (HTST):** This is referred to as the flash method. In this milk is pasteurized at 72 °C for 15 seconds, which completely kills pathogenic bacteria in milk, rendering it safe to drink for up to three weeks if continually refrigerated. Dairies print best before dates on each container, after which stores remove any unsold milk from their shelves.
- **Ultra Heat Treatment Method (UHT):** Ultra High Temperature is another thermal temperature. It is achieved by heating at temperatures of 135-140 degree C for a few second.

UHT is a type of milk processing where all bacteria are destroyed with high heat to extend its shelf life for up to 6 months, as long as the package is not opened. Milk is firstly homogenized and then is heated to 138 degree C for 1–3 seconds. The milk is immediately cooled down and packed into a sterile container. As a result of this treatment, all the pathogenic bacteria within the milk are destroyed, unlike when the milk is just pasteurised. The milk will now keep for up for 6 months if unopened. UHT milk does not need to be refrigerated until the package is opened, which makes it easier to ship and store. But in this process there is a loss of vitamin B1 and vitamin C and there is also a slight change in the taste of the milk.

Disadvantages

- Survival of heat-resistant proteinases and lipases produced during growth of psychrotrophic bacteria, such as *Pseudomonas*, *Alcaligenes* and *Flavobacterium* in the raw milk. Some of these enzymes have much greater heat resistance than highly heat-resistant spores. For example, proteinases of fluorescent pseudomonads had residual activities ranging from 14 to 51% at 140 degree C for 5s. Quite low levels of residual proteinase can cause a reduction of shelf-life of UHT milk by causing bitterness and gelation.
- A side effect of the heating of pasteurization is that some vitamin and mineral content is lost. Soluble calcium and phosphorus decrease by 5%, thiamin and vitamin B12 by 10%, and vitamin C by 20%.
Filtration

- Microfiltration is a process that partially replaces pasteurization and produces milk with fewer microorganisms and longer shelf life without a change in the taste of the milk. In this process, cream is separated from the skimmed milk and is pasteurized in the usual way, but the skimmed milk is forced through ceramic microfilters that trap 99.9% of microorganisms in the milk (as compared to 99.999% killing of microorganisms in standard HTST pasteurization). The skimmed milk then is recombined with the pasteurized cream to reconstitute the original milk composition.

- Ultrafiltration uses finer filters than microfiltration, which allow lactose and water to pass through while retaining fats, calcium and protein. As with microfiltration, the fat may be removed before filtration and added back in afterwards. Ultrafiltered milk is used in cheese making, since it has reduced volume for a given protein content, and is sold directly to consumers as a higher protein, lower sugar content, and creamier alternative to regular milk.

Check Your Progress

1. Why are fermented milks popular?
2. What is the raw ingredient and fermenting organisms of Kefir?
3. Give the various set of tests applied to judge the quality of Yoghurt.
4. How is defect in yoghurt classified?
5. List the applications of yoghurt for therapeutic benefits.
6. Write in brief about curd.
7. Give the nutritional value of curd.
8. Name the type of Koumiss.
9. Define the term buttermilk.

8.3 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. The fermented milks are popular because of following reasons:
   - Preservation: Fermented milk has higher shelf-life than normal raw milk. This is because fermentation of milk results in a product that is hostile to undesirable bacteria.
   - Organoleptic Properties: A variety of flavours in fermented milk, refreshing taste, and specific consistency and viscosity favourably influence the consumption of these products.
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- Production of varieties: It is possible to make several modifications in fermented milks to have varieties like sweet or sour, salted or spiced, beverage or gel, etc.
- Ideal for Probiotics: Fermented milk is an ideal medium for carrying probiotic microorganisms and also makes probiotic and symbiotic foods.

2. Kefir: Raw Ingredient is milk and fermenting organism/starter cultures are two bacteria (Lactobacillus helveticus and Lactococcus lactis) and one yeasts (S. cerevisiae) isolated from kefir grain and combined with two yoghurt strains (Lactobacillus delbrueckii subsp bulgaricus, and Streptococcus thermophilus).

3. Various set of tests applied to judge the quality of Yoghurt are given below:
   - Physical Tests: Physical appearance, free whey, presence of gas slits, package conditions, etc.
   - Organoleptic Tests: Colour and appearance, body and texture, flavour.
   - Chemical Tests: Titratable acidity, pH, presence of flavour compounds, flavour, etc.
   - Microbiological Tests: Microscopic examination, starter bacterial count, coli form count, Yeast-mold count, etc.
   - Tests for Specific Properties: If any specific property or function is claimed from the product, a test to justify is required to be done.

4. Defects in yoghurt can be classified as:
   - Appearance Defects
   - Body and Texture Defects
   - Flavor Defects
   - Acidification Defects
   - Storage Related Defects

5. Some of the applications of yoghurt for therapeutic benefits are listed below:
   - Used as Therapeutic Agents in Gastro-Intestinal Disorders: Yoghurt flora is known to inhibit several food spoilage organisms and intestinal pathogens due to presence of several inhibitory substance and low pH. This helps in prevention of infections and control of several gastro-intestinal tract illness including diarrhea and constipation.
   - Can be digested by Lactose Intolerant People: Yoghurt has lower lactose due to fermentation and it also provides lactase from autolysed bacteria in gut and hence it helps in digestion of lactose.
May have Hypocholesterolemic Activity: There are some reports that yoghurt flora can reduce cholesterol in the body by different mechanisms. This can help in reducing the risk of heart diseases.

Anticarcinogenic Activities: Yoghurt, especially having probiotic cultures are known to have some tumor inhibiting properties. They also reduce the activity of enzymes that convert pro-carcinogen to carcinogen in gut which reduces the possibility of colon cancer.

6. Curd is considered the oldest Indian fermented milk product and is equivalent to Western Yoghurt. It is believed that curd has valuable therapeutic properties and helps curing gastrointestinal disorders. In the Indian system of medicine curd has been recommended for curing dyspepsia, dysentery, and other intestinal disorders. Curd is prepared and consumed in the household on a day to day. Recently several commercial dairies have come up with production of curd on large scale and are having good market.

7. Nutritional value of curd is as follows:
   - Fat ranging from 5 to 8 per cent.
   - Protein 3.3 to 3.4 per cent.
   - Ash 0.75 to 0.79 per cent.
   - Lactic acid .5 to 1.1 per cent.
   - Diacetyl is the major flavor compound in curd.

8. Based on different concentration of lactic acid and alcohol, three types of koummiss can be prepared.
   - Weak Koummiss: (0.7% Lactic Acid, 1.0 % Alcohol).
   - Ordinary Koummiss: (1.1% Lactic Acid, 1.8% Alcohol).
   - Strong Koummiss: (1.8 % Lactic Acid, 2.5% Alcohol).

9. Buttermilk is the milk that remains after cream is churned for the production of butter.

**8.4 SUMMARY**

- Milk is a highly unstable food as it is a very good medium for the growth of many microorganisms.
- Fermentation of milk primarily aims at preserving the same by converting into more stable, nutritious and desirable products such as cheese, yoghurt, butter milk and butter.
- The microorganisms used in the industrial scale of fermentation of cow’s milk include mainly lactic acid producing bacteria such as the mesophilic.
Fermented Milk Products

Lactococcus and Leuconostoc species with optimum growth temperatures in the range of 20 to 30°C and thermophilic Lactobacillus and Streptococcus species used at temperatures up to 45°C.

- Milk from cows, sheep, goats and other mammals may be used directly for fermentation or after preliminary treatment such as pasteurization, ultrafiltration or lactose hydrolysis.
- The fermentation may be initiated spontaneously by native microflora or by addition of specific starter cultures or material from previous fermentation.
- The fermented milk may undergo further processing to yield a more concentrated product or may be blended with other ingredients such as salts, fruits, herbs, spices, sugars and natural colourants to change flavour, appearance and texture of such products.
- Microbial growth, either of natural or inoculated populations, causes chemical and/or textural changes to form a product that can be stored for extended periods.
- The fermentation process also is used to create new, pleasing food flavors and odors.
- Pasteur’s work enabled the development of pure LAB starter cultures and the industrialization of milk fermentation.
- LAB includes species belonging to the genera Lactobacillus, Lactococcus, Leuconostoc, and Streptococcus. These bacteria are low G +C Gram-Positives that tolerate acidic conditions, are non-sporing, and are aerotolerant with a strictly fermentative metabolism.
- Fermented milk has higher shelf-life than normal raw milk. This is because fermentation of milk results in a product that is hostile to undesirable bacteria.
- A variety of flavors in fermented milk, refreshing taste, and specific consistency and viscosity favourably influence the consumption of these products.
- Fermented milk is an ideal medium for carrying probiotic microorganisms and also makes probiotic and symbiotic foods.
- Yoghurt is the most popular and widespread fermented milk product in the United States and is produced commercially and at home with yogurt-making kits.
- Milk contains well-balanced macronutrients including carbohydrate, fat, and protein and micronutrients including calcium, phosphorus, magnesium, and zinc.
- Fermentation improves the digestibility of the proteins. It helps in formation of softer curd and its digestibility is facilitated by presence of lactic acid.
• Yoghurt and related products have been used for health properties since very longtime.
• The normal yoghurt cultures do not survive passage through digestive system and do not implant in intestine and hence it do not give the benefits of ingestion of live probiotics.
• Curd is considered the oldest Indian fermented milk product and is equivalent to Western Yogurt. It is believed that curd has valuable therapeutic properties and helps curing gastrointestinal disorders.
• A good quality curd made from whole milk has a creamy layer on the top, the rest being made up of a homogenous body of curd and the surface being smooth and glossy, while the cut surface should be firm and free from cracks of gas bubbles.
• The milk should be fresh, clean and free from developed acidity and any off flavours. It should be free from antibiotic residues and inhibitory substances which can adversely affect the starter activity.
• The chemical composition, status of fat, protein, minerals, enzymes and vitamins, etc. of curd can vary with the type of milk used in its manufacture.
• Milks of different species of mammals have been used for the production of curd depending on the availability.
• Milk with abnormal composition like colostrum, late lactation milk and mastitic milk should not be used as it adversely affects the growth of starter culture which subsequently results into poor quality product.
• Mishti curd is an indigenous sweetened fermented milk product popular in the eastern parts of the India.
• Mishti curd has creamish to light brown colour, firm consistency, smooth texture, and pleasant aroma.
• Lactobacilli dominate in sour curd due to their higher acid resistance, while streptococci dominate in sweet curd.
• Kefir is a viscous, acidic, and mildly alcoholic (2%) milk beverage produced by Yeast-lactic fermentation of milk. This unique fermented milk originated in the Caucasus Mountains and it is produced east into Mongolia.
• Kafir grains are coagulated lumps of casein that contain yeasts, lactic acid bacteria, and acetic acid bacteria.
• Kefir grains resemble small cauliflower florets: they measure 1-3 cm in length, are lobed, irregularly shaped, white to yellow-white in colour, and have a slimy but firm texture.
• Koumiss is similar to kefir except that mare’s milk is used, the culture organisms do not form grains, and the alcohol may reach 2%.
• Mare’s milk does not coagulate at the isoelectric point of casein and hence koumiss is not considered as a curdled product.
• Buttermilk, as the name suggests, is the milk that remains after cream is churned for the production of butter.
• True buttermilk is the fluid remaining after cream is churned into butter.
• Acidophilus milk is a sour product produced by the inoculation of an intestinal implantable strain of Lactobacillus acidophilus into sterile skim milk.
• Kishk, is a typical wheat milk mixture fermented food popular in Egypt and most of the Arab World. It consists of small, round or irregular pieces, yellowish brown in colour, which have a rough surface and hard texture.
• Yakult is a probiotic fermented milk product which originated from Japan almost 70 years before. It is made with the help of a culture of Lactobacillus casei strain Shirota, which is named after the inventor Prof. Minoru Shirota.
• Yakult is originally made from skim milk and sweetener agents like sucrose, glucose, liquid sugar and starch syrup. It may contain fruit juices and flavouring essences.
• Milk quality plays a key role not only for its wide consumption but also because milk is involved in a large series of derived products, such as yogurt, butter, cheese, and ice cream.
• Pasteurization was given by Louis Pasteur in 1864. It is used to kill harmful pathogenic bacteria resent in milk.
• Pasteurization reduces the natural microflora of milk. It helps to kill some pathogens in milk, for example Mycobacterium tuberculosis, the Q fever agent, Coxiella burnetti.
• Ultra High Temperature is another thermal temperature. It is achieved by heating at temperatures of 135-140 degree C for a few second.
• Microfiltration is a process that partially replaces pasteurization and produces milk with fewer microorganisms and longer shelf life without a change in the taste of the milk.
• Ultrafiltration uses finer filters than microfiltration, which allow lactose and water to pass through while retaining fats, calcium and protein.

8.5 KEY WORDS

• Kefir: Kefir is a viscous, acidic, and mildly alcoholic (2%) milk beverage produced by Yeast-lactic fermentation of milk.
• Kafir grains: Kafir grains are coagulated lumps of casein that contain yeasts, Lactic Acid Bacteria, and Acetic Acid Bacteria.
• **Microfiltration**: Microfiltration is a process that partially replaces pasteurization and produces milk with fewer microorganisms and longer shelf life without a change in the taste of the milk.

• **Buttermilk**: Buttermilk is the milk that remains after cream is churned for the production of butter.

### 8.6 SELF ASSESSMENT QUESTIONS AND EXERCISES

#### Short Answer Questions

1. What is yoghurt? Give its nutritional value.
2. How is yoghurt commercially produced?
4. Give the nutritional value and manufacturing procedure of Kefir.
5. Explain the bacterial and fungal spoilage of butter.

#### Long Answer Questions

1. Discuss about fermented milk products in detail.
2. Explain with the help of table different types of fermentation methods that are involved in making fermented milk products.
3. Write a note on acidophilus milk and bifidus milk.
4. How is cheese manufactured? Explain.
5. Explain with the help of table various milk based acidophilus and bifidus products.

### 8.7 FURTHER READINGS


Fermented Milk Products

NOTES


UNIT 9 MICROBIAL FOOD FERMENTATION

Structure
9.0 Introduction
9.1 Objectives
9.2 Microbial Food Fermentation
   9.2.1 Fermentation and Its Concept
   9.2.2 Fermentation Types and Uses
9.3 Answers to Check Your Progress Questions
9.4 Summary
9.5 Key Words
9.6 Self Assessment Questions and Exercises
9.7 Further Readings

9.0 INTRODUCTION

Fermentation is one of the most important food processing technologies. Many fermented products are preserved with extension of shelf life. In addition to being more shelf stable products and removal of anti-nutritional components, all fermented foods have aroma and flavor characteristics that result directly or indirectly from the fermenting microorganisms. The most common groups of microorganisms involved in food fermentation are bacteria, yeasts, and molds. Microbial enzymes also play an important role in food fermentation. Fermented foods play an important role in improving food security, increasing income and employment, enhancing livelihoods and improving the nutrition and social well being of millions of people around the world, and others. A fermentation is influenced by numerous factors, including moisture, temperature, dissolved O₂ concentration, and dissolved CO₂. Variation of these factors may affect the rate of fermentation, the organoleptic properties of the product, nutritional quality, and other physicochemical properties. Fermentation preserves perishable raw materials.

Food fermentation is a food processing technology that utilizes the growth and metabolic activity of microorganisms for the stabilization and transformation of food materials. Fermentation was primarily developed for the stabilization of perishable agricultural produce. Notwithstanding, the technology has evolved beyond food preservation into a tool for creating desirable organoleptic, nutritional, and functional attributes in food products. Fermented food products still make up a significant portion of the diet in developing countries and the Far East, whereas that is no longer the case in the developed West. Nevertheless, there is a renewed interest in fermented food products in recent times mainly driven by the purported health benefits of such products. The current trend is set to continue into the future...
in light of the increasing prevalence of metabolic syndromes such as obesity, various food allergies, and intolerances, lifestyle choices such as vegetarianism and veganism, and increasing interest by consumers in everything perceived natural and that promotes health and longevity.

In this unit, you will study about microbial food fermentation, fermentation in food processing and role of microorganism in food fermentation in detail.

9.1 OBJECTIVES

After going through this unit, you will be able to:
- Understand about microbial food fermentation
- Explain the role of fermentation in food processing
- Discuss about the role of microorganism in food fermentation

9.2 MICROBIAL FOOD FERMENTATION

Fermentation is a technology that utilizes the growth and metabolic activities of microorganisms for the preservation and transformation of food materials. During food fermentation, the growth of spoilage and pathogenic organisms is inhibited by the metabolites generated by the fermenting organisms, thereby extending the shelf life of perishable produce. For instance, during lactic acid fermentation, Lactic Acid Bacteria synthesize metabolites such as lactic acid, acetic acid, carbon dioxide, ethanol, hydrogen peroxide, bacteriocins, and antimicrobial peptides, which synergistically suppress the survival and growth of pathogenic and spoilage microorganisms.

Besides preservation, fermentation imparts characteristic aroma, flavor, texture, and nutritional profile into food. Thus, although ancient civilizations developed fermentation primarily as a way of preserving perishable agricultural produce, the technology has evolved beyond preservation into a tool for creating desirable organoleptic profiles in foods and improving their palatability. Bread is a classic example for this case, where the primary function of dough fermentation is to create the characteristic structure, texture, and organoleptic profile of bread after the baking process. Fermentation also helps to remove anti-nutritional factors and toxins in food materials and improve their nutritional profile. For instance, fermentation of soybean into products such as tempeh (fermented dehulled soybean with meat like flavor and texture), natto (a fermented soybean dish from Japan with strong smell and flavor and a slimy texture), and soy sauce (a dark brown condiment made from fermentation of soybean, wheat, and salt) leads to reduction of anti-nutritional factors such as phytic acid and trypsin inhibitors and results in the hydrolysis of complex soy proteins into more digestible and bioavailable peptides and amino acids.
9.2.1 Fermentation and Its Concept

Fermentation helps to preserve the food and results in distinctive new food products. It makes new products, and their preservative effects are by chance. Fermentation can be carried out by yeasts, bacteria, moulds or by combinations of these organisms.

- Yeasts produce bread, beer, wine and distilled liquors
- Bacteria produce fermented milk products
- Moulds produce various types of cheese and oriental food
- Yeasts and bacteria produce vinegar

So, fermentation can be defined as an energy yielding anaerobic and metabolic process in which the organisms convert nutrients typically carbohydrates in alcohols and acids (acetic acid and lactic acid).

Fermentation is the process of conversion of carbohydrates such as sugars into acids and alcohols. Moreover, fermentation refers to the use of yeast to change sugar into alcohol or the use of bacteria to create lactic acid into certain foods. So a food is said to be fermented when one or more constituents are acted upon by microorganisms to produce a considerable final products acceptable for human use.

The term fermentation refers to both anaerobic and aerobic breakdown of carbohydrate and carbohydrate-like materials. Thus, the conversion of lactose to lactic acid by *Streptococcus lactis* which takes place under anaerobic conditions and conversion of ethyl alcohol to acetic acid by the bacteria named *Acetobacter aceti* under aerobic conditions are both referred to as fermentation. The earliest use of fermentation was likely to create alcoholic beverages such as beer and wine. But, nowadays, there are so many products which are prepared by using fermentation.

Following are the types of fermentation that are mostly used:

- **Alcoholic Fermentation:** It basically produces beer and wine.
  
  \[
  \text{Sugar} \xrightarrow{\text{Yeast}} \text{Ethyl Alcohol + Carbon Dioxide}
  \]
  
  Anaerobic Conditions

- **Acetic Acid Fermentation:** It basically produces vinegar.
  
  \[
  \text{Ethyl alcohol} + \text{O}_2 \xrightarrow{\text{Acetobacter Aceti}} \text{Acetic Acid} + \text{H}_2\text{O}
  \]
  
  Aerobic Conditions

- **Lactic Acid Fermentation:** It basically produces sauerkraut and pickles.
  
  \[
  \text{Lactose} \xrightarrow{\text{Lactobacilli}} \text{Lactic Acid}
  \]
Table 9.1 shows some products fermentation of which requires various organisms.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Substrate</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid Bacteria</td>
<td>Cabbage</td>
<td>Sauerkraut</td>
</tr>
<tr>
<td></td>
<td>Cucumber</td>
<td>Pickles</td>
</tr>
<tr>
<td></td>
<td>Red meat</td>
<td>Sausages, pepperoni and thuringer</td>
</tr>
<tr>
<td></td>
<td>Cream</td>
<td>Sour cream</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>Yoghurt, buttermilk (unripened)</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>Cheese (cottage cheese and paneer)</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>Cheese ripened (Cheddar)</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>Shrikhand</td>
</tr>
<tr>
<td>Acetic Acid Bacteria</td>
<td>Fermented jaggery, molasses and wine</td>
<td>Vinegar</td>
</tr>
<tr>
<td>Lactic Acid Bacteria and Yeast</td>
<td>Flour (dough)</td>
<td>Bread</td>
</tr>
<tr>
<td>Yeast and Moulds</td>
<td>Beans</td>
<td>Vermicelli</td>
</tr>
<tr>
<td></td>
<td>Soya bean</td>
<td>Soya sauce</td>
</tr>
<tr>
<td></td>
<td>Malt</td>
<td>Beer and lager</td>
</tr>
<tr>
<td></td>
<td>Fruit</td>
<td>Wine and cider</td>
</tr>
<tr>
<td></td>
<td>Wine</td>
<td>Brandy (distilled from wine)</td>
</tr>
<tr>
<td></td>
<td>Molasess</td>
<td>Rum</td>
</tr>
<tr>
<td></td>
<td>Grain mash</td>
<td>Whisky</td>
</tr>
<tr>
<td></td>
<td>Flour dough</td>
<td>Bread, naan</td>
</tr>
<tr>
<td></td>
<td>Rice and dals</td>
<td>Idli, dosa, dhokla</td>
</tr>
</tbody>
</table>

Factors Required for Healthy Fermentation

Following factors are required for healthy fermentation:

- **Sugar**: Sugar is the most important food that yeast needs in order to survive. It consumes the sugar and changes it in alcohol and carbon dioxide. Yeast can use several kinds of sugar. Yeast can digest simple sugars, like glucose and fructose as well as maltose and sucrose very easily, but it does not use larger molecules such as dextrins and starch.

- **Oxygen**: For fermentation to get a good start, oxygen is needed for the initial growth. If there is not enough oxygen at the start of the fermentation then it will take longer time to ferment.

- **Nutrients**: Yeast needs certain nutrients, like soluble nitrogen, vitamins and minerals. If you are planning to add a lot of sugar, you might need additional nutrients.

- **Cleanliness**: In planning home fermentation, it is very necessary that everything is kept clean.
A Brief History of Fermentation in the West

In 1675, a Dutch merchant named Anton van Leeuwenhoek, saw and reported one-celled organisms, which he termed as ‘animacules.’ Today those organisms are called ‘protozoa.’ This discovery electrified the scientific world of that era. In 1680, with the help of a microscope which increased the diameter of every object 300-fold, he looked at yeast and discovered that they consist of tiny spheroids. While the protozoa were alive, the yeast did not appear to be so. No connection was formed between these tiny organisms and the well known process of fermentation.

Early 1800s saw that in Europe there was a huge increase of interest in microbiology. The scientific period started with great advances in the subject of botany, increased interest in microscopy and willingness to experiment about individual organisms. The two main problems that challenged the greatest researchers in the new field of microbiology were related with the basic nature of the fermentation and enzymes.

In the late 1700s, Lavoisier showed that in the process of changing sugar to alcohol and carbon dioxide (as in wine), the weight of alcohol was consumed in the process which equaled the weight of the latter produced. In 1810 J.L. Guy-Lussac summarized the process with the famous equation $C_6H_{12}O_6 = 2CO_2 + 2CH_3CH_2OH$. The whole process was thought to be a simple chemical reaction and yeast (which was till then not even classified as a definite substance, was instrumental to fermentation) was expected to play a physical rather than a chemical role. It was said that either the catalytic action at the yeast cell or the molecular vibration from the decomposing organic matter arising from the death of the cells sparked the chemical changes resulted in fermentation. Putrefaction, which spoilage and fermentation were all thought to be processes of death and not of life.

The first living nature evidence of yeast came between 1837 and 1838 when three publications by C. Cagniard de la Tour, T. Swann and F. Kuetzing came out, each of which independently concluded that as a result of microscopic investigations yeast was a living organism which reproduced by budding. Origin of the word ‘yeast’ traces back to the Sanskrit word and it means ‘boiling.’ It was mainly as wine, beer and bread were the basic food of Europe that most of the early studies on fermentation were concentrated on yeast, with which they were made. Soon bacteria was also discovered. The term was first used in English in the late 1840s, and it did not come into general use until the 1870s, and then also it was largely in connection with the new germ theory of disease.

The idea that fermentation was a process which was initiated by living organisms soon aroused huge criticism from the finest chemists of the day, especially Justus von Liebig, J.J. Berzelius and Friedrich Wohler.

In 1857, Pasteur showed that lactic acid fermentation was caused by living organisms. In 1860, he demonstrated that bacteria, caused the milk to sour a process which was earlier thought to be merely a chemical change and his work in
identifying the work of microorganisms in food spoilage led to the discovery of the process of pasteurization. In 1877, while working to improve the French brewing industry, Pasteur published his famous papers on fermentation, *Études sur la Bière*, which were translated into English in 1879 as *Studies on Fermentation*.

He defined fermentation (incorrectly) as ‘Life without air,’ but correctly showed that particular types of microorganisms cause particular types of fermentations.

In 1877, the era of modern medical bacteriology started when Koch (a German physician; 1843–1910) and Pasteur showed that the anthrax bacillus was the cause of the infectious disease called anthrax. This discovery to Pasteur’s general germ theory of infectious disease, which says that each disease is caused by a specific microorganism. Koch also made very significant discovery of the method for isolating microorganisms in pure culture.

Interestingly, until his death in 1873, the eminent German chemist J. von Liebig continued to criticise Pasteur’s work on fermentation, putrefaction and infectious diseases. He recognized the similarity of these processes but he did not believed that living organisms were the main causative agents. He felt fermentation was basically a chemical rather than a biological process. It has now been broned that neither Pasteur was entirely right nor Liebig entirely wrong.

The work of Pasteur and his many colleagues and predecessors opened up the doors to a vast new vista in the field of biochemistry, microbiology and fermentation. The term ‘biochemistry’ was first used in English in 1869, but this new science of the application of chemistry to biology was usually called ‘physiological chemistry’ until the early 1900s. The two outstanding pioneers of the subject were Liebig and Pasteur. The term ‘microbiology’ was first used in English in 1885, long after Pasteur’s major discoveries.

Basic knowledge of the study of minute living organisms closely related to human activity or welfare did not begin to enter the popular consciousness until the early 1900s. Around 1900s the scientific breakthroughs of the 1870s and 1880s had begun to initiate a change in people’s conception of the world around them so completely that it can be called revolutionary. Food microbiology was set on a scientific foundation, based on the specific microorganisms action. A rational theory of infectious diseases (which were earlier not differentiated from one another) set people’s minds free from the fear of vengeance from an unknowable and invisible disease that caused entity, and the ancient theory of spontaneous generation of lower life forms, that said they could arise de novo and in a fully formed way from decomposing matter. It was replaced by the verifiable theory of biogenesis. For the first time people began to accept the truth that they shared their environment with millions of minute organisms that exerted an ongoing powerful influence on human life. This new world view, amongst other things, provided a huge stimulus for new researches on fermented foods.

Although by showing that fermentation was basically the result of the action of living microorganisms as an epic breakthrough, it did not fell about the basic
nature of the fermentation process, or prove that fermentation was caused by the microorganisms that were generally always present. In 1700s, it had been proved that there was another type of chemical change which resembled the yeast fermentation in certain ways. This was the sort of change which occurred, for example, in the digestion of food. In 1752, Reamur, while studying the digestive processes of a falcon, showed that its digestive juices could dissolve meat. In 1785, William Irvine discovered that aqueous extracts of sprouted barley led to liquefaction of starch. The first clear recognition of 'enzymes' came in 1833 when two French chemists, A. Payen and J.F. Persoz, made a more detailed investigation of the method of solubilizing starch with a malt extract to form a sugar which they called 'maltose.' They called the agent that was responsible for this transformation 'diastase' and they showed that it could be destroyed or inactivated by boiling and that without undergoing permanent change itself, a little amount of diastase could convert a huge amount of starch to sugar, and that it could be concentrated and purified by precipitation with alcohol.

In 1835 the German naturalist Swann isolated a substance from gastric juice which could dissolve meat but which was not an acid. He named it 'pepsin' from a Greek word which meant 'digestion.' It soon became novel to call organic catalysts like, diastase and pepsin 'ferments,' as digestion and fermentation, which were both allied with life, seemed to be somewhat identical processes.

Under the vitalist's influence, ferments were grouped into two types: those which were involved with life process they were called 'organized ferments' and those which were not (like pepsin) they were merely 'unorganized ferments.' Many suspected that there was relation between the two types of ferments. Traube came forward with the theory that all fermentations were due to ferments which were definite chemical substances that were related to proteins and were produced in the cells by the organism. In 1876, for reducing the confusion that existed concerning the two types of ferments, the German physiologist Wilhelm Kuehne suggested that an unorganized ferment, in the absence of life, was to called an 'enzym' after the Greek word 'in yeast.' In 1881 this term was renamed as to 'enzyme' by William Roberts, and it begun to become popular by the 1890s.

Many scientists, including Pasteur, attempted unsuccessfully to extract the fermentation enzyme from yeast. Success finally came in 1897 when the German chemist Eduard Buechner ground up yeast and extracted a juice from them and then found to his surprise that this 'dead' liquid could ferment a sugar solution which formed carbon dioxide and alcohol just like living yeasts. The so-called unorganized ferments behaved just like the organized ones. From that time onwards the term 'enzyme' came to be applied to all ferments. The term 'ferment' went out of the scientific vocabulary altogether and the vitalist position collapsed. After this, it was agreed that only one set of laws will apply to all the things, both animate and inanimate, and that there was no special vital force that characterized living things and acted under different laws. Hence, it was finally understood that fermentation was caused by enzymes that are produced by microorganisms. In 1907, Buechner...
won the Nobel Prize in chemistry for his work, and it opened a new era in the field of enzymes and fermentation.

The science of microbiology, biochemistry, fermentation technology, mycology and bacteriology all share a deep interest in the nature and working of enzymes. Yet, by the early 1900s no one knew exactly what enzymes were or how they acted. As the agricultural microbiologist Conn asked in 1901, 'How can they produce chemical actions without being acted upon or entering into the reactions? Are the enzymes fully lifeless or semi-living? We still do not know the fundamental mystery of fermentation.' Slowly, an understanding of enzymes and catalysts came. In 1905, Harden and Young discovered co-enzymes, the agents that were necessary for the action of enzymes. In 1926, the American biochemist J.B. Sumner first purified and crystallized an enzyme (urease) and showed that it was a protein, more specifically a protein catalyst. Eventually, enzymes came to be called as the key catalysts in all the life processes, each highly specialized in its catalytic action and mainly responsible for only one small step in the complex, multi-step biochemical reactions. Enzymes are still produced only by living organisms—both animals and plants. They have never been synthesized till now.

Improvements in microbiology and fermentation technology have continued steadily up till the present. For example, in late 1930s it was discovered that microorganisms could be mutated with better physical and chemical treatments so that they became higher yielding, faster growing, tolerant of less oxygen and are able to use a more concentrated medium. Strain selection and hybridization developed as well that affected most of the modern food fermentation.

A Brief History of Fermentation in East Asia

Traditional fermented food played an unusually extensive role in East Asia food system. The main use of moulds in the process of making koji (mould-fermented grains and/or soybeans), served as a source of more than 50 enzymes in subsequent fermentation in almost the same way that, in the West, the enzymes of malt (steeped and sprouted barley or other cereal grains) were used to make alcoholic beverages.

The nature of koji exactly like the word with which it is written. In the more traditional form—used with most miso koji and especially with barley koji—the ideographs for ‘barley’ and ‘chrysanthemum’ are placed together. But, nowadays, the ideographs for ‘rice’ and ‘flower’ are conjoined. Its first form is said to have originated in China, whereas the new form has been developed in Japan about 1,000 years ago. In both, the idea the grain covered with a bloom of mould is completely expressed.

Since, ancient times the koji making process has been limited to East Asia, where it has been used in the making of fermented foods such as soy sauce, miso, sake, soy nuggets, shochu (spirits) and rice vinegar (yonezu). The only traditional East Asian fermented soyfood not prepared with mould is Japan’s natto, and its relatives thua-nua in Thailand and kinema in Nepal, which are made by bacterial fermentations. Some have suggested that moulds are widely used since they grow
well in areas that have a humid climate and long rainy season during the warm months. In the West, mould fermented foods are limited basically to a number of cheeses characterized by their strong flavours and aromas, for example, Camembert, Blue and Brie. Owing to the widespread use of mould-fermented foods in East Asia, the word ‘mould’ has a rather positive notion, something like ‘yeast’ in the West.

The koji-making process can be traced back to at least 300 BC in China and to the third century AD in Japan. In East Asia, it was probably thought that fermentation was a life process long before it was thought of in the West. By the sixth century AD, as recorded in the Ch’i-min yao-shu (the earliest encyclopedia of agriculture), the Chinese had various names for two types of moulds that were used in fermented soyfoods. Aspergillus was then called ‘yellow robe’ and Rhizopus was termed as ‘white robe.’ These cultures were carefully distinguished and propagated every year. By the tenth century, a koji starter or inoculum was deliberately being used in the preparation of koji for fermented foods.

Before 1870, makers of the East Asian fermented foods did not know about the basic nature of the fermentation process of microorganisms, enzymes and their respective interactions. Makers of koji did not know what caused the grains and/or soybeans to become covered with a fragrant white mycelium after several days of incubation in a warm koji room, or what later transformed the koji almost like magic into a delicious, savoury seasonings such as miso, shoyu or soy nuggets, or into heady beverages such as sake. The microscope was completely unknown in East Asia prior to the 1880s.

Improvements in food fermentation from the exchange of people and ideas was most provided in Japan. The first generation of European scientists plunged into their investigations of fermented foods with a lot of curiosity and enthusiasm there. One of their initial subjects of research was the koji mould, now called as Aspergillus oryzae and the various foods in which it was used, especially sake and shoyu. Tradition described the introduction of sake brewing in Japan to some emigrants who come from Korea towards the end of the third century AD who doubtless learned the process from China, where it had long been practiced. One of the earliest description of sake being produced by a Westerner came in 1874 when Dr. J.J. Hoffmann a German professor in the medical school of today’s Tokyo University, published a translation of an article on sake from a Japanese encyclopedia of 1714. In the same year he wrote a long description of rice koji process. Although Hoffmann described accurately the process of how to make rice koji, he did not use the word ‘koji.’

In 1878, Korschelt, who was also a German gave an exact and detailed description of exactly how to make koji and koji starter. He was the first Western scientist to use the words koji and tane koji (koji starter) and used them frequently and accurately. In reality, the term koji appeared a little earlier in Hepburn’s famous Japanese-English Dictionary, that translated it (inaccurately) as ‘barm or yeast.’ In 1884, Ferdinand J. Cohn, a Polish botanist and microbiologist, first gave the koji mould its present day name, which is Aspergillus oryzae.
Atkinson was another pioneer in the field of *koji* research. He was a professor of analytical and applied chemistry at Tokyo University. In 1878, after he visited sake factories, he wrote ‘On Sake Brewing,’ which had a preliminary description of how to make *koji* and mentioned the word ‘*koji*.’ In 1889 Dr. Oscar Kellner and his Japanese co-workers published important studies on *koji*, *shoyu* and *miso*. Later on, in 1895 and 1901, C. Wehmer, who taught mycology at Hannover, described the *koji* mould in greater detail. He also stated (1895) that *koji* was being made in America at a large Japanese sake brewery on U Street in Peoria, Illinois, the same area that later on become America’s leading center of research on *koji* and *miso*, in the 1960s. As Western researchers studied *koji*, they quickly understand that it has almost the same relationship with *shoyu* and *miso* fermentation that malt has with Western alcoholic grain fermentations.

One of the first Japanese to make an important commercial use of the new knowledge of microbiology and fermentation science was Jokichi Takamine. After hearing of the malting process in the West, he ambitiously decided to try to introduce the *koji* process in its place. After increasing the diastatic activity of the *koji* mould, he went to America in 1880, but there he met with opposition from the malt makers. He then started a new project of extracting the enzymes from the *koji* mould for commercial uses. In 1894 he got two US patents for the process of making diastatic enzyme, which was a very successful product under the name Takadiastase. This product, had a rich variety of enzymes and it was used widely in enzymology. It brought international popularity to both him and the *koji* mould. Takamine was future seen in recognizing the potential industrial significance of enzymes, even in time when the knowledge of enzymes was very less.

K. Saito was another early leader in the field of microbiology and fermented soyfoods. He did excellent early investigations on *shoyu* fermentation, and he was an authority on yeasts and moulds. Similarly, K.N. Yabe also did a lot of important work in bacteriology and in natto fermentation.

During the 20th century, Japanese microbiologists made many important contributions to the development of microbiology and these developments, included the manufacture of fermented soyfoods, until recently, their strength was more in the area scientific knowledge than in pioneering basic scientific and microbiological breakthroughs. From the early 1900s, major studies on the *koji* mould and its enzymes were done by Japanese scientists. Important advances in enzymology, with much of the work was done on *koji* moulds. By the 1950s, Japanese scientists had isolated various protease and amylase enzymes, which induced mutations and used them commercially. They also developed the technology for the microbial production of *L*-glutamic acid and Mono Sodium Glutamate (MSG), lysine and other amino acids, flavour enhancing nucleotides such as inosinic acid and organic acids. They used the *koji* mould *Aspergillus oryzae* for the commercial production of enzymes including amylases, proteases and amyloglucosidase lipase. They made many product such as microbial rennet. Actually, after World War II, Japan became the world leader in the field of industrial fermentations.
From 1960s, East Asian fermented soyfoods (especially soy sauce or *shoyu*, *miso* and *tempeh*), began to be popularly used in the West. This basically happen due to the growing general interest in soyfoods, the cultural and religious movement toward meatless and vegetarian diets, the increasing interest in nutritious foods with less animal fats, the awareness these foods as a good vegetarian source of vitamin B12, the growing worldwide travel stimulating interest in foreign foods, the increase of East Asian refugees to the West and the increased interest in microbiology and enhanced image of fermented foods.

**Concept of Fermented Foods**

In general, food facilitates growth of different types of microorganisms. Fermentations are enzyme-induced chemical changes in food where the enzymes involved may either be produced by microorganisms or are indigenous to the food. All the fermentation processes are very complex.

The typical character of a fermented food is determined by the nature and quality of the food itself, the changes that occur due to the action of its inherent enzymes, the changes that occur due to microbial fermentations and the interactions occurring between the products of these actions and the constituents of foods. When desirable microorganisms cause fermentations, they impart flavour, aroma and texture to the end products. The keeping quality is due to high acidity, low pH and low oxidation-reduction potential which provide inhibition of other organisms and other undesirable chemical changes. Similar effect is obtained by the production of ethanol, carbon dioxide and absence of oxygen.

Pure culture fermentation is rare in foods; mostly mixed fermentations occur due to the availability of common constituents of yeasts and bacteria in most of the cases.

- **Homofermentative** bacteria produce pure culture fermentations of vegetables and yeasts ferment fruit juices which are characterized by conversion of simple sugars to lactic acid and of sugars to ethanol and carbon dioxide in vegetable and fruit fermentations, respectively. Some amounts of other products are also produced and some changes in proteins, lipids and other nutrients are there but are less evident.

- **Heterofermentative** bacteria initiate complex fermentations and are continued by the homofermenters and are most common in vegetables. The major end products of these heterofermentative actions are lactic acid, acetic acid, ethanol, carbon dioxide and sometimes mannitol and/or dextran, while homofermentation produce lactic acid. Heterofermentation also utilizes pentose sugars. The changes in proteins, lipids and other nutrients also occur but they have limited influence.

- **Mould fermentation** are complex as these are mainly oxidative and may involve different stages of utilization of the food components.

Sugars are the most readily available source of energy. Sugars are essential for metabolic processes but the proteins, lipids, vitamins, nucleic acid and minerals
Microbial Food Fermentation

Related notes on how to properly ferment foods for optimal results.

Notes

Microbial Food Fermentation

are essential for the synthesis of cell protoplasm. These nutrients need to be supplied for growth because the fermentative organisms, particularly bacteria are poor in synthetic abilities. Most of the foods contain sufficient quantities of these nutrients which allow active growth of microorganisms during fermentations.

The protein content is changed but is minor, while in lipids sufficient hydrolysis occur which supply essential components for protoplasm synthesis. The enzyme activity which is already present in food can not be ignored during fermentation, as this activity is associated with curing, ripening and ageing rather than fermentation. The curing of meat is usually the result of the presence of enzymes in the meat, but it is also due to enzymes produced by growth of moulds on the surface of meat. The sausage preparation involves meat enzymes during lactic acid bacterial fermentation. The changes occurring in coffee beans, cacao, tea and other foods involve both the changes, i.e., by microbial enzymes and enzymes present in the produce.

A range of products is available from simple lactic acid bacterial fermentation which depends on high acidity and low oxidation-reduction potential to the cured cheese varieties including those which require activity of other bacterial species or moulds. The action of growth of one species over another and the presence of inherent enzymes is not well defined, but it is proved that aging improves many fermented products, due to some chemical changes.

It is emphasized that there are several species of microorganisms which can produce desirable changes as well undesirable changes. This emphasizes the need for establishing desirable practices.

The desired characteristic flavour, aroma and texture of fermented foods depends on the nature of food itself, the changes resulting from the activity of microbial enzymes, enzymes inherent to the food and the interactions occurring during the fermentation and during further aging and curing.

Benefits of Fermentation

Following are the benefits of fermentations:

- Fermentation enhance the taste and acceptability value of the food. Although the taste and food acceptability are not nutritive factors, these foods have specific flavours and aromas that represent diacetyl acetic acid and butyric acid which makes them more appetizing.
- Fermentation reduces the viscosity and energy intake of food. Effective increases in the energy density are related with the reduction in viscosity, and fermentation as it reportedly reduces the viscosity of certain foods. Porridge made from fermented cassava flour has a lower viscosity as compared with the product from unfermented flour. This reduction is basically due to the activities of amylase-producing microorganisms which break down the starch into simpler sugars the release bound water and thus reduces viscosity.
- Fermentation increases the amino acid and protein content of the food. The affect of the fermentation helps in improving the concentration of lysine,
methionine and tryptophan during corn meal fermentation. In another study it was discovered that riboflavin and niacin increased with the fermentation of maize. Fermentation specifically improved the percentage relative nutritive value (protein quality) as well as the level of lysine in maize, sorghum, millet, etc. A natural lactic acid fermentation of rice meals produced huge increases in isoleucine and lysine and in the relative nutritive value of riboflavin.

- Fermentation makes iron and other di- and trivalent cations available in food. Cereals have high levels of phytates usually, which binds cations such as iron and zinc with mineral phytates. These are complexes of low solubility and therefore they reduce the availability of these essential minerals. White sorghum-based slurries which were subjected to lactic acid fermentation showed increases in iron availability. The level of iron increased when dehulled flour was fermented. A six-fold increase happened when a combination of germination and fermentation was used. These increases correlated with the reductions in phytates.
- Microorganisms, in addition to breaking down complex compounds, synthesize several vitamins and other growth factors during fermentation.
- Fermented foods are enzyme-rich foods that have lots of microorganisms. These foods help the beneficial microflora to colonize in the intestine, which help in maintaining health and fight against infections.
- Fermented foods help in lowering serum cholesterol levels and also show anticancer property.
- Fermentation process reduces the toxicity of some foods.
- Fermentation, especially by moulds, breaks down the indigestible coating of cellulosic and hemicellulosic structures of carbohydrates and proteins enclosed in grains and seeds as moulds are rich in cellulosic splitting enzymes.

Development of Fermented Foods

Human beings made fermented foods since ancient times. The earliest types were beer, wine and leavened bread (primarily made from yeasts) and cheeses (which was made by bacteria and moulds). These were later followed by East Asian fermented foods, yogurt and other fermented milk products, pickles, sauerkraut, vinegar (soured wine), butter and a host of traditional alcoholic beverages. Now, moulds have been used in industrial fermentation to make vitamins B2 (riboflavin) and B12, textured protein products (from Fusarium and Rhizopus in Europe) antibiotics (such as penicillin), citric acid and gluconic acid. Bacteria are now used for the making of amino acids such as lysine and glutamic acid. Single-celled protein foods like nutritional yeast and microalgae (spirulina, chlorella) are also made in modern industrial fermentation.

In early societies, the change of basic food into fermented food was a mystery and a miracle, as they had no idea what caused the unusually sudden, dramatic and welcomed transformation. Some societies attributed this change to divine
intervention. The Egyptians praised Osiris for the brewing of beer and the Greeks established Bacchus as the God of wine. Similarly, at many early Japanese miso and shoyu breweries, a small temple occupied a central place and was worshipped daily. In ancient times fermentation joined smoking, drying and freezing as basic and widely practiced food preservation technique. Wang and Hesseltine (1979) said that ‘Probably the first fermentation were discovered accidentally when salt was incorporated with the food material, and the salt selected certain harmless microorganisms that fermented the product to give a nutritious and acceptable food.’ The process was went further by the early Chinese who first inoculated with the basic foods with moulds, and it created enzymes in salt-fermented soyfoods like miso, soy sauce, soy nuggets and fermented tofu, these also aided salt-tolerant yeasts and bacteria.

9.2.2 Fermentation Types and Uses

Food fermentation basically refers to the process of converting carbohydrates to alcohols and carbon dioxide or organic acids using bacteria, yeasts or molds, under anaerobic conditions. Another definition of food fermentation is the chemical conversion of sugars into ethanol. The fermentation science is known as zymology. The fermentation process is used in the production of cider, beer and wine. It is used as a technique of preservation, so as to create lactic acid in sour foods such as yogurt, vinegar, dry sausages.

Anaerobic fermentation

Although respiration and breathing are often thought as similar processes, they are quite different in nature. While breathing is the exchange of gases between the external environment and the organisms, respiration is a process that occurs within the living cells. This process involves breaking down of bonds of organic molecules and releasing energy that can be used by the cells.

\[
\text{Enzymes} + \text{ATP} + \text{C}_{6}\text{H}_{12}\text{O}_{6} + 6\text{O}_{2} \rightarrow 6\text{CO}_{2} + \text{Energy}
\]

We are not familiar with the process of fermentation taking place in comparatively less complex organisms like bacteria and yeasts. The reactions of fermentation in case of microbes are anaerobic, and happen in the absence of oxygen. These reactions involve cellular food products and glucose sugar as their reactants. In the absence of oxygen they produce combination of ethyl alcohol, carbon dioxide and lactic acid as the by-products. Since long, man has been using the byproducts of such anaerobic respiration to his advantage.

Fermentation process has been considered to be the most important method of preserving food for centuries now. Today, most products that are consumed are prepared or preserved by the process of fermentation. Yeasts are used as tiny factories of fermentation, producing carbon dioxide and alcohol. Some bacteria and molds are able to ferment milk, thereby producing lactic acid and carbon dioxide.
Fermentations were earlier considered to be a result of bacteria, yeasts, molds and other such microbes. However, it has been now realized that the changes that occur are actually caused by the enzymes released by these microorganisms. Some foods are fermented by the enzymes that are already present naturally in the foods.

The enzymes that cause major changes during fermentation process of the foods can be categorized as follows:
- The enzymes that are native to the food.
- The enzymes that are present on unfermented foods and are produced by the flora of microbes.

A good fermentation is one in which the enzymes produced by the fermentative microorganisms play the primary role. Fermented foods are buttermilk, sour cream, butter, cheese, yogurt, pickles, sausages, breads, pretzels, grape nuts which have been produced by using bacteria, yeasts and molds.

Specific Uses of Microbes
- The most common microbes used in sausages for preservation and flavour include the following:
  - Pediococcus cerevesiae
  - Lactobacillus plantarum
  - Micrococcus spp.
- The enzyme chymosin which is extracted from the stomach of milk-fed veal calves is used to replace rennet in cheese. Recombinant chymosin is produced by E. coli.
- Glucose isomerase is used to convert or isomerize glucose to fructose, which is a sweeter product.
- Amylase is used to break starch into separate glucose components. It is used in the brewing industry for malting and baking.
- The enzyme pectinase is used to remove particulate matter or clarify fruit juices.
- The enzyme glucose oxidase is used in the production of dried egg whites. While drying egg whites, the glucose present in the white is most likely to react with amines and cause the white to turn brown. The addition of glucose oxidase breaks down the glucose and prevents it from reacting and causing the change in color.

Fermented Products
Following are some of the fermented products:

Dairy Products
Milk is a very good medium of growth for a variety of microorganisms. Milk fermentation primarily aims at preventing it from getting spoilt, by converting it into a more stable, nutritive and desirable product, for example yogurt, cheese,
butter milk and butter. After preliminary treatment, such as, pasteurization, ultra filtration or lactose hydrolysis, milk from cows, goats, sheep and other mammals may be used. The process of fermentation can be started spontaneously, by the native micro flora or by adding specific starter cultures. Sometimes, material from previous fermentation can also be used.

In order to obtain a more concentrated product, the milk that has been fermented may undergo further processing. It may also be blended with other ingredients such as spices, sugars, fruits, salts, herbs, etc. to change flavour, appearance and color of the products.

In the industrial scale of fermentation of cow’s milk, the microorganisms that are commonly used are mainly bacteria that produce lactic acid, such as, the mesophilic Lactococcus and Leuconostoc species. The optimum temperature for growth required by them is in the range of 20°C to 30° C. Sometimes, thermophilic bacteria such as Corynebacterium, Propionibacterium and yeasts such as Saccharomyces and molds such as P. camemberti and P. candidum are also used.

For example, for making sour cream, Streptococcus cremoris or S. lactis lactic acid and Leuconostoc cremoris are used. Yogurt is made by fermenting milk with a mixture of Lactobacillus, bulgaricus and Streptococcus, thermophilus at 40°C. Cheese is produced using the enzyme rennin (casein coagulase or chymosin) and lactic acid bacterial starter cultures. Natural production of cheese involves lactic acid fermentation, with various mixtures of Streptococcus and Lactobacillus spp. used as starter cultures. Different flavours of the cheese are achieved by using different microbial starter cultures and varying incubation times. Swiss cheese formation involves a late propionic acid fermentation with ripening done by Propionibacteria shermanii. Various fungi are also used in the ripening of different cheeses. The unripened cheese is inoculated with fungal spores.

**Meat Products**

On slaughtering, the sterile tissues of animals get contaminated by spoilage and pathogenic Gram-Negative Bacteria, for example, E. coli, species of Salmonella, species of Pseudomonas and Clostridium perfringens. Sometimes some Gram-Positive microbes also contaminate the meat products. For the preservation of meat and related products, the water activity is reduced either by salting or curing that is done by adding nitrite or drying.

In order to enhance the stability, texture, color and flavour of the product, acid fermentation of meat is carried out. For this purpose, appropriate starter cultures are used. For manufacturing fermented meat products, Pediococcus cerevisiae, Lactobacillus plantarum and Micrococi are used. The fermented products may be classified into two categories. The first category is of dry sausages with 25 to 45 per cent moisture content, and the second category is of semi-dry sausages with 50 per cent moisture content.

Compounds for meat curing and starter cultures are mixed and stuffed in cases, at low temperatures of about 5°C and incubated at temperature between
20°C and 40°C. The fermented product is dried at 10°C to 20°C. The formation of acid during fermentation quickens the process of drying. Examples of dry sausages are pepperoni and salami, and those of semi-dry are bologna and summer sausages. Poultry sausages that are fermented include dry and semi-dry turkey sausages.

**Vegetable Products**

Major fermented vegetable products include sauerkraut from cabbage, and pickles from cucumber, carrots, olives, green tomatoes and mixed vegetables. Vegetables have large numbers of Gram-Negative Bacteria, but low lactic acid. While the repression of Gram-Negative Bacteria is carried out by salting, the growth of lactic acid is stimulated through anaerobiosis and reduced water activity during fermentation.

The naturally present lactic acid bacteria on cabbage are heterolactic. The activity of the type of lactic acid bacteria in the primary and secondary stages of fermentation process is controlled by temperature and concentration of salt. By fermenting at higher concentrations of salt, that is in the range of 5 to 8 per cent, cucumber and olive pickles are made. This fermentation is mainly carried out by the homolactic, *Lactobacillus plantarum*.

**Oriental Food Products**

The fermentations of oriental foods are mainly based on the solid state or solid substrate fermentation. The solid substrates of wheat, soyabeans or rice are aerobically fermented in the absence of free water. Depending on the water binding properties of the substrate, the water content varies from 20 to 80 per cent. The nutrients available in the substrate are in complex forms of starch and proteins, and thus only those organisms that are capable of degrading these substances, can bring growth in them.

With their enzymes of amylases and proteases, fungi are capable of utilizing the nutrients in such substrates. The different fermentation types of solid state are generally divided into five categories on the basis of the steps involved. They are as follows:

- Fungal fermentation that is followed by brine processing as in soya sauce.
- Bacterial fermentation that is used to produce pastes with meat flavour such as natto.
- Acid soaking and then fungal fermentation as done in case of tempeh production from coconut, peanut or soya.
- Lactic acid bacterial fermentation of doughs as done in case of idli or dosa from rice.
- Fermentation using yeast or other fungi to yield alcoholic foods.

Apart from these food products, bread and beverages are also included in the category of fermented food products. Baker’s yeast is used for leavening.
and getting aroma in breads. This happens due to the formation of carbon dioxide and alcohol while making bread. The process of manufacturing beverages like tea, coffee, and cocoa and alcoholic beverages, such as wine, beer and ale, involves fermentation spontaneously or by the use of specific starter cultures.

**Fermentation For Flavour Production**

These days, specific organisms are used solely for flavour production in foods. Table 9.2 lists the name of some common microorganisms and the resultant flavour compounds produced.

<table>
<thead>
<tr>
<th>Food</th>
<th>Microorganism</th>
<th>Flavour Compounds Produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buttermilk</td>
<td>Streptococcus lactis</td>
<td>Small amounts of acetaldehyde, lactic acid and diacetyl</td>
</tr>
<tr>
<td></td>
<td>Streptococcus cremoris</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lactobacillus bulgaricus</td>
<td></td>
</tr>
<tr>
<td>Yogurt</td>
<td>Streptococcus thermophilus</td>
<td>Acetaldehyde</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus bulgaricus</td>
<td></td>
</tr>
<tr>
<td>Alcoholic Fermented Milk</td>
<td>Saccharomyces sp., Lactobacillus sp.</td>
<td>Acetaldehyde and diacetyl</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetoin</td>
</tr>
<tr>
<td>Sauerkraut</td>
<td>Mixed cultures of Lactobacillus brevis, Leuconostoc mesenteroides, Lactobacillus plantarum</td>
<td>Ethanol, Acetoin and diacetyl</td>
</tr>
<tr>
<td>Soybean Milk</td>
<td>Lactobacillus sp.</td>
<td>Acetate and small amounts of short chain fatty acids</td>
</tr>
<tr>
<td></td>
<td>Streptococcus thermophilus</td>
<td></td>
</tr>
<tr>
<td>Soya Sauce</td>
<td>Aspergillus oryzae</td>
<td>Aldehydes including pentanal</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus sp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saccharomyces rouxii</td>
<td></td>
</tr>
<tr>
<td>Tempeh</td>
<td>Rhizopus sp.</td>
<td>Organic acids, alkyl phenols and pyrazines</td>
</tr>
<tr>
<td>Cocoa</td>
<td>Saccharomyces sp., Lactobacillus sp., Acetobacter sp.</td>
<td>Fatty acids</td>
</tr>
<tr>
<td>Cheese</td>
<td>Mucor melehi</td>
<td>Fatty acids and aromatic acids</td>
</tr>
<tr>
<td>Swiss Cheese</td>
<td>Propionobacterium shermanii</td>
<td>Propionic acids</td>
</tr>
<tr>
<td>Bread</td>
<td>Saccharomyces cerevisiae</td>
<td>Ethanol</td>
</tr>
</tbody>
</table>
Check Your Progress

1. What happens during food fermentation?
2. What does yeasts, bacteria, moulds produce?
3. List the factors that are required for healthy fermentation.
4. What does homofermentative bacteria produce?
5. List the major changes that are caused by enzymes during fermentation process of the foods.
6. Name the common microbes used in sausages for preservation and flavour.
7. Give the different fermentation types of solid state.

9.3 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. During food fermentation, the growth of spoilage and pathogenic organisms is inhibited by the metabolites generated by the fermenting organisms, thereby extending the shelf life of perishable produce. For instance, during lactic acid fermentation, Lactic Acid Bacteria synthesize metabolites such as lactic acid, acetic acid, carbon dioxide, ethanol, hydrogen peroxide, bacteriocins, and antimicrobial peptides, which synergistically suppress the survival and growth of pathogenic and spoilage microorganisms.

2. Fermentation can be carried out by yeasts, bacteria, moulds or by combinations of these organisms.
   - Yeasts produce bread, beer, wine and distilled liquors
   - Bacteria produce fermented milk products
   - Moulds produce various types of cheese and oriental food
   - Yeasts and bacteria produce vinegar

3. Following factors are required for healthy fermentation:
   - Sugar: Sugar is the most important food that yeast needs in order to survive. It consumes the sugar and changes it in alcohol and carbon dioxide. Yeast can use several kinds of sugar. Yeast can digest simple sugars, like glucose and fructose as well as maltose and sucrose very easily, but it does not use larger molecules such as dextrins and starch.
   - Oxygen: For fermentation to get a good start, oxygen is needed for the initial growth. If there is not enough oxygen at the start of the fermentation then it will take longer time to ferment.
   - Nutrients: Yeast needs certain nutrients, like soluble nitrogen, vitamins and minerals. If you are planning to add a lot of sugar, you might need additional nutrients.
4. Homofermentative bacteria produce pure culture fermentations of vegetables and yeasts ferment fruit juices which are characterized by conversion of simple sugars to lactic acid and of sugars to ethanol and carbon dioxide in vegetable and fruit fermentations, respectively. Some amounts of other products are also produced and some changes in proteins, lipids and other nutrients are there but are less evident.

5. The enzymes that cause major changes during fermentation process of the foods can be categorized as follows:
   - The enzymes that are native to the food
   - The enzymes that are present on unfermented foods and are produced by the flora of microbes

6. The most common microbes used in sausages for preservation and flavour include the following:
   - *Pediococcus cerevisiae*
   - *Lactobacillus plantarum*
   - *Micrococcus spp.*

7. The different fermentation types of solid state are generally divided into five categories on the basis of the steps involved. They are as follows:
   - Fungal fermentation that is followed by brine processing as in soya sauce.
   - Bacterial fermentation that is used to produce pastes with meat flavour such as natto.
   - Acid soaking and then fungal fermentation as done in case of tempeh production from coconut, peanut or soya.
   - Lactic acid bacterial fermentation of doughs as done in case of idli or dosa from rice.
   - Fermentation using yeast or other fungi to yield alcoholic foods.

9.4 SUMMARY

- Fermentation is a technology that utilizes the growth and metabolic activities of microorganisms for the preservation and transformation of food materials.
- During food fermentation, the growth of spoilage and pathogenic organisms is inhibited by the metabolites generated by the fermenting organisms, thereby extending the shelf life of perishable produce.
- During lactic acid fermentation, Lactic Acid Bacteria synthesize metabolites such as lactic acid, acetic acid, carbon dioxide, ethanol, hydrogen peroxide,
bacteriocins, and antimicrobial peptides, which synergistically suppress the survival and growth of pathogenic and spoilage microorganisms.

- Besides preservation, fermentation imparts characteristic aroma, flavor, texture, and nutritional profile into food.
- Although ancient civilizations developed fermentation primarily as a way of preserving perishable agricultural produce, the technology has evolved beyond preservation into a tool for creating desirable organoleptic profiles in foods and improving their palatability.
- Bread is a classic example for this case, where the primary function of dough fermentation is to create the characteristic structure, texture, and organoleptic profile of bread after the baking process.
- Fermentation also helps to remove anti-nutritional factors and toxins in food materials and improve their nutritional profile.
- Fermentation of soybean into products such as tempeh, natto, and soy sauce leads to reduction of anti-nutritional factors such as phytic acid and trypsin inhibitors and results in the hydrolysis of complex soy proteins into more digestible and bioavailable peptides and amino acids.
- Fermentation helps to preserve the food and results in distinctive new food products. It makes new products, and their preservative effects are by chance.
- Fermentation can be carried out by yeasts, bacteria, moulds or by combinations of these organisms.
- Yeasts produce bread, beer, wine and distilled liquors.
- Bacteria produce fermented milk products.
- Moulds produce various types of cheese and oriental food.
- Yeasts and bacteria produce vinegar.
- In 1675, a Dutch merchant named Anton van Leeuwenhoek, saw and reported one-celled organisms, which he termed as ‘animacules’.
- Early 1800s saw that in Europe there was a huge increase of interest in microbiology. The scientific period started with great advances in the subject of botany, increased interest in microscopy and willingness to experiment about individual organisms.
- The first living nature evidence of yeast came between 1837 and 1838 when three publications by C. Cagniard de la Tour, T. Swann and F. Kuetzing came out, each of which independently concluded that as a result of microscopic investigations yeast was a living organism which reproduced by budding’.
- In 1857, Pasteur showed that lactic acid fermentation was caused by living organisms. In 1860, he demonstrated that bacteria, caused the milk to sour.
Microbial Food Fermentation

NOTES

• a process which was earlier thought to be merely a chemical change and his work in identifying the work of microorganisms in food spoilage led to the discovery of the process of pasteurization.

• Interestingly, until his death in 1873, the eminent German chemist J. von Liebig continued to criticize Pasteur’s work on fermentation, putrefaction and infectious diseases.

• Although by showing that fermentation was basically the result of the action of living microorganisms as an epic breakthrough, it did not fall about the basic nature of the fermentation process, or prove that fermentation was caused by the microorganisms that were generally always present.

• In 1835 the German naturalist Swann isolated a substance from gastric juice which could dissolve meat but which was not an acid. He named it ‘pepsin’ from a Greek word which meant ‘digestion.’

• Traditional fermented food played an unusually extensive role in East Asia food system. The main use of moulds in the process of making koji served as a source of more than 50 enzymes in subsequent fermentation in almost the same way that, in the West, the enzymes of malt were used to make alcoholic beverages.

• Food facilitates growth of different types of microorganisms. Fermentations are enzyme-induced chemical changes in food where the enzymes involved may either be produced by microorganisms or are indigenous to the food.

• Homofermentative bacteria produce pure culture fermentations of vegetables and yeasts ferment fruit juices which are characterized by conversion of simple sugars to lactic acid and of sugars to ethanol and carbon dioxide in vegetable and fruit fermentations, respectively.

• Mould fermentation are complex as these are mainly oxidative and may involve different stages of utilization of the food components.

• Microorganisms, in addition to breaking down complex compounds, synthesize several vitamins and other growth factors during fermentation.
Fermented foods help in lowering serum cholesterol levels and also show anticancer property.

Food fermentation basically refers to the process of converting carbohydrates to alcohols and carbon dioxide or organic acids using bacteria, yeasts or moulds, under anaerobic conditions.

A good fermentation is one in which the enzymes produced by the fermentative microorganisms play the primary role.

Fermented foods are buttermilk, sour cream, butter, cheese, yogurt, pickles, sausages, breads and grape nuts which have been produced by using bacteria, yeasts and moulds.

Milk is a very good medium of growth for a variety of microorganisms.

Milk fermentation primarily aims at preventing it from getting spoil, by converting it into a more stable, nutritive and desirable product, for example yogurt, cheese, butter milk and butter.

On slaughtering, the sterile tissues of animals get contaminated by spoilage and pathogenic gram-negative bacteria, for example, *E. coli*, species of *Salmonella*, species of *Pseudomonas* and *Clostridium perfringens*.

Major fermented vegetable products include sauerkraut from cabbage, and pickles from cucumber, carrots, olives, green tomatoes and mixed vegetables.

**9.5 KEY WORDS**

- **Fermentation**: Fermentation is a technology that utilizes the growth and metabolic activities of microorganisms for the preservation and transformation of food materials.

- **Microbe**: A microorganism, or microbe, is a microscopic organism, which may exist in its single-celled form or in a colony of cells.

**9.6 SELF ASSESSMENT QUESTIONS AND EXERCISES**

**Short Answer Questions**

1. Define the term fermentation.
2. What are the types of fermentation mostly used?
3. Explain with the help of table different types of fermented food products.
4. What are Heterofermentative bacteria?
5. How is fermentation useful?
Long Answer Questions

1. Write a note on fermentation and explain how fermentation is carried out by different microbes.
2. What are the factors that are required for healthy fermentation?
3. Discuss about the history of fermentation.
4. Explain the concept of fermented food.
5. How did development of fermented food started?
6. Explain the types of fermentation.
7. Give the use of different microbes.
8. Discuss about fermented products.

9.7 FURTHER READINGS

UNIT 10 MICROBIAL PRODUCTS OF FOOD

Structure
10.0 Introduction
10.1 Objectives
10.2 Single Cell Protein (SCP)
   10.2.1 Mushrooms
   10.2.2 Oriental Foods Fermented Beverages
10.3 Answers to Check Your Progress Questions
10.4 Summary
10.5 Key Words
10.6 Self Assessment Questions and Exercises
10.7 Further Readings

10.0 INTRODUCTION

Microorganisms themselves may serve as food fit for human consumption or animal feed. In addition, they find use in the production of chemicals, proteins and enzymes required in food processing industries. The term Single Cell Protein (SCP) refers to microbial cells grown and harvested primarily for use as human or animal food. The development of a microorganism as a direct primary food for human consumption is based on the conversion of the abundant carbohydrates (starch and sugar) of plants into protein. SCP offers a possible solution to meet a shortage of protein under unusual conditions when agriculture and fishing may not be able to produce sufficient quantities of protein so as to meet the demand. Any new protein food must satisfy the conditions of safety and organoleptic characteristics fit for human consumption. Quorn- a single cell protein product is marketed as a meat substitute since 1984. The product is some 45 per cent protein, 14 per cent fat and 26 per cent fibre by dry weight. It is 11 per cent protein, 3 per cent available carbohydrate, 6 per cent fibre, 3 per cent fat, 2 per cent ash and 75 per cent water by wet weight. It is sold in a variety of commercial forms, for example, pieces and minced. Nutritionally, it stacks up very well against other foods. It possesses a complete complement of essential amino acids and is a particularly good source of threonine, which tends to be the limiting amino acid in meat.

A number of microorganisms have been identified as potential food for humans and animals. Bacteria, yeasts, moulds and algae may be used for production of SCP, but till today, only yeasts have been produced commercially and used as food. Recently Spirulina maxima, an algae is being grown commercially as a
nutrient rich food. Organisms may be grown directly for the purpose of food (primary organisms) or they may be recovered as by-product of a fermentation (secondary organisms). Advantages of SCP include the inherent high protein content (about 60 to 70 per cent of the cell), rapid increase in cells in a short time and independence from climatic conditions for growth.

Microorganisms themselves can serve as an important food source. Mushrooms (Agaricus bisporus) are one of the most important fungi used as a food source. Spirulina, a Cyanobacterium, also is a popular food source sold in specialty stores.

In this unit, you will study about microbial products of food; SCP, mushrooms, oriental foods fermented beverages (fruit and cereal based) in detail.

10.1 OBJECTIVES

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

- Understand about microbial products of food
- Explain about SCP and mushrooms
- Discuss the oriental foods fermented beverages

10.2 SINGLE CELL PROTEIN (SCP)

The term Single-Cell Protein (SCP) describes the protein-rich cell mass derived from microorganisms grown on a large scale for either animal or human consumption. During World war second, when there were shortages of protein and vitamins in the diet, the German produced yeasts and a mould (Geotrichum candidum) in some quantity for food. After the war the British established a plant in Jamaica for the production of food yeast.

Rationale for SCP Production

The interest in SCP was generated in the wake of protein deficiency, especially in developing countries. SCP may be used directly as human food supplement or it may be used in animal feed. It is imperative that new food sources should be found in order that future generations are adequately fed. A food source that is nutritionally complete and requires a minimum of land, time, and cost to produce is highly desirable. It can be used for protein supplementation of a staple diet by replacing costly conventional source like soymeal and fishmeal to minimize the problem of food scarcity.
Production of SCP

Process of SCP production from any microorganism or substrate would have the following basic steps:

- Establishment of active yeast growth in the fermenter. The optimal temperature, pH, the amounts and kind of inorganic nutrients to be added depend on the substrates
- Provision of carbon source, nitrogen, phosphorus and other nutrients needed to support optimal growth of selected microorganisms
- Prevention of contamination by maintaining sterile conditions
- Selected microorganism is inoculated in pure state
- Application of optimal aeration and agitation
- Liberation of cell proteins by destruction of indigestible cell walls. Microbial biomass recovery from the medium
- Reduction of nucleic acid content
- Processing of biomass and enhancing its usefulness and storability
- The yeast cells are centrifuged from the medium, washed, concentrated and dried. All food yeasts are killed before use
- Special pre-treatments are required before some of the raw materials can be used to grow yeast. Most of the sulphur diode must be removed from the spent sulphite liquor by steam stripping or by aeration and treatment with lime. Wood hydrolysate similarity requires pre-treatment.

Microorganisms as Source of SCP

Microorganisms are an excellent source of SCP over plant and animal source of proteins because of the following reasons:

- Microorganisms have rapid growth rate because they have a very short generation time and can thus provide a rapid mass increase.
- Microorganisms can be easily modified genetically to produce cells that bring about desirable results.
- Microorganism’s ability to use very inexpensive raw materials readily available in large quantities.
- SCP production can be carried out in continuous culture and thus be independent of climatic changes.
- SCP has a good nutritional value (high content of protein containing all the essential amino acids, some SCPs are good source of vitamins, particularly B group of vitamins).
- The greater speed and efficiency of microbial protein production compared to plant and animal sources.
Table 10.1 Comparison of Nutritive Values and Other Parameters for SCP Production from Algae, Fungi and Bacteria

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Algae</th>
<th>Yeast</th>
<th>Mould</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aerobic Rate</strong></td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>Lower than bacteria and yeast</td>
</tr>
<tr>
<td><strong>Substrate</strong></td>
<td>Light, CO₂</td>
<td>Wide range except CO₂</td>
<td>Light, CO₂</td>
<td>Wide range</td>
</tr>
<tr>
<td><strong>pH Range</strong></td>
<td>4-7</td>
<td>5-7</td>
<td>3-8</td>
<td>5-7</td>
</tr>
<tr>
<td><strong>Contamination</strong></td>
<td>High and serious</td>
<td>Low</td>
<td>Low</td>
<td>Precautions needed</td>
</tr>
<tr>
<td><strong>Sodium Content</strong></td>
<td>60-90% crude protein, rich in essential amino acids</td>
<td>50-60%</td>
<td>80%</td>
<td>55-65%</td>
</tr>
<tr>
<td><strong>Nutritional Value</strong></td>
<td>Rich in proteins, fats, B-carotene, vitamin K and minerals such as Ca, Fe, Mg, Mn, Cu, Zn, Mn</td>
<td>Rich in proteins and B complex vitamins</td>
<td>Deficient in vitamin B12</td>
<td>High in protein and certain essential amino acids. Rich in methionine</td>
</tr>
<tr>
<td><strong>SulfurContaining Amino Acids</strong></td>
<td>Low level</td>
<td>Deficient in methionine and cysteine</td>
<td>Deficient in methionine</td>
<td>Deficient in methionine</td>
</tr>
<tr>
<td><strong>Nucleic Acid Removal</strong></td>
<td>Very low nucleic acid content</td>
<td>Low level of nucleic acid contamination</td>
<td>Low level of nucleic acid contamination</td>
<td>Nucleic acid content high, especially RNA. Nucleic acid removal is required</td>
</tr>
<tr>
<td><strong>Time</strong></td>
<td>Suitable for animal feed as protein-rich supplement. Digestibility of algae is lower since cell wall is undigestible, taste can be unpleasant</td>
<td>SCP used both for animal and human feed supplement.</td>
<td>Better public acceptance</td>
<td>Easy harvesting because of size and concentration</td>
</tr>
<tr>
<td><strong>Factors</strong></td>
<td>SCP used both for animal and human feed supplement.</td>
<td>Home public acceptance.</td>
<td>Easy harvesting because of size and concentration</td>
<td>Hydrogen-utilizing bacterial cell wall is undigestible, energy by the oxidation of hydrogen, and CO₂ as sources of carbon. These include Pseudomonas facilis, P. saccharophila, P. flava, Alcaligens eutrophus, A. paradoxus, Cellulomas spp, Rhodobacter capsulatus. Methane or Methanol-Utilizing Bacteria: Methanomonas spp. can be grown at 56-74 degree C. Carbons, C. Pseudolentis.</td>
</tr>
</tbody>
</table>

**NOTES**

**Microbial Products of Food**

**Table 10.1 Comparison of Nutritive Values and Other Parameters for SCP Production from Algae, Fungi and Bacteria**

<table>
<thead>
<tr>
<th>Parameter</th>
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</tr>
</tbody>
</table>

**Table 10.1 Comparison of Nutritive Values and Other Parameters for SCP Production from Algae, Fungi and Bacteria**

**Disadvantages of SCP**

Although SCP shows very attractive features as a nutrient for humans, however there are some problems that deter its adoption on global basis:

- Fast growing microorganisms such as bacteria and yeast have a high concentration of nucleic acid, notably RNA. Nucleic acid removal is not necessary from animal feeds but is from human foods. During purification, nucleic acids must be removed.
- Similar to plant cells, the cell wall of some microorganisms such as algae and yeast contain non-digestible components, such as cellulose. The cells...
Microbial Products of Food

10.2.1 Mushrooms

Mushrooms have been consumed since earliest history; ancient Greeks believed that mushrooms provided strength for warriors in battle, and the Romans perceived them as the Food of the Gods. Mushrooms are a group of filamentous fungi that form large, edible fruiting bodies. The fruiting body is called the mushroom and is formed through the association of a large number of individual fungal hyphae to form a mycelium.

The mushroom commercially grown in most parts of the world is the basidiomycete *Agaricus bisporus*, and it is generally cultivated in ‘mushroom farms’. The organism is grown in special beds, usually in buildings where temperature and humidity are carefully controlled and exposure to light is severely limited. Beds are prepared by mixing soil with a material rich in organic matter and are then inoculated with a pure culture of the mushroom fungus that has been grown in large bottles on an organic-rich medium. In the mushroom bed, it begins as a small button-shaped structure underground growing into the typical mushroom shape with a stalk and cap. The appearance of mushrooms on the surface of the bed is called a flush. Mushrooms must be collected immediately upon flushing. After collection they are packaged and kept cool until brought to market.

Another cultured mushroom is the Shiitake, *Lentinus edulus*. Shiitake mushrooms are cellulose-digesting fungi that grow on hardwood trees. They are cultivated on small logs. The logs are hydrated by soaking in water. Plugs of mushroom culture are inoculated into small holes drilled in the logs. The fungus grows through the log, and after about a year forms a flush of fruiting bodies, the edible mushroom. Shiitake mushrooms are considered to have a superior taste to *Agaricus bisporus*, and therefore are more expensive.

Common Edible Mushrooms

There are approximately 14,000 different species of mushroom, many of which are inedible *Ganoderma* the ‘mushroom of immortality’. The most cultivated...
mushroom worldwide is *Agaricus bisporus*, followed by *Lentinus edodes*, *Pleurotus spp.*, and *Flammulina velutipes*. Mushroom production continuously increases, China being the biggest producer around the world. Cultiva.

Other cultured mushrooms are:

- **Crimini**: Similar in appearance to whites, but have a light tan to rich brown cap and a firmer texture.
- **Portabella**: Relative of crimins, have tan or brown cap and measure up to 6 inches in diameter.
- **Oyster (*Pleurotus spp.*)**: They can be gray, pale yellow or even blue with a velvety texture.
- **Enoki**: Have tiny, button shaped caps and long, spindly stems.
- **Beech**: Beech mushrooms are petite with either all white or brown caps.
- **Maitake**: Appears rippling and tan-shaped, without caps. They are also called “Hen of the Woods”.

Some other species of mushrooms are also edible and possess health benefits, i.e., as follows:

- **Trametes versicolor** has been shown to promote chemopreventive potential; it inhibits growth of several human cancer cell lines, acts as adjuvant in breast cancer prevention.
- **Grifola frondosa** is promoted as anticancer agent, particularly on human gastric carcinoma.
- **Cordyceps militaris** has several beneficial effects. It acts as an antitumor, anti-proliferative, anti-metastatic, insecticidal, and antibacterial compound. Besides, it is also a cancer preventive material and is effective against chronic bronchitis, influenza A, and viral infections.
- **Cordyceps sinensis** contains substances called cordycepin, cordycepic acid, with therapeutic applications like the effects of increased oxygen utilization, ATP production, and stabilization of blood sugar metabolism. Besides, it has antibacterial function, reduces asthma, and lowers blood pressure. On the other hand, it has been reported as organ protector, as well as with a protective effect for heart, liver, and kidney diseases. Also, *C. sinensis* has sedative effect on the central nervous system.
- **Antrodia cinnamomea** is a medicinal mushroom native to Taiwan. Different commercial products are made with this mushroom and it has been used to treat food and drug intoxication, diarrhoea, abdominal pain, hypertension, skin itching, and cancer.
- **Panellus serotinus** (*Mukitake*) is extremely appreciable in Japan as one of the most delicious edible mushrooms. The use of this fungus helps to prevent the development of non-alcoholic fatty liver disease.
- **Auricularia** species are edible and are grown commercially in China. *A. polytricha* has potential medicinal properties and is considered effective to
reduce LDL cholesterol and aortic atherosclerotic plaque; it also has antitumor and anticoagulant activities. *A. auricula-judae* is a popular ingredient in many Chinese dishes; it has been used as a blood tonic and has shown antitumor, hypoglycemic, anticoagulant, and cholesterol-lowering properties.

- *Flammulina velutipes* is available as fresh or canned product and it is traditionally used for soups in China. It contains biologically active components such as dietary fiber, polysaccharides, and antioxidants, which reduce blood sugar, blood pressure, and cholesterol.

**Nutritional Value of Mushrooms**

Mushrooms are popular valuable foods because they are low in calories, carbohydrates, fat, and sodium: also, they are cholesterol-free. Mushrooms are a good source of proteins, and fiber. Besides, mushrooms provide important nutrients such as the B vitamins riboflavin, pantothenic acid, and niacin. They also provide several important minerals such as selenium, ergothioneine, copper, and potassium.

**Mushrooms Farming**

Growing mushrooms requires careful preparation of the growth medium and control of environmental conditions. The mushroom bed is a carefully developed compost, which can be steam sterilized to improve mushroom growth. Large caves provide optimal conditions for the production of this delicacy (Refer Figure 10.1).

*Fig. 10.1* Mushroom Farming

The general process for farming mushrooms involves six sequential steps, outlined below for the *Agaricus* species.

- **Phase I Composting**: Making the compost: The first phase of composting begins by mixing and wetting the bulk compost ingredients on a large
concrete slab called a wharf. Bulk compost ingredients may be natural (manure) or synthetic. A compost turner is used to mix and aerate the compost, and water is added as the bulk ingredients are mixed. The mixture is then stacked in piles with tight sides and loose centers, or ricks. Phase I composting usually occur outdoors and take 7-14 days to complete.

- **Phase II Composting**: Finishing the compost. During this phase, which lasts 10-14 days, the compost is pasteurized to kill any pests present in the compost and remove ammonia formed during Phase I composting.

- **Spawning**: During this phase, the compost is inoculated with commercially produced mycelium (mushroom spawn) and held under controlled conditions to allow the spawn to colonize the substrate. This period, called the spawn run, generally takes 14-21 days.

- **Casing**: In this step, casing (a top-dressing of clay-loam field soil mixed with peat moss, ground limestone and, occasionally, spent, reclaimed mushroom substrate) is applied to the spawn-run compost and the crop is heavily watered. The casing acts as a water reservoir and is where thicker mycelia (called rhizomorphs) form. After casing, the compost is kept at approximately 75°F for 5 days with high relative humidity, and then the temperature is lowered by 2°F per day until young mushrooms (pins) form.

- **Pinning**: When the rhizomorphs change from the vegetative growth stage to the fruiting growth stage, young mushrooms, called pins, push up through the casing layer. Pins develop in response to the introduction of fresh air into the growing room, which lowers the ambient concentration of carbon dioxide.

- **Cropping**: During this phase, mature mushrooms are harvested in 3-5 day periods called ‘breaks’. A complete harvest usually takes 30-42 days but can go on for up to 150 days.

The process is similar for other species, with the following slight process differences by species. Oyster mushrooms are farmed by a similar process but require more humidity and fresh air and grow in tubular plastic bags rather than horizontal racks. Shiitake mushrooms are farmed using either natural oak logs or synthetic ‘logs’ made from oak sawdust packed in poly bags. Enoki mushrooms are farmed in plastic bottles at colder temperatures (45°F) and only produce one set of fruiting bodies per crop. Beech mushrooms are farmed similarly to Enoki mushrooms but at higher temperatures (60-64°F). Maitake mushrooms are farmed on synthetic ‘logs’, similar to shiitake mushrooms, and only produce one set of fruiting bodies per crop.

**Foodborne Outbreaks of Mushrooms**

Not all mushrooms are edible. Some wild mushrooms are very poisonous and can even cause death. Cultivated mushrooms are usually cooked, which kills any harmful bacteria that may have contaminated them from soil, insect pests, rodents or handling.
Microbial Products of Food during processing. The greatest risks are from fecal bacteria, *Staphylococcus aureus* and bacterial spores from the soil. However commercial mushroom production is carried out with great attention to hygiene and there have been few reported cases of food poisoning from this product.

There are few documented cases of foodborne illness due to pathogens associated with mushrooms in the U.S., i.e., as follows:

- In 1989, a multistate outbreak of *Staphylococcal* food poisoning was associated with canned mushrooms imported from the People’s Republic of China. *Staphylococcal* enterotoxin was isolated from unopened cans in several establishments. Spoilage prior to processing likely created an anaerobic environment which inhibited the normal microbiota present on mushrooms, allowing *Staphylococcus aureus* to grow and produce the enterotoxin. The toxin was heat stable and thus survived the canning process which kills bacteria.

- A restaurant-associated outbreak of Salmonella Heidelberg was linked to improperly handled mushrooms. The previously canned mushrooms may have come in contact with raw meat and poultry products.

Food Safety

There are several pathogens that are of concern in mushroom production.

- *Campylobacter*, a leading cause of bacterial enteritis, was isolated from mushrooms sampled in retail markets.

- *Clostridium botulinum* is of particular concern in mushroom production and improper home preservation has been linked multiple times to illness and death. Mushrooms’ high rate of respiration can rapidly create an anaerobic environment within sealed plastic packages which favours growth of *C. botulinum* and, ultimately, production of botulinal toxin.

- The spore-forming bacterium can thrive in improperly packaged mushrooms. The general recommendation for consumers is to store mushrooms in the refrigerator, unwashed in a paper bag, to prevent trapping moisture which will promote spoilage.

10.2.2 Oriental Foods Fermented Beverages

The fermented food industry in the Orient is large. It includes traditional fermented foods made in the Orient, including Japan, Indonesia, India, Pakistan, Thailand, Philippines, Taiwan, China, Korea, and the encompassing areas. These foods were produced long before written history; some of these processes are so little known that even today one can only guess as to the organisms used. In Korea, 35% of the 442,803 metric tons of soybeans produced is fermented. Indonesia uses about 75,600 tons of soybeans in making tempeh. Recent data from the Soycrafters Association of North America state that a total of 926,640 pounds of tempeh was produced in the United States in 1980 using *Rhizopus oligosporus* NRRL 2710, originally isolated from Indonesian tempeh.
Main fermentation processes involved are as follows:

- **Alcohol Fermentation** results in the production of ethanol, and yeasts are the predominant organisms, for example wines and beers.
- **Lactic Acid Fermentation**, for example fermented milks and cereals, is mainly carried out by lactic acid bacteria.
- **Acetic Acid Fermentations** in which Acetobacter convert alcohol to acetic acid in the presence of excess oxygen.
- **Alkali Fermentation** often takes place during the fermentation of fish and seeds, popularly used as condiment.

Relatively few genera and species of microorganisms are employed in the Oriental fermentations. In the Near East and Africa, molds seem to be little or never utilized; instead lactic acid bacteria and yeasts are the rule in the preparation of fermented foods. Table 10.2 lists a number of Oriental fermented foods, the microorganisms used in their manufacture, and appropriate strain numbers of useful cultures.

The microorganisms involved in a fermented food can be divided into the following categories:

**Monoculture**: Fermentations in which one species of microorganism is necessary to produce the product;

- The Indonesian tempeh fermentation is an example: only *Rhizopus* is necessary to make the soybean food.
- Natto is a food made by fermentation of soybeans with *Bacillus natto*; the whole fermented soybean.
- Ang-kak is a fermented rice product used for coloring other foods. The pigments are known and are water soluble. The only microorganisms used are selected cultures of *Monascus purpurea*.
- Other processes requiring only one microorganism include the ontjom (Neurospora intermedia) and sufu (*Actinomucor elegans*) fermentations.

**Multiculture**: In these fermentations, more than one microorganism is required, belonging taxonomically to different species. An example of this is ragi, in which two fungi (*Amylomyces* and *Rhizopus*), yeast and a bacterium are required to make the starter. (Ragi is not itself a food but is used as a starter for several food fermentations.)

**Unimulticulture**: These are fermentations in which two or more strains of the same species are used together. Soybean yogurt is an example in which two strains of *Lactobacillus acidophilus* are employed, with each strain contributing to the final product: flavor from one strain and acid from the other. In this fermentation, as in the multiculture fermentations above, two or more strains may be used.

**Polyculture**: These are food fermentations in which different microorganisms are many and the species specifically required to make the product are unknown. An example is the mixture of microorganisms found in silage fermentation and in Indochinese fish fermentations.
Except for the examples described above, in which only a single microorganism is employed even though there are usually several contaminating microorganisms present, most Asian food fermentations are carried out by more than one microorganism. The essential microbial cultures may be introduced simultaneously or they may be inoculated in sequence. To make lao chao, the starter culture containing the mold and the yeasts is added at one time. Sequential inoculation of microorganisms is exemplified in the shoyu fermentation, with koji first prepared using *Aspergillus oryzae* cultures, followed by a yeast bacterial inoculation and then fermentation by the latter organisms. At this time the *Aspergillus oryzae* strains from the koji are killed.

### Table 10.2: Oriental Foods and Beverages

<table>
<thead>
<tr>
<th>Alcoholic Beverages</th>
<th>Raw Material</th>
<th>Microorganism</th>
<th>Country of Use</th>
<th>Appearance and Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rice Wine</strong></td>
<td>Rice</td>
<td><em>S. cerevisiae</em></td>
<td>China</td>
<td>Alcoholic clear beverage</td>
</tr>
<tr>
<td><strong>Chongju</strong></td>
<td>Rice</td>
<td><em>S. cerevisiae</em></td>
<td>Korea</td>
<td>Clear liquid, rice wine</td>
</tr>
<tr>
<td><strong>Sake</strong></td>
<td>Rice</td>
<td><em>Saccharomyces sake</em></td>
<td>Japan</td>
<td>Alcoholic clear drink</td>
</tr>
<tr>
<td><strong>Rice Beer</strong></td>
<td>Rice, wheat</td>
<td><em>Lactic acid bacteria</em></td>
<td>Korea</td>
<td>Alcoholc turbid drink</td>
</tr>
<tr>
<td><strong>Tapuy</strong></td>
<td>Rice</td>
<td><em>Mucor indicus</em></td>
<td>Philippines</td>
<td>Dark brown liquid, sour, alcoholic</td>
</tr>
<tr>
<td><strong>Brembali</strong></td>
<td>Glutinous rice</td>
<td><em>Hansenula anomala</em></td>
<td>Indonesia</td>
<td>Sweet, alcoholic paste mixed with water</td>
</tr>
<tr>
<td><strong>Alcoholic Rice Paste</strong></td>
<td>Rice</td>
<td><em>Rhizopus oligosporus</em></td>
<td>India/Sikkim</td>
<td>Sweet-sour alcoholic paste, used as condiment</td>
</tr>
<tr>
<td><strong>Miso</strong></td>
<td>Rice, soybeans or rice, barley, wheat</td>
<td><em>Aspergillus oryzae</em>, <em>Aspergillus sojae</em>, <em>Saccharomyces rouxii</em>, <em>Candida etchellsii</em>, <em>Pediococcus halophilus</em>, <em>Torulopsis etchellsii</em>, <em>Lactobacillus delbrueckii</em></td>
<td>Japan, China</td>
<td>Paste used as seasoning</td>
</tr>
<tr>
<td><strong>Tapai pulut</strong></td>
<td>Rice</td>
<td><em>Chlamydomucor Endomycopsis Hansenula</em></td>
<td>Malaysia</td>
<td>Sweet, alcoholic paste, at breakfast or snack</td>
</tr>
<tr>
<td><strong>Tape-ketan</strong></td>
<td>Rice</td>
<td><em>Saccharomyces cerevisiae</em>, <em>Hansenula anomala</em>, <em>Rhizopus oryzae</em>, <em>Chlamydomucor oryzae</em>, <em>Mucor, Endomycopsis fibuliger</em></td>
<td>Indonesia</td>
<td>Sweet-sour alcoholic soft paste</td>
</tr>
<tr>
<td><strong>Lao-chao</strong></td>
<td>Rice</td>
<td><em>Rhizopus chinensis</em>, <em>Amylomyces rouxii</em>, <em>Rhizopus oryzae</em>, <em>Saccharomycopsis fibuligera</em>, <em>Saccharomycopsis malanga</em></td>
<td>China, Indonesia</td>
<td>Paste, used as dessert or combined with eggs, seafood</td>
</tr>
</tbody>
</table>

### Alcoholic Beverages

<table>
<thead>
<tr>
<th>Alcohol Beverages</th>
<th>Raw Material</th>
<th>Microorganism</th>
<th>Country of Use</th>
<th>Appearance and Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rice Wine</strong></td>
<td>Rice</td>
<td><em>S. cerevisiae</em></td>
<td>China</td>
<td>Smooth, clear, alcoholic drink</td>
</tr>
<tr>
<td><strong>Chongju</strong></td>
<td>Rice</td>
<td><em>S. cerevisiae</em></td>
<td>Korea</td>
<td>Clear, rice wine</td>
</tr>
<tr>
<td><strong>Sake</strong></td>
<td>Rice</td>
<td><em>Saccharomyces sake</em></td>
<td>Japan</td>
<td>Clear, rice wine, alcoholic drink</td>
</tr>
<tr>
<td><strong>Rice Beer</strong></td>
<td>Rice, wheat</td>
<td><em>Pediococcus halophilus</em>, <em>Lactobacillus delbrueckii</em></td>
<td>Korea</td>
<td>Alcoholic turbid drink</td>
</tr>
<tr>
<td><strong>Tapuy</strong></td>
<td>Rice</td>
<td><em>Mucor indicus</em></td>
<td>Philippines</td>
<td>Dark brown liquid, sour, alcoholic</td>
</tr>
<tr>
<td><strong>Brembali</strong></td>
<td>Glutinous rice</td>
<td><em>Candida</em></td>
<td>Indonesia</td>
<td>Sweet, alcoholic paste mixed with water</td>
</tr>
<tr>
<td><strong>Alcoholic Rice Paste</strong></td>
<td>Rice</td>
<td><em>Rhizopus oligosporus</em></td>
<td>India/Sikkim</td>
<td>Sweet-sour alcoholic paste, used as condiment</td>
</tr>
<tr>
<td><strong>Miso</strong></td>
<td>Rice, soybeans or rice, barley, wheat</td>
<td><em>Aspergillus oryzae</em>, <em>Aspergillus sojae</em>, <em>Saccharomyces rouxii</em>, <em>Candida etchellsii</em>, <em>Pediococcus halophilus</em>, <em>Torulopsis etchellsii</em>, <em>Lactobacillus delbrueckii</em></td>
<td>Japan, China</td>
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</tr>
<tr>
<td><strong>Tapai pulut</strong></td>
<td>Rice</td>
<td><em>Chlamydomucor Endomycopsis Hansenula</em></td>
<td>Malaysia</td>
<td>Sweet, alcoholic paste, at breakfast or snack</td>
</tr>
<tr>
<td><strong>Tape-ketan</strong></td>
<td>Rice</td>
<td><em>Saccharomyces cerevisiae</em>, <em>Hansenula anomala</em>, <em>Rhizopus oryzae</em>, <em>Chlamydomucor oryzae</em>, <em>Mucor, Endomycopsis fibuliger</em></td>
<td>Indonesia</td>
<td>Sweet-sour alcoholic soft paste</td>
</tr>
<tr>
<td><strong>Lao-chao</strong></td>
<td>Rice</td>
<td><em>Rhizopus chinensis</em>, <em>Amylomyces rouxii</em>, <em>Rhizopus oryzae</em>, <em>Saccharomycopsis fibuligera</em>, <em>Saccharomycopsis malanga</em></td>
<td>China, Indonesia</td>
<td>Paste, used as dessert or combined with eggs, seafood</td>
</tr>
</tbody>
</table>

### Fermented Breads, Noodles and Porridges

<table>
<thead>
<tr>
<th>Alcohol Beverages</th>
<th>Raw Material</th>
<th>Microorganism</th>
<th>Country of Use</th>
<th>Appearance and Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Idli</strong></td>
<td>Rice grits, black gram</td>
<td><em>L. mesenteroides</em>, <em>S. fecalis</em>, <em>T. candida</em>, <em>T. pullulans</em></td>
<td>South India/Sri Lanka</td>
<td>Steamed cake for breakfast or snack</td>
</tr>
<tr>
<td><strong>Dosa</strong></td>
<td>Rice, Bengal gram, black gram powder</td>
<td><em>L. mesenteroides</em>, <em>S. fecalis</em>, <em>T. candida</em>, <em>T. pullulans</em></td>
<td>India</td>
<td>Griddled cake for breakfast or snack</td>
</tr>
</tbody>
</table>

Self-Instructional Material

NOTES

Microbial Products of Food
<table>
<thead>
<tr>
<th>Country</th>
<th>Food Type</th>
<th>Microbial Species</th>
<th>Shelf Life</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>Dhokla</td>
<td>Rice, Wheat, Bengal gram</td>
<td>Leuconostoc, Streptococcus, Lactobacillus fermenti</td>
<td>1-3 months</td>
</tr>
<tr>
<td>Nepal</td>
<td>Tasty Rice and curries</td>
<td>Rice, wheat</td>
<td>Hansenula anomala</td>
<td>Semi-solid</td>
</tr>
<tr>
<td>Nepal</td>
<td>Nepal Jalebies</td>
<td>Rice, wheat flour</td>
<td>Saccharomyces bayanus</td>
<td>Yeast leavened</td>
</tr>
<tr>
<td>Nepal</td>
<td>Kanji</td>
<td>Rice and carrots</td>
<td>Hansenula anomala, Lactobacillus</td>
<td>Liquid added</td>
</tr>
<tr>
<td>Indonesia</td>
<td>Kecap</td>
<td>Rice, wheat, soybeans</td>
<td>Aspergillus, Lactobacillus, Hansenula</td>
<td>Fermented</td>
</tr>
<tr>
<td>Thailand</td>
<td>Khanomjien</td>
<td>Rice</td>
<td>Lactobacillus sp., Streptococcus sp</td>
<td>Yeast leavened</td>
</tr>
<tr>
<td>China</td>
<td>Puto</td>
<td>Rice, sugar</td>
<td>L. mesenteroides, S. faecalis, yeast</td>
<td>Yeast leavened</td>
</tr>
<tr>
<td>China</td>
<td>Rabdi</td>
<td>Maise and buttermilk</td>
<td>Penicillium acidilactici, Bacillus</td>
<td>Semi-solid</td>
</tr>
<tr>
<td>India</td>
<td>Kichudok</td>
<td>Rice, wheat flour</td>
<td>Hansenula anomala</td>
<td>Liquid seasoning</td>
</tr>
<tr>
<td>Malaysia</td>
<td>Mantou</td>
<td>Unbleached wheat flour</td>
<td>Saccharomyces cerevisiae</td>
<td>Yeast leavened</td>
</tr>
<tr>
<td>India</td>
<td>Vada</td>
<td>Cereal/legume</td>
<td>Pediococcus, Streptococcus, Leuconostoc</td>
<td>Semi-solid</td>
</tr>
<tr>
<td>India</td>
<td>Torani</td>
<td>Rice, wheat flour</td>
<td>Hansenula anomala, Candida, Lactobacillus</td>
<td>Liquid seasoning</td>
</tr>
<tr>
<td>Turkey</td>
<td>Tarhana</td>
<td>Parboiled wheat meal and yoghurt (2:1)</td>
<td>LAB</td>
<td>Solid powder</td>
</tr>
<tr>
<td>Vietnam</td>
<td>Me</td>
<td>Rice</td>
<td>Lactic acid bacteria</td>
<td>Sour food ingredient</td>
</tr>
<tr>
<td>Afghanistan</td>
<td>Nab</td>
<td>Unbleached wheat flour</td>
<td>Saccharomyces cerevisiae, LAB</td>
<td>Semi-solid</td>
</tr>
</tbody>
</table>

**Traditional Wheat Based Fermented Foods**

**Soy Sauce (Shoyu)**

Soy sauce is a fermented dark brown liquid, made from a blend of soybeans and wheat. It is popular in Japan, China and used as seasoning. Soy sauces have a salty taste. For the preparation of soya sauce cooked soybeans are mixed with coarse wheat with initial moisture content 55%. The mixture is inoculated by molds, and after 3 days of fermentation mixture is called koji at this stage. Koji is mixed with 20-25% brine solution in 1:3 w/v. This brine solution containing koji is known as moromi. The moromi is left to ferment for a period of 1–12 months. After
fermentation is completed, soy sauce is separated, filtered, pasteurised and bottled. The microorganisms involved in fermentation include fungi such as Aspergillus oryzae and A. soyae involved in the koji production, halotolerant lactic acid bacteria and yeast strains such as Zygosaccharomyces rouxii and Candida species responsible for the moromi fermentation. The characteristic aroma and flavour of Soy sauce is due to the enzymatic activities of yeasts and some lactic acid bacteria.

Good quality Koikuchi shoyu has the following characteristics: 1.5-1.8% total nitrogen (one half must be free amino acids, 3-5% reducing sugar (mainly glucose); 2-2.5% ethanol; 1-1.5% polyalcohol (primarily glycerol); 1-2% organic acid (primarily lactic); 4.7-4.8 pH; 17-18% sodium chloride

Tarhana

Tarhana (Trahanas) is prepared by mixing wheat flour, sheep milk yoghurt, yeast and a variety of cooked vegetables and spices (tomatoes, onions, salt, mint, paprika) followed by fermentation for 1–7 days. The fermented matter is dried and stored in the form of biscuits. The fermentation process and the type of product obtained is very similar to kishk. The sheep milk yoghurt contains Streptococcus thermophilus and Lactobacillus bulgaricus as the major fermenting organisms.

Tarhana has an acidic and sour taste with a strong yeasty flavour, and is a good source of protein and vitamins. While tarhana soup can be used as a part of any meal, it is often eaten for breakfast. The practical nutritional importance of tarhana is the improvement of the basic cereal protein diet by adding dairy protein in a highly acceptable form. The low pH (3.8–4.2) and low moisture content (6–9%) make tarhana a poor medium for pathogens and spoilage organisms. In addition, tarhana powder is not hygroscopic and can be stored for 1–2 years without any sign of deterioration.

Tempe or tempeh is a traditional Southeast Asian soy product, originating from Indonesia. It is made by a natural culturing and controlled fermentation process that binds soybeans into a cake form. Here a special fungus is used, which has the Latin name Rhizopus oligosporus, usually marketed under the name Tempeh starter. It is especially popular on the island of Java, where it is a staple source of protein. Like tofu, tempe is made from soybeans, but it is a whole soybean product with different nutritional characteristics and textural qualities. Tempe’s fermentation process and its retention of the whole bean give it a higher content of protein, dietary fiber, and vitamins. It has a firm texture and an earthy flavor, which becomes more pronounced as it ages.
Indigenous Rice Based Fermented Foods

Dhokla

Dhokla is cereals based fermented product. It is also similar to Idli except that Bengal gram dhal is used instead of black gram dhal in its preparation. It is famous in south India and Sri Lanka. A mixture of rice and chickpea flour is also used as the substrate for the fermentation. The fermented batter is poured into a greased pie tin and steamed in an open steamer as in Idli preparation. As in other indigenous fermented foods, a significant improvement in the biological value and net protein utilisation of Dhokla due to fermentation has been reported. *Leuconostoc mesenteroides*, *Streptococcus faecalis*, *Torulopsis candida*, *T. pullulans* are the major microorganisms involved in Dhokla fermentation. It is used as steamed cake for breakfast or as snack food.

Idli

Idli is a traditional fermented food, prepared and consumed in South India and in many parts of Sri Lanka. It is normally consume with chutney and sambar. Idli made up of a blend of rice and dehulled black gram. For Idli preparation rice and black gram are soaked in water and then the rice is coarsely ground and the black gram is finely ground. Then the rice and the black gram batters are mixed together in 2:1 ratio with addition of a little amount of salt and leave it for fermentation overnight at about 30 degree C. Finally, the fermented batter is steamed for 5-8 min in. Lactic acid bacteria *Leuconostoc mesenteroides*, *Lactobacillus fermenti*, *Streptococcus faecalis*, *Lactobacillus delbrueckii*, *Lactobacillus lactis* and *Pediococcus cerevisiae* are the significant microorganisms responsible for Idli fermentation. Some yeast like *Torulopsis holmi*, *Geotrichum candidum*, and *Trichosporon pullulans* are also helps in Idli fermentation. Idli is a low caloric food used as breakfast and snacks. Fermentation increase essential amino acid in Idli and decrease antinutrients. In Idli preparation, instead of rice, kodri, millet and soybean or green gram instead of black gram are also used.

Dosa

In Dosa preparation, wheat, bajra, maize or kodri instead of rice and sprouted peas, cowpea, field beans, soybeans or groundnut oilcake instead of black gram are also used. Dosa batter is similar to Idli batter but the dosa batter is thicker. A dosa batter is prepared by grinding wet rice and wet black gram individually with water and the two suspensions are then mixed and allowed to undergo fermentation, usually for 8-20 h. After fermentation, the fermented Dosa batter is heated with little oil on hot pan as thin, semi soft to crisp pancake and eaten with chutney and Sambar.
Traditional Cereal Based Fermented Foods

Some of the traditional cereal based fermented foods are as follows:

Miso

Miso can be classified into three types, based on the substrate:

- Rice miso made from rice, soybeans, and salt.
- Barley miso made from barley, soybeans, and salt.
- Soybean miso, made only from soybeans and salt.

**Fermentation Method:** Unlike shoyu, whole soybeans are used because defatted flakes make an inferior product. Soybeans are soaked in water, and then cooked in water at 115 degree C for 20min. The koji is made from cleaned milled rice that is soaked in water and then steamed in an open cooker for 40 min. The cooled rice is then sprayed with tane koji spores of *A. oryzae*. Tane koji refers to the dry spores often prepared by a factory engaged only in inoculum production. In some instances, only one strain of *A. oryzae* or *A. sojae* is used, but in many instances, as in the shoyu koji, as many as three strains of mold are blended together. The inoculum is conidia only, since no other means of reproduction is known in these species. The koji is fermented for 40-48 h in a variety of different types of koji fermentors. Salt and cooked soybeans are added to the rice koji; the salt, plus the anaerobic conditions developing in the second fermentation, kills the molds. As is not the case in preparation of shoyu, in that of miso no water is added; therefore, the fermenting mash is a thick paste. At the time of mashing, a pure culture of yeast and lactic bacteria is added. Fermentation proceeds slowly at 30 degree C and continues for one to three months, depending on the variety of miso desired. Since the mash is a paste, it cannot be stirred with air, but rather the immature miso is removed from one vat to another at least twice to improve fermentation. Typically, the yeasts are *Saccharomyces rouxii*, *Candida versatillis*, and *C. etchellsii*; the lactic bacteria are *Pediococcus halophilus* and *P. pentosaceus*.

**The final product has the following characteristics:**

60% of the protein is water soluble, including amino acids and polypeptides; 75% of the carbohydrate is reducing sugar; and trypsin inhibitors and hemagglutinins are inactivated. The product is safe to store for long periods because of the high salt content (6-13%). However, in making miso soup, it is a practice to add ten times the weight of miso with hot water to give a salt content of about 1.2%. After aging, the miso is packaged and sold as a paste, but it also may be dehydrated.
Sake
Sake is the traditional cereal based fermented alcoholic beverage of Japan. It is also called as rice wine and popular in Japan and China. To prepare the Sake rice is firstly polished then steamed and a steamed portion is used to grow Aspergillus oryzae, which activate different types of enzymes required for sake brewing. The seed mash is traditionally obtained by natural lactic acid fermentation involving various aerobic bacteria, wild yeasts, Lactic Acid Bacteria, and Sake yeasts.

Production of Other Alcoholic Beverages

Wine and Champagnes
Once grapes are pressed, the sugars in the juice (the must) can be immediately fermented to produce wine. Must preparation, fermentation, and aging are critical steps. Production of wine is detailed in Figure 10.2. Wine can be dry or sweet, depending on the level of free sugar that remains at the end of the alcoholic fermentation.

Many processing variations can be used during wine production. The wine can be distilled to make a 'burned wine' or brandy. Acetobacter and Gluconobacter can be allowed to oxidize the ethanol to acetic acid and form wine vinegar. In the past an acetic acid generator was used to recirculate the wine over a bed of wood chips, where the desired microorganisms developed as a surface growth. Today the process is carried out in large aerobic submerged cultures under much more controlled conditions.

Natural Champagnes are produced when the fermentation, resulting in CO₂ formation, is continued in the bottles to produce a naturally sparkling wine. Sediments that remain are collected in the necks of inverted champagne bottles after the bottles have been carefully turned. The necks of the bottles are then frozen and the corks removed to disgorge the accumulated sediments. The bottles are refilled with clear champagne from another disgorged bottle, and the product is ready for final packaging and labeling.

Beers and Ales

Beer and ale are produced from cereals and grains. Starches in these substrates are hydrolyzed, in the processes of malting and mashing, to produce fermentable wort as discussed diagrammatically in Figure 10.2 and 10.3. Saccharomyces cerevisiae is a major yeast used in the production of beer and ales.
### Microbial Products of Food

#### Fig. 10.2 Wine Making

<table>
<thead>
<tr>
<th>Process Step</th>
<th>Microbial Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grape pressing</td>
<td>Once grapes are pressed, the sugars in the juice (the must) can be immediately fermented to produce wine.</td>
</tr>
<tr>
<td>Sterilization Yeast addition</td>
<td>First must is treated with a mixture of yeast and a desired strain of the yeast Saccharomyces cerevisiae or S. ellipsoidea is added.</td>
</tr>
<tr>
<td>Fermentation of must</td>
<td>Alcohol production from sugars</td>
</tr>
<tr>
<td>Setting vat</td>
<td>Malolactic fermentation</td>
</tr>
<tr>
<td>Aging</td>
<td>Development of final wine bouquet</td>
</tr>
</tbody>
</table>

#### NOTES

- **Wine Making**
Health Benefits of Fermented Foods

As a Probiotics: *Lactobacillus plantarum* has been linked to reduced inflammatory bowel, small bowel bacterial overgrowth in children, reduced problems for sufferers of irritable bowel syndrome, and had a positive effect on the immune systems of those suffering from HIV. *Bifidobacteria* has been linked to decrease cases of neonatal necrotizing enterocolitis. Many strains of probiotics...
Microbial Products of Food

have been directly linked to reduced bouts of digestive complaints including diarrhea. *Lactobacillus acidophilus* also shown a preventative effect for polyps, adenomas, and colon cancer.

**Source of Nutrition:** Fermentation increase the digestibility and nutritional value of cereals based foods. Cereals based fermented foods protect the body against age-related diseases such as diabetes and cardiovascular diseases. Cereals also contain micronutrients such as vitamin E, folates, phenolic acids, zinc, iron, selenium, copper, manganese, carotenoids, betaine, choline, sulphur amino acids, phytic acid, lignins, lignans, and alkylresorcinols which provide various types of health benefits.

Fermented cereals can also contain a high mineral content and generally have a lower fat percentage than their dairy-based counterparts, but grains are generally lacking in essential amino acids. These forms of beverages can also naturally provide plant-based functional components, such as fibre, vitamins, minerals, flavonoids and phenolic compounds, which can affect oxidative stress, inflammation, hyperglycemia and carcinogenesis.

**Flatulence Reducing Effect:** During fermentation of the beans for preparation of tempe, the trypsin inhibitor is inactivated, and the amount of several oligosaccharides which usually cause flatulence are significantly reduced.

**Anticancerogenic Effect:** Anticarcinogenic effect of fermented foods showing potential role of lactobacilli in reducing or eliminating procarcinogens and carcinogens in the alimentary canal.

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**Check Your Progress**

1. What is SCP?
2. Write a short note on rationale for SCP production.
3. Give a short note on microorganisms as source of SCP.
4. What are mushrooms? Name some of the common edible mushrooms.
5. List the nutritional value of mushrooms.
6. What is multiculture fermentation?

---

**10.3 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS**

1. The term Single-Cell Protein (SCP) describes the protein-rich cell mass derived from microorganisms grown on a large scale for either animal or human consumption.

2. The interest in SCP was generated in the wake of protein deficiency, especially in developing countries. SCP may be used directly as human food supplement.
or it may be used in animal feed. It is imperative that new food sources should be found in order that future generations are adequately fed. A food source that is nutritionally complete and requires a minimum of land, time, and cost to produce is highly desirable. It can be used for protein supplementation of a staple diet by replacing costly conventional source like soymeal and fishmeal to minimize the problem of food scarcity.

3. Microorganisms are an excellent source of SCP over plant and animal source of proteins because of the following reasons:
   - Microorganisms have rapid growth rate because they have a very short generation time and can thus provide a rapid mass increase.
   - Microorganisms can be easily modified genetically to produce cells that bring about desirable results.
   - Microorganism’s ability to use very inexpensive raw materials readily available in large quantities.
   - SCP production can be carried out in continuous culture and thus be independent of climatic changes.
   - SCP has a good nutritional value (high content of protein containing all the essential amino acids, some SCPs are good source of vitamins, particularly B group of vitamins).
   - The greater speed and efficiency of microbial protein production compared to plant and animal sources.

4. Mushrooms have been consumed since earliest history; ancient Greeks believed that mushrooms provided strength for warriors in battle, and the Romans perceived them as the Food of the Gods. Mushrooms are a group of filamentous fungi that form large, edible fruiting bodies. The fruiting body is called the mushroom and is formed through the association of a large number of individual fungal hyphae to form a mycelium.

There are approximately 14,000 different species of mushroom, many of which are inedible Ganoderma the ‘mushroom of immortality’. The most cultivated mushroom worldwide is Agaricus bisporus, followed by Lentinus edodes, Pleurotus spp., and Flammulina velutipes. Mushrooms production continuously increases, China being the biggest producer around the world Cultiva.

5. Mushrooms are popular valuable foods because they are low in calories, carbohydrates, fat, and sodium: also, they are cholesterol-free. Mushrooms are a good source of proteins, and fiber. Besides, mushrooms provide important nutrients such as the B vitamins riboflavin, pantothenic acid, and niacin. They also provide several important minerals such as selenium, ergothioneine, copper, and potassium.

6. In multiculture fermentations, more than one microorganism is required, belonging taxonomically to different species. An example of this is ragi, in which two fungi (Amylomyces and Rhizopus), yeast and a bacterium are required to make the starter.
10.4 SUMMARY

- The term Single-Cell Protein (SCP) describes the protein-rich cell mass derived from microorganisms grown on a large scale for either animal or human consumption.
- During World War II, when there were shortages of protein and vitamins in the diet, the German produced yeasts and a mold (Geotrichum candidum) in some quantity for food.
- The interest in SCP was generated in the wake of protein deficiency, especially in developing countries.
- SCP may be used directly as a human food supplement or it may be used in animal feed. It is imperative that new food sources should be found in order that future generations are adequately fed.
- A food source that is nutritionally complete and requires a minimum of land, time, and cost to produce is highly desirable.
- Fast-growing microorganisms such as bacteria and yeast have a high concentration of nucleic acid, notably RNA. Nucleic acid removal is not necessary from animal feeds but is from human foods. During purification, nucleic acids must be removed.
- Similar to plant cells, the cell wall of some microorganisms such as algae and yeast contain non-digestible components, such as cellulose. The cells of such kind of SCP should be broken up in order to liberate the cell interior and allow complete digestion.
- Mushrooms have been consumed since earliest history; ancient Greeks believed that mushrooms provided strength for warriors in battle, and the Romans perceived them as the Food of the Gods.
- Mushrooms are a group of filamentous fungi that form large, edible fruiting bodies. The fruiting body is called the mushroom and is formed through the association of a large number of individual fungal hyphae to form a mycelium.
- The mushroom commercially grown in most parts of the world is the basidiomycete Agaricus bisporus, and it is generally cultivated in ‘mushroom farms’.
- The appearance of mushrooms on the surface of the bed is called a flush. Mushrooms must be collected immediately upon flushing.
- Another cultured mushroom is the Shiitake, Lentinus edulis. Shiitake mushrooms are cellulose-digesting fungi that grow on hardwood trees. They are cultivated on small logs.
- There are approximately 14,000 different species of mushroom, many of which are inedible Ganoderma the ‘mushroom of immortality’.
The most cultivated mushroom worldwide is *Agaricus bisporus*, followed by *Lentinus edodes*, *Pleurotus* spp., and *Flammulina velutipes*.

Mushrooms production continuously increases, China being the biggest producer around the world. 

*Cordyceps sinensis* contains substances called cordycepin, cordycepic acid, with therapeutic applications like the effects of increased oxygen utilization, ATP production, and stabilization of blood sugar metabolism.

*Antrodia cinnamomea* is a medicinal mushroom native to Taiwan. Different commercial products are made with this mushroom and it has been used to treat food and drug intoxication, diarrhoea, abdominal pain, hypertension, skin itching, and cancer.

Mushrooms are popular valuable foods because they are low in calories, carbohydrates, fat, and sodium; also, they are cholesterol-free.

Cultivated mushrooms are usually cooked, which kills any harmful bacteria that may have contaminated them from soil, insect pests, rodents or handling during processing.

In 1989, a multistate outbreak of Staphylococcal food poisoning was associated with canned mushrooms imported from the People’s Republic of China.

*Campylobacter*, a leading cause of bacterial enteritis, was isolated from mushrooms sampled in retail markets.

Alcohol Fermentation results in the production of ethanol, and yeasts are the predominant organisms, for example wines and beers.

Lactic Acid Fermentation, for example fermented milks and cereals is mainly carried out by lactic acid bacteria.

Acetic Acid Fermentations in which *Acetobacter* convert alcohol to acetic acid in the presence of excess oxygen.

Alkali Fermentation often takes place during the fermentation of fish and seeds, popularly used as condiment

Monoculture is the fermentations in which one species of microorganism is necessary to produce the product.

In multiculture fermentations, more than one microorganism is required, belonging taxonomically to different species.

In unimulticulture fermentations two or more strains of the same species are used together.

Polyculture are food fermentations in which different microorganisms are many and the species specifically required to make the product are unknown.

Soy sauce is a fermented dark brown liquid, made from a blend of soybeans and wheat. It is popular in Japan, China and used as seasoning. Soy sauces have a salty taste.
Tarhana (Trahanas) is prepared by mixing wheat flour, sheep milk yoghurt, yeast and a variety of cooked vegetables and spices (tomatoes, onions, salt, mint, paprika) followed by fermentation for 1–7 days.

Idli is a traditional fermented food, prepared and consumed in South India and in many parts of Sri Lanka. It is normally consumed with chutney and sambar. Idli made up of a blend of rice and dehulled black gram.

Sake is the traditional cereal based fermented alcoholic beverage of Japan. It is also called as rice wine and popular in Japan and China.

Natural champagnes are produced when the fermentation, resulting in CO₂ formation, is continued in the bottles to produce a naturally sparkling wine.

Beer and ale are produced from cereals and grains. Starches in these substrates are hydrolyzed, in the processes of malting and mashing, to produce fermentable wort.

### 10.5 KEY WORDS

- **Single-Cell Protein**: The term Single-Cell Protein (SCP) describes the protein-rich cell mass derived from microorganisms grown on a large scale for either animal or human consumption.
- **Mushrooms**: Mushrooms are a group of filamentous fungi that form large, edible fruiting bodies.
- **Flush**: The appearance of mushrooms on the surface of the bed is called a flush.
- **Monoculture**: Fermentations in which one species of microorganism is necessary to produce the product.
- **Multiculture**: In multiculture fermentations, more than one microorganism is required, belonging taxonomically to different species.
- **Unimulticulture**: In unimulticulture fermentations two or more strains of the same species are used together.
- **Polyculture**: Polyculture are food fermentations in which different microorganisms are many and the species specifically required to make the product are unknown.

### 10.6 SELF ASSESSMENT QUESTIONS AND EXERCISES

**Short Answer Questions**

1. What is SCP?
2. Give short note on production of SCP.
3. How are microorganisms considered as source of SCP?
4. Write in short about some of the species of mushrooms that are edible.
5. Briefly discuss about the steps involved in mushroom farming.
6. Discuss briefly about traditional cereal based fermented foods.
7. How are fermented food beneficial for health?

Long Answer Questions
1. Explain with the help of table comparison of nutritive values and other parameters for SCP production from algae, fungi and bacteria.
2. Discuss about SCP in detail. Also write about its advantages and disadvantages.
3. Write in detail about mushrooms. Also mention the nutritive value of mushroom and its cultivation.
4. Give a note on food safety.
5. What are oriental foods fermented beverages? Explain in detail.
6. Explain the procedure of production of alcoholic beverages.

10.7 FURTHER READINGS

Enzymes are the biocatalysts synthesized by living cells. They are complex protein molecules that bring about chemical reactions concerned with life. It is fortunate that enzymes continue to function when they are separated from the cells, i.e., in-vitro. Basically, enzymes are non-toxic and biodegradable. They can be produced in large amounts by microorganisms for industrial applications.

Enzyme technology broadly involves production, isolation, purification and use of enzymes for the ultimate benefit of humankind. In addition, recombinant DNA technology and protein engineering involved in the production of more efficient and useful enzymes are also a part of enzyme technology. The commercial production and use of enzymes is a major part of biotechnology industry. The specialties like microbiology; chemistry and process engineering, besides biochemistry have largely contributed for the growth of enzyme technology.

Enzymes have wide range of applications. These include their use in food production, food processing and preservation, washing powders, textile manufacture, leather industry, paper industry, medical applications, and improvement of environment and in scientific research.

In this unit, you will study about industrial production of enzymes: cellulases, amylases, proteases, phytases, pectinases, lipases and glucose isomerases in detail.
11.1 OBJECTIVES

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

- Understand the industrial production of enzymes
- Discuss about various enzymes like cellulases, amylases, proteases, phytases, pectinases, lipases and glucose isomerases

11.2 INDUSTRIAL PRODUCTION OF ENZYMES

Enzymes are macromolecular biological catalysts. Enzymes accelerate chemical reactions. The molecules upon which enzymes may act are called substrates and the enzyme converts the substrates into different molecules known as products. Almost all metabolic processes in the cell need enzyme catalysis in order to occur at rates fast enough to sustain life. Metabolic pathways depend upon enzymes to catalyze individual steps. The study of enzymes is called enzymology and a new field of pseudoenzyme analysis has recently grown up, recognizing that during evolution, some enzymes have lost the ability to carry out biological catalysis, which is often reflected in their amino acid sequences and unusual ‘pseudocatalytic’ properties.

Enzymes are known to catalyze more than 5,000 biochemical reaction types. Most enzymes are proteins, although a few are catalytic RNA molecules. The latter are called ribozymes. Enzymes’ specificity comes from their unique three-dimensional structures.

The protein biocatalysts, called enzymes, used by living cells are responsible for numerous metabolic processes of the cell. The use of microbial enzymes, has been going on for centuries. Since, microorganisms are responsible for the fermentations, of beer, wine, bread, cheese and various vegetables, all these processes are examples of cell-mediated conversions or applications of enzymes. Current technology makes it possible to isolate, purify and even immobilize (bind to a fixed support) the specific enzyme needed for a desired function.

The protein bio-catalyst is responsible for numerous metabolic process of the cell. Fermentations of beer, wine, bread, cheese and various vegetables are examples of microbial cell mediated conversions or application of enzymes.

I. Cellulases

Cellulases break down the cellulose molecule into monosaccharides (simple sugars), such as β-glucose, or shorter polysaccharides and oligosaccharides. Cellulose breakdown is of considerable economic importance, because it makes a major constituent of plants available for consumption and use in chemical reactions. The specific reaction involved is the hydrolysis of the 1,4-β-D-glycosidic linkages in
cellulose, hemicelluloses, lichenin, and cereal β-D-glucans. Most mammals have only very limited ability to digest dietary fibres, such as cellulose by themselves. In many herbivorous animals, such as ruminants like cattle and sheep and hindgut fermenters like horses, cellulases are produced by symbiotic bacteria. Cellulases are produced by a few types of animals, such as some termites, snails and earthworms.

Five general types of cellulases based on the type of reaction catalyzed:

- **Endocellulases** randomly cleave internal bonds at amorphous sites that create new chain ends.
- **Exocellulases or Cellobiohydrolases** cleave two to four units from the ends of the exposed chains produced by endocellulase, resulting in tetrasaccharides or disaccharides, such as cellobiose. Exocellulases are further classified into type I that work processively from the reducing end of the cellulose chain, and type II, that work processively from the non-reducing end.
- **Cellobiases or Beta-Glucosidases** hydrolyse the exocellulase product into individual monosaccharides.
- **Oxidative Cellulases** depolymerize cellulose by radical reactions, as for instance.

Cellulose hydrolyses cellulose to cellulodextrins and glucose. It is widely used for producing fermentable sugar in brewer’s mashes, clarifying orange and lemon juices and concentrates, and tenderizing green beans.

#### II. Amylases

The amylases hydrolyse the starches. Starch is composed of two fractions: amyllose (20–25%), a linear polymer of α-1,4-linked glucose units, and amylopectin (75–80%), a branched polymer containing linear chains of α-1, 4-linked glucose units with branches introduced by occasional α-1, 6-linkages.

**Types of Amylases**

Depending on how they act on the starch molecules, amylases include:

- **α-amylase** are endoenzymes, which randomly hydrolyzed the α-1,4 glucoside linkages (between of glucose units) of amyllose or amylopectin but not the 1,6 - glucosidic linkages of amylopectin resulting in complete degradation, through dextrins to glucose.
- **β-amylases**, are exoenzyme, which splits only the second α-1,4 glucoside linkages from the non-reducing chain ends, detaching one molecule of maltose at a time from the chain and finally leaving dextrins; and
- **Amyloglucosidase** which hydrolyse both 1, 6 and 1, 4 glucoside linkages to produce glucose without intermediate dextrins and maltose. Maltose or α-glucosidase (not an amylase) hydrolyzed maltose to glucose.
Depending on microbial source, amylases include:

**Fungal Amylases**
- **Production by *Rhizopus delemar, Mucor roucii***: In Amylo process, a starchy grain mash is saccharified by amylases produced by the mould growing on it. This process is used mainly in the preparation of mashes for alcoholic fermentation by yeasts.
- **Production by *Aspergillus oryzae* and *A. niger***: Moistened, steamed wheat or rice brand is used for the production of amylase from *A. oryzae* by the tray method, in which the mould is grown on thin layers of the medium in trays, or the drum method, in which the bran is tossed loosely in a rotating drum. A maximal yield is obtained by the tray method in 40 to 48 hour at about 30 degree C in an atmosphere with a high humidity and adequate ventilation.

**Uses**
In the food industries fungal amylases have been employed:
- To remove starch from fruit extracts, for example in the production of pectin from Apple pomace.
- To clarify starch turbidites in wines, beer and fruit juice.
- To convert acid modified starches to sweet syrups.
- To substitute for malt in bread making in aiding leavening and improving dough consistency and hence gas retention.
- To saccharify starch in mashes for alcoholic fermentation.

**Bacterial Amylases**
*Bacillus subtilis* has been the principal bacterium used for the production of amylases. Most bacteria produce more α-amylase than β-amylase. Various culture media or mashes are used or the production of amylases, ranging from a complex medium, such as thin stillage, a byproduct of alcohol production from grains, to a simpler medium consisting of soluble or hydrolyzed starch, ammonium salts, and buffers.

Incubation temperatures from 25 to 37 degree C from 2-6 days have been employed in the tray method, and 30 to 40 degree C from 24-48 hour in the submerged method. The optimum pH for the fermentation medium is 7.0. Calcium carbonate is used to maintain the pH near neutrality, since the amylase gets denatured below pH 6.

Bacterial amylases are purified and concentrated by dialysis, condensation and fractional precipitation.
Uses
Bacterial amylase, chiefly the alpha type, has been used:

- In brewing to produce dextrins of low fermentability and also to clarify the beer.
- In the manufacture of corn syrup and chocolate syrup to prevent thickening by dextrinizing the starch present.

III. Proteases
The proteolytic enzymes or protease is the mixtures of proteinases which catalyse the hydrolysis of the protein molecule into large polypeptides and the peptidases which further hydrolyze these polypeptides fragments to amino acids.

Bacterial Protease
Cultures: Bacillus Subtilis: A high yield strain is selected, and
Method of Production: Submerged culture method
The Medium or Mash: special culture media containing fairly high content of carbohydrate (2 to 6 percent), protein, and also mineral salts, is employed.
Incubation: 3 to 5 days at about 37°C with adequate degree of aeration to favour the production of protease over that of amylase.
Recovery of the Enzyme: The filtrate from the culture is concentrated and the enzymes are used in this form, purified further, or absorbed into some inert material, such as saw dust. The enzyme mixture also contains varying amounts of amylases.

Uses: The proteases are used primarily for their proteinase activity. Bacterial proteases have been applied to the digestion of fish livers to liberate fish oil, to the tenderization of meat and to the clarification and maturing of malt beverages.

Fungal Protease
Culture: Aspergillus oryzae is a good source of protease.
Medium: Many different media containing wheat bran, soybean cake, alfalfa meal, middlings, yeast and other materials.
Method of Production: Either semi solid culture or submerged culture method.
Recovery of the Enzyme: By extraction, concentration and precipitation.
Use: Fungal proteases are active in the manufacture of soy sauce and other Oriental mould fermented foods and may be added to bread dough, where along with amylase, they help improve the consistency of the dough, they also may be used for chill-proofing beer and ale by removal of protein haze, for the tenderization of meat, for thinning egg white so that it can be filtered before drying, and for the hydrolysis of the gelatinous protein material in fish waste and press water to facilitate concentration and drying.
IV. Pectinases

Pectin is methylated polygalacturonic acid, which is important in food industries because of its ability to form gels with sugar and acid. In jellies this characteristic is desirable, but it’s not desirable in fruit juices.

**Enzymes Involved in the Hydrolysis of Pectin:** Pectinesterases which hydrolyze pectin to methanol and polygalacturonic acid (pectic acid) and polygalacturonase, which hydrolyze polygalacturonic acid to monogalacturonic acid. Further hydrolysis would yield sugars and other products.

**Pectinase from Moulds:** Only the fungal type of pectinase is produced industrially from moulds, such as species of *Aspergillus*, *Penicillium* and other genera.

**Medium:** These fungi grow on beet sugar molasses because this material contains pectins.

**Culture Method:** Semisolid culture can be used by employing *A. niger*.

**Medium Used:** The mycelium is developed on a medium containing pectin or a pectin like compound; a nitrogen source, such as plant, yeast, or malt extract, ammonia, peptone, etc. and mineral salts.

**Recover of Enzyme:** The mycelium is harvested, softened and extracted, and the crude enzyme mixture thus obtained may be precipitated and concentrated.

**Pectinase from Bacteria:** Pectinase is yielded by various bacteria, including the clostridia. However, Pectin is produced mostly by moulds.

**Uses:** Used in the food industries for the clarifications of fruit juice, wines, vinegars, syrups, and jellies that may contain suspected pectic material. Treatment of fruit juices with pectinase helps prevent jelling of the juices upon concentration. The addition of pectinase to crushed fruit, for example grapes aids in the expression of juice and results in wines that clarify readily.

V. Lipases

Lipases (glycerol ester hydrolases) split fats (glycerol esters) into di- or monoglycerides and fatty acids.

These are usually extracellular. Most of lipases are produced adaptively in the presence of oils and fats. However, some organisms like *Penicillium roqueforti* are reported to secrete lipase constitutively and in the presence of fats its enzymes production is repressed. Species of *Aspergillus*, *Mucor*, *Rhizopus*, *Penicillium*, *Geotrichum* and yeasts (*Torulopsis* and *Candida*) are reported to be good producers.

Similarly species of *Pseudomonas*, *Achromobacter* and *Staphylococcus* are good producers of lipases. Lipases are generally bound to the cells and hence inhibit on over production, but addition of cation such as magnesium ion liberates the lipase and leads to a higher enzyme titer in the production process. Catalyze the hydrolysis of fats (lipids).
**Uses**

Microbial lipase removes fat from yolk residues in dried egg albumen, assists mould spores in product of blue cheese flavour in spreads and adds to the flavour of milk chocolate. Use in applications, such as baking, laundry detergents and even as biocatalysts in alternative energy strategies to convert vegetable oil into fuel.

High enzyme activity lipase can replace traditional catalyst in processing biodiesel

**VI. Glucose Isomerase**

Glucose isomerase causes the isomerization of glucose to fructose. The reaction is reversible and a mixture of glucose and fructose is produced. The ratio of these products depends on the enzyme used and reaction conditions such as incubation time, pH and temperature. The enzyme has become of commercial value because price of sugar has increased as compared to that of starch.

Converts glucose to fructose and is used in the corn milling industry. Commercially, Streptomyces or Bacillus coagulans is used.

**Fermentative Production**

Glucose isomerase is used in production of High Fructose Syrup (HFCs) or isosyrup must fulfill the following criteria:

- Low pH optimum to avoid side reactions.
- High specific activity.
- High temperature optima.

Some of the Glucose isomerase producers are as follows:

- *Bacillus coagulans*
- *Streptomyces phaeochromogenes*
- *Streptomyces rubiginosus*
- *Streptomyces olivochromogenes*
- *Arthrobacter spp.*
- *Actinoplanes missouriensis*
- *Microbispora rosea*
- *Micromonospora coerula*
- *Nocardia asteroides*
- *Nocardia dassonvillei*
- *Brevibacterium imperiale*
- *Flavobacterium arborescens*
Industrial Production of Enzymes

Check Your Progress

1. Define the term enzyme.
2. On what does metabolic pathway depend?
3. Give the types of cellulases based on the type of reaction catalysed.
4. List the types of amylases.
5. What is the use of fungal amylases?
6. Give the culture, medium, production method and recovery of enzyme of fungal protease.
7. How is glucose isomerase used in production?

11.3 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Enzymes are macromolecular biological catalysts. Enzymes accelerate chemical reactions. The molecules upon which enzymes may act are called substrates and the enzyme converts the substrates into different molecules known as products.

2. Metabolic pathways depend upon enzymes to catalyze individual steps.

3. Five general types of cellulases based on the type of reaction catalyzed:
   - Endocellulases randomly cleave internal bonds at amorphous sites that create new chain ends.
   - Exocellulases or Cellobiohydrolases cleave two to four units from the ends of the exposed chains produced by endocellulase, resulting in tetrascarbohydres or disaccharides, such as cellobiose. Exocellulases are further classified into type I that work processively from the reducing end of the cellulose chain, and type II, that work processively from the non-reducing end.
   - Cellobiases or Beta-Glucosidases hydrolyse the exocellulase product into individual monosaccharides.
   - Oxidative Cellulases depolymerize cellulose by radical reactions, as for instance.

4. Depending on how they act on the starch molecules, amylases include:
   - α-amylase are endoenzymes, which randomly hydrolyzed the α-1,4 glucoside linkages (between of glucose units) of amyllose or amylopectin but not the 1,6 - glucosidic linkages of amylpectin resulting in complete degradation, through dextrins to glucose.
Industrial Production of Enzymes

5. In the food industries fungal amylases have been employed:
   - To remove starch from fruit extracts, for example in the production of pectin from Apple pomace.
   - To clarify starch turbidites in wines, beer and fruit juice.
   - To convert acid modified starches to sweet syrups.
   - To substitute for malt in bread making in aiding leavening and improving dough consistency and hence gas retention.
   - To saccharify starch in mashes for alcoholic fermentation.

6. Culture: *Aspergillus oryzae* is a good source of protease.
   - Medium: Many different media containing wheat bran, soybean cake, alfalfa meal, middlings, yeast and other materials.
   - Method of Production: Either semi solid culture or submerged culture method.
   - Recovery of the Enzyme: By extraction, concentration and precipitation.

7. Glucose isomerase is used in production of High Fructose Syrup (HFCs) or isosyrup must fulfill the following criteria:
   - Low pH optimum to avoid side reactions.
   - High specific activity.
   - High temperature optima.

11.4 SUMMARY

- The protein biocatalysts, called enzymes, used by living cells are responsible for numerous metabolic processes of the cell. The use of microbial enzymes, has been going on for centuries.
- Current technology makes it possible to isolate, purify and even immobilize the specific enzyme needed for a desired function.
- The protein bio-catalyst called is responsible for numerous metabolic process of the cell.
- Fermentations of beer, wine, bread, cheese and various vegetables are examples of microbial cell mediated conversions or application of enzymes.
Industrial Production of Enzymes

NOTES

- Cellulases break down the cellulose molecule into monosaccharides (simple sugars), such as \( \beta \)-glucose, or shorter polysaccharides and oligosaccharides.
- Cellulose breakdown is of considerable economic importance, because it makes a major constituent of plants available for consumption and use in chemical reactions.
- The specific reaction involved is the hydrolysis of the 1, 4-\( \beta \)-D-glycosidic linkages in cellulose, hemicelluloses, lichenin, and cereal 1, 4-\( \beta \)-D-glucans.
- Cellulases are produced by a few types of animals, such as some termites, snails and earthworms.
- Endocellulases randomly cleave internal bonds at amorphous sites that create new chain ends.
- Exocellulases or Cellobiohydrolases cleave two to four units from the ends of the exposed chains produced by endocellulase, resulting in tetrasaccharides or disaccharides, such as cellobiose.
- Exocellulases are further classified into type I that work processively from the reducing end of the cellulose chain, and type II, that work processively from the non-reducing end.
- Cellobiases or Beta-Glucosidases hydrolyse the exocellulase product into individual monosaccharides.
- Oxidative Cellulases depolymerize cellulose by radical reactions, as for instance.
- \( \alpha \)-amylase are endoenzymes, which randomly hydrolyzed the \( \alpha \)-1,4 glucoside linkages (between of glucose units) of amylose or amylpectin but not the 1, 6 - glucosidic linkages of amylopectin resulting in complete degradation, through dextrins to glucose.
- \( \beta \)-amylases, are exoenzyme, which splits only the second \( \alpha \)-1,4 glucoside linkages from the non-reducing chain ends, detaching one molecule of maltose at a time from the chain and finally leaving dextrin.
- Amyloglucosidase which hydrolyse both 1, 6 and 1, 4 glucoside linkages to produce glucose without intermediate dextrins and maltose. Maltose or \( \alpha \)-glucosidase (not an amylase) hydrolyzed maltose to glucose.
- In Amylo process, a starchy grain mash is saccharified by amylases produced by the mould growing on it. This process is used mainly in the preparation of mashess for alcoholic fermentation by yeasts.
- Moistened, steamed wheat or rice brand is used for the production of amylase from \( A.\text{oryzae} \) by the tray method, in which the mould is grown on thin layers of the medium in trays, or the drum method, in which the bran is tossed loosely in a rotating drum.
- A maximal yield is obtained by the tray method in 40 to 48 hour at about 30 degree C in an atmosphere with a high humidity and adequate ventilation.
• *Bacillus subtilis* has been the principal bacterium used for the production of amylases. Most bacteria produce more α-amylase than β-amylase.

• The proteolytic enzymes or protease is the mixtures of proteinases which catalyse the hydrolysis of the protein molecule into large polypeptides and the peptidases which further hydrolyze these polypeptides fragments to amino acids.

• Pectin is methylated polygalacturonic acid, which is important in food industries because of its ability to form gels with sugar and acid. In jellies this characteristic is desirable, but it’s not desirable in fruit juices.

• Lipases (glycerolesterhydrolases) split fats (glycerolesters) into di-or monoglycerides and fatty acids.

• Microbial lipase removes fat from yolk residues in dried egg albumen, assists mould spores in product of blue cheese flavour in spreads and adds to the flavour of milk chocolate.

• Glucose isomerase causes the isomerization of glucose to fructose. The reaction is reversible and a mixture of glucose and fructose is produced. The ratio of these products depends on the enzyme used and reaction conditions such as incubation time, pH and temperature.

• The enzyme has become of commercial value because price of sugar has increased as compared to that of starch.

11.5 KEY WORDS

• **Enzymes**: Enzymes are macromolecular biological catalysts. Enzymes accelerate chemical reactions.

• **Substrates**: The molecules upon which enzymes may act are called substrates.

• **Products**: The enzyme converts the substrates into different molecules known as products.

• **Enzymology**: The study of enzymes is called enzymology.

11.6 SELF ASSESSMENT QUESTIONS AND EXERCISES

**Short Answer Questions**

1. What are enzymes?
2. How many types of cellulases are there?
3. Write a short note on bacterial amylases.
4. What is fungal protease?
Industrial Production of Enzymes

5. Give a short note on glucose isomerase.
6. Write about the fermentative production of glucose isomerase.

Long Answer Questions

1. Discuss in detail about industrial production of enzymes.
2. Explain about cellulases enzyme in detail.
3. What is amylases enzyme? What are the types of amylases?
4. Elaborate a note on proteases.
5. Explain in detail about pectinases. What are the enzymes involved in the hydrolysis of pectin? Also, give the uses pectinases.
6. Define the term lipases and write about it in detail. Also give its different uses.

11.7 FURTHER READINGS

UNIT 12 FOOD BORNE DISEASES

Structure
12.0 Introduction
12.1 Objectives
12.2 Food Borne Diseases Outbreak
   12.2.1 Outbreak Control Team (OCT)
   12.2.2 Investigation of Foodborne Disease Outbreaks
   12.2.3 Interpretation of Results
12.3 Answers to Check Your Progress Questions
12.4 Summary
12.5 Key Words
12.6 Self Assessment Questions and Exercises
12.7 Further Readings

12.0 INTRODUCTION

Foodborne illnesses are infections or irritations of the GastroIntestinal (GI) tract caused by food or beverages that contain harmful bacteria, parasites, viruses, or chemicals. The GI tract is a series of hollow organs joined in a long, twisting tube from the mouth to the anus. Common symptoms of foodborne illnesses include vomiting, diarrhea, abdominal pain, fever, and chills. Most foodborne illnesses are acute, meaning they happen suddenly and last a short time, and most people recover on their own without treatment. Rarely, foodborne illnesses may lead to more serious complications. Climatic factors influence the growth and survival of pathogens, as well as transmission pathways. Higher ambient temperatures increase replication cycles of food-borne pathogens, and prolonged seasons may augment the opportunity for food handling mistakes - in 32% of investigated food-borne outbreaks in Europe ‘temperature misuse’ is considered a contributing factor.

Foodborne illness is caused by consuming contaminated foods or beverages. Many different disease-causing microbes or pathogens can contaminate foods, so there are many different types of foodborne illnesses. Most foodborne diseases are infections caused by a variety of bacteria, viruses, and parasites. Other diseases are poisonings caused by harmful toxins or chemicals that have contaminated food. Of note many foodborne pathogens also can be acquired through recreational or drinking water, from contact with animals or their environment, or through person-to-person spread. Common symptoms of foodborne illness are diarrhea and/or vomiting, typically lasting 1 to 7 days. Other symptoms might include abdominal cramps, nausea, fever, joint/back aches, and fatigue.

In this unit, you will study about food borne disease outbreaks, objectives of investigation, field investigation, lab testing and preventive measures in detail.
12.1 OBJECTIVES

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

- Understand what food borne disease outbreaks are
- Explain the objectives of investigation
- Discuss about lab testing and preventive measures

12.2 FOOD BORNE DISEASES OUTBREAK

Food-borne diseases are usually caused by ingestion of infected food which may or may not exhibit symptoms of food decay. These diseases have somewhat similar symptoms and are, at times, mistaken for each other. Generally, they are classified into two groups—food infections and food intoxications.

Food Infections

When pathogenic microorganisms or parasites are present in the food at the time of consumption and they grow in the body of host to cause illness, the condition is known as food infection. For example, salmonellosis is a food infection which results due to the ingestion of food (usually meat and poultry) infected with *Salmonella*. Table 12.1 tabulates the various food infections based on its characteristics, etc. The characteristics of various food infections, the causative organism, symptoms and the food involved are also being discussed in Table 12.1 as follows.

<table>
<thead>
<tr>
<th>Type of Food Infection</th>
<th>Causative Organism</th>
<th>Source or Food Involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonellosis</td>
<td><em>Salmonella</em></td>
<td>Faeces of infected animals and human beings</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em> Infection</td>
<td><em>Clostridium perfringens</em></td>
<td>Raw foods, cooked meat</td>
</tr>
<tr>
<td><em>Bacillus cereus</em> gastroenteritis</td>
<td><em>Bacillus cereus</em></td>
<td>Custard, puddings, sauces</td>
</tr>
<tr>
<td><em>Escherichia coli</em> Infection</td>
<td><em>Escherichia coli</em></td>
<td>Coffee substitutes, cheese</td>
</tr>
<tr>
<td><em>Yersinia</em></td>
<td><em>Yersinia pseudotuberculosis</em></td>
<td>Pork, raw milk</td>
</tr>
<tr>
<td>Shigellosis</td>
<td><em>Shigella</em></td>
<td>Milk, macaroni salads, potato, tuna, turkey</td>
</tr>
<tr>
<td>Scarlet Fever, Sore Throat</td>
<td><em>Streptococcus pyogenes</em></td>
<td>Milk, ice cream, eggs, custard, pudding</td>
</tr>
<tr>
<td>Listeriosis</td>
<td><em>Listeria monocytogenes</em></td>
<td>Milk, soft cheese</td>
</tr>
</tbody>
</table>

**Salmonellosis**

Salmonellosis is one of the most common bacterial food infections. In addition, consumption of *Salmonella* can cause two other diseases—typhoid fever, and paratyphoid fever. The salmonellae are Gram-Negative, non-spore-forming bacteria.
that can grow over a wide range of temperature, pH and aw in a suitable medium. They are able to grow at a temperature range of 7ºC – 45ºC, and the optimum temperature required for their growth is 37ºC. They can survive at a pH ranging from 4.1 to 9.0 and the minimum aw needed for their growth is 0.93 – 0.95.

Sources of Salmonella include humans and animals that contaminate the food with these bacteria either directly or indirectly. The major food sources of salmonellae are poultry, their eggs and rodents. There is evidence to show that meat and poultry products, including eggs, form nearly one-third of food involved in Salmonella infections. The risk of contamination for these foods, further, increases if they are kept unrefrigerated for long periods. Fresh meat and meat products like sausages and cured meats when held at room temperature also encourage the growth of Salmonella.

The initial symptoms of Salmonella gastrointestinal infection are headache and chills followed by nausea, vomiting, abdominal pain and diarrhea. Other signs of the disease include watery greenish foul smelling stools, muscular weakness, fever and drowsiness. However, the intensity of infection may vary from mild (as a discomfort) to moderate (as diarrhea) or it may prove fatal in extreme cases.

The preventive measures for Salmonella gastrointestinal infection include the following:

- Avoiding food contamination from sources like infected human beings and animals, carriers and salmonellae contaminated food items.
- Killing the bacteria with thermal processes.
- Preventing the growth of bacteria by use of low temperature. In addition, proper sanitation and hygiene must be maintained.

Clostridium perfringens Gastroenteritis

The prevalence of this illness is very low. It is caused by Clostridium perfringens, which is type A, gram-positive, anaerobic, spore-forming bacteria. Though the optimum temperature needed for its growth is 43ºC – 47 ºC, it can grow at a temperature range of 15ºC – 55ºC and a pH range of 5.0 – 9.0. Common salt and sodium nitrate have an inhibiting action on this bacterium.

Spores of Clostridium perfringens are mostly present in raw foods, soil, sewage and animal faeces. A high Clostridium perfringens count in a food indicates that it has not been handled hygienically. Cooked meat kept unrefrigerated is at risk of contamination by these bacteria.

Symptoms of the disease include acute abdominal pain, diarrhea and flatulence. In rare cases fever, nausea and vomiting also appear. However, these symptoms appear only on the ingestion of millions of viable cells of these bacteria which release a toxin, called enterotoxin, during sporulation in the intestines.

For preventing Clostridium perfringens gastroenteritis, some measures need to be followed. These are as follows:

- Proper and speedy cooling of cooked foods, especially meat and meat products.
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- Keeping hot food hot.
- Reheating of leftover food.
- Following hygienic practices in food handling.

**Vibrio parahaemolyticus Infection**

*Vibrio parahaemolyticus* is a gram-negative, straight or curved motile rod. It requires sodium chloride (common salt) in a concentration of 1 – 3 per cent for its growth and can continue to grow even at a 7 per cent concentration of salt. The optimal temperature for its growth is 35ºC – 37ºC; but it remains active at a temperature range of 10ºC – 44ºC and a pH range of 5.0 – 11.0. Main sources of this microorganism are uncooked seafood, saltwater fish, shellfish, crustacea, and fish products. Incubation period for the infection is generally 12 hours, but it may range from 2 – 48 hours. Symptoms of the disease include pain in the abdomen, watery stools with blood and mucus, nausea vomiting, low grade fever, chills and headache. However, the recovery is fast and it takes only 2 – 5 days to recover from this infection.

The seafood must be cooked properly and held at low temperature for preventing contamination with *Vibrio parahaemolyticus*. In addition, the equipment used should be sanitized properly. Cross contamination from saltwater fish to other foods must be prevented and one must refrain from using seawater for rinsing foods or for cleaning purposes.

**Escherichia coli Infection**

*E. coli* is generally considered as a normal intestinal flora for human beings and many animals yet, it can result in diarrheal diseases. Some of its strains produce an enterotoxin and cause cholera-like symptoms, such as rice-water stools, vomiting, dehydration and shock in humans. Others consist of invasive strains that produce cytotoxin and result in dysentery-like syndrome with symptoms like fever, chills, headache, cramps in abdomen and watery diarrhea. The incubation period for this infection is 8 to 24 hours with an average 11 hours for the invasive type and 26 hours for the enterotoxigenic type. However, the ingested food (like salmon, cheese, coffee substitute) needs to be highly contaminated for the symptoms to appear. The desired temperature for *E. coli* ranges from 10ºC – 40ºC, the optimum being 37ºC. It can grow at a pH ranging from 4.0 – 8.5, but the optimum growth takes place at a pH between 7.0 – 7.5.

**Food Intoxications**

Food intoxications, also known as food poisoning, are caused by consuming food contaminated with toxins. The toxic substances are usually produced in food before consumption as by-products of microorganisms. Food poisoning may result from bacterial, fungal, viral, parasitic or chemical sources. The characteristics of some food intoxications along with the causative organism, symptoms and the food involved are described in Table 12.2.
### Table 12.2 Characteristics of Some Food Intoxications

<table>
<thead>
<tr>
<th>Type of Food Intoxication</th>
<th>Causative Organism</th>
<th>Source or Food Involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Botulism</td>
<td>Clostridium botulinum</td>
<td>Canned food</td>
</tr>
<tr>
<td>Staphylococcal Intoxication</td>
<td>Staphylococcus aureus</td>
<td>Custard and cream filled bakery products</td>
</tr>
<tr>
<td>Mycotoxins (produced by fungi)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aflatoxin</td>
<td>Aspergillus flavus</td>
<td>Barley, corn, millet, oats, peanuts, rice, soybeans</td>
</tr>
<tr>
<td>Patulin</td>
<td>Pencillium</td>
<td>Apple sap, apple cider, apple juice</td>
</tr>
<tr>
<td>Ochratoxin</td>
<td>Aspergillus</td>
<td>Corn, wheat, barley, bread, eggs</td>
</tr>
<tr>
<td>Luteoskyrin</td>
<td>Pencillium toxiciscidii</td>
<td>Mold, rice flour</td>
</tr>
<tr>
<td>Penicillic acid</td>
<td>Pencillium</td>
<td>Dried beans, tobacco</td>
</tr>
<tr>
<td>Alimentary Toxic Aleukia (ATA)</td>
<td>Cladosporium, Pencillium, Mucor, Fusarium</td>
<td>Grain,</td>
</tr>
<tr>
<td>Roquefortite</td>
<td>Pencillium roqueforti</td>
<td>Blue cheese, Roquefort cheese</td>
</tr>
<tr>
<td>Viruses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infectious Hepatitis</td>
<td>Infectious hepatitis virus</td>
<td>Milk, other beverages</td>
</tr>
<tr>
<td>Poliomyelitis</td>
<td>Poliovirus types I, II, III</td>
<td>Milk, other beverages</td>
</tr>
<tr>
<td>Rickettsias</td>
<td>Coxiella burnett</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>Infections due to Food-Borne Parasites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoebias</td>
<td>Endamoeba histolytica</td>
<td>Water, food contaminated with human feces</td>
</tr>
<tr>
<td>Tapeworm</td>
<td>Taenia saginata</td>
<td>Insufficiently cooked beef</td>
</tr>
<tr>
<td>Trichinosis</td>
<td>Trichinella spiralis</td>
<td>Insufficiently cooked pork and products</td>
</tr>
<tr>
<td>Seafood Toxicants</td>
<td>Gonyaulax catenella</td>
<td>Toxic algae</td>
</tr>
<tr>
<td>Poisoning by Chemicals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arsenic, Lead</td>
<td>Fruit sprays</td>
<td>Fruits</td>
</tr>
<tr>
<td>Flouride</td>
<td>Insecticide sodium fluoride</td>
<td>Flour, dry milk, baking powder</td>
</tr>
</tbody>
</table>

**12.2.1 Outbreak Control Team (OCT)**

When two or more people get the same illness from the same contaminated food or drink, the event is called a foodborne outbreak. Outbreaks provide important...
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Self-Instructional Material

insights into how pathogens (germs, chemicals, or toxins) are spread, which food and pathogen combinations make people sick, and how to prevent food poisoning. Each year, Centre for Disease Control (CDC) summarizes foodborne disease outbreak data in an annual surveillance report and makes it available to the public through the NORS (National Outbreak Reprtng System) Dashboard. Highlights from annual surveillance report for foodborne disease outbreaks, United States, 2016 are given below:

- In 2016, 839 foodborne disease outbreaks were reported, resulting in 14,259 illnesses, 875 hospitalizations, 17 deaths, and 18 food recalls.
- Norovirus was the most common cause of confirmed, single-etioloogy outbreaks, accounting for 145 (36%) outbreaks and 3,794 (42%) illnesses.
- Salmonella was the next most common cause, accounting for 132 (33%) outbreaks and 3,047 (33%) illnesses, followed by Shiga toxin-producing Escherichia coli, which caused 25 (6%) confirmed, single-etioloogy outbreaks and 366 (4%) illnesses.
- Fish (26 outbreaks), mollusks (21), and dairy (19) were the most common single food categories implicated. The most outbreak-associated illnesses were from mollusks (529 illnesses), pork (438), and grains and beans (383).
- As reported in previous years, restaurants (459 outbreaks, 61% of outbreaks reporting a single location of preparation), specifically restaurants with sit-down dining (363, 48%), were the most commonly reported locations of food preparation associated with outbreaks.

Outbreak Control Team

A Multidisciplinary Outbreak Control Team (OCT) is involved in such an outbreak. The role of the OCT is to coordinate all the activities involved in the investigation and control of an outbreak. This may involve:

- Deciding whether there is really an outbreak.
- Deciding on the type of investigations to be conducted.
- Case-finding and interviews.
- Planning the appropriate clinical and environmental sampling.
- Ensuring that all collaborators use a complementary methodology.
- Conducting an environmental investigation of suspected food premises.
- Agreeing and implementing control measures to prevent the further spread by means of exclusions, withdrawal of foods, closure of premises, etc.
- Working in concert with local medical providers to make recommendations on treatment and/or prophylaxis.
- Organizing ongoing communications among OCT members about the outbreak.
• Producing reports, including lessons learned, for health authorities and other interested parties.
• Requesting external assistance, for example secondment of a national investigation team.

Members of an Outbreak Control Team

Outbreak control team comprises of a field group, a laboratory group and a person in charge.
• The field group interviews persons, both ill and healthy, who consumed the suspected foods, physicians and nurses who are treating the victims, personnel at the place of exposure to the disease; collects samples of suspected foods and transmit them to the laboratory; collects specimens from patients or foods handlers; inspects the premises where the foods were stored, prepared and served; ascertains where suspected foods were purchased and the conditions there; and fills out appropriate reports on these activities, to be made available to person in charge and to the laboratory staff.
• The Laboratory group makes such microbiological and chemical tests as are indicated by the reports of the field group and the nature of the suspected food and records its findings on appropriate report blanks.
• A Person in charge is a public health practitioner or epidemiologist then can interpret the data from all sources to determine the cause and the source of the disease outbreak.

At times cooperation may be needed between the epidemiologist, the health officer, physicians, nurses, clinician, veterinarian; sanitarians, sanitary engineers, laboratory technicians, statisticians in an investigation.

12.2.2 Investigation of Foodborne Disease Outbreaks

Foodborne disease outbreaks are investigated to prevent both ongoing transmissions of disease and similar outbreaks in the future.

Objectives of Investigation
• Control of ongoing outbreaks.
• Detection and removal of implicated foods.
• Identification of specific risk factors related to the host, the agent and the environment.
• Identification of factors that contributed to the contamination, growth, survival and dissemination of the suspected agent.
• Prevention of future outbreaks and strengthening of food safety policies.
Acquisition of epidemiological data for risk assessment of food borne pathogens.

Stimulation of research that will help in the prevention of similar outbreaks.

Field Investigation

Preliminary Assessment of the Situation

Investigation of a potential outbreak starts with the reporting of an outbreak to the health department. The assessment of all available information to confirm the existence of an outbreak must be initiated quickly (because samples or specimens may be available for only a short time) and completed promptly (in order to prevent further illnesses), and should include:

- Checking the validity of the information.
- Identifying cases and obtaining information about them.
- Ensuring the collection of appropriate clinical specimens and food samples.

Gathering Information

Once the validity of the reporting source has been verified, a group of the initial cases are interviewed as soon as possible. The interviews should include questions about:

- Clinical details, including date of onset, duration and severity of symptoms.
- Contact with other ill persons.
- Food consumption history.
- The respondent’s thoughts on what caused their illness.
- Whether the respondent knows others with the same or a similar illness.
- Potential common exposures among those who have the same or a similar illness.

Collection of Specimens

Samples from Human Sources (Clinical Samples)

Diagnosis of most infectious diseases can be confirmed only if the etiological agent is isolated and identified from ill persons. Clinical samples should be taken from ill persons as well as food handlers. Whenever possible, they should be taken from individuals who have not received antibiotic treatment for their illness. Cultures from the nose or throat or from skin lesions of food handlers are made to test for staphylococci able to produce enterotoxin. Faecal samples are used to test Salmonella and Shigella spp., capable of causing enteric infections; the tests might be to find carriers among food handlers or to identify the cause of illness in patients. Blood samples from patients for serological tests for the identification and typing of certain pathogens, for example Salmonella spp. Vomitus may be
tested when chemical poisoning is suspected. Specimens should be collected from persons who have been interviewed so that a link can be made between the laboratory and the epidemiological investigations. Clinical specimens should be collected at the time of first contact because many of the pathogens and toxins that cause foodborne disease remain in the intestinal tract for only a short time after the onset of illness.

Collection of Food Samples

Samples of all leftover foods and beverages served at the suspected meal or meals should be taken aseptically into sterile, and samples of perishable foods should be refrigerated immediately and kept cold in transit to the laboratory. If a packaged food item is suspected of being involved in an outbreak, entire packages of foods may be taken. This can help to establish whether the food was contaminated before its receipt at the site of preparation. If no foods are left from a suspect meal such as food scraps from plates on which the food was served or samples from serving container, then any ingredients and raw items that are still available can be sampled, rinsings, garbage or food handled in the same manner as the suspected food can also be sampled. If a canned food is involved, part of the used can of food is taken, but only a sample from the same lot must be taken. Brand and lot number should be obtained from the can of the commercially canned food, and the method of canning and heating of the home-canned food should be ascertained. It is essential to label each sample to give information as to the type of food, the place and time of sampling, reason for its collection, the organism or chemical suspected.

During a foodborne disease outbreak, investigation of a food establishment will often require:

- Interviewing any employees who may have had a role in the processing or preparation of suspected foods.
- A review of employee records (to determine whether some were out ill during the period of interest).
- A review of the overall operations and hygiene.
- A specific assessment of procedures undergone by a suspect food.
- A review of food worker health and hygiene, including specimens for analysis.
- An assessment of the water system and supply.
- Measurement of temperatures, pH and water activity ($a_w$) with appropriate equipment.

Appropriate food and environmental samples should be taken as early as possible since the amount of physical evidence will diminish with time. The laboratory should be alerted in advance of sample collection and can provide sampling materials appropriate to the type and quantity of specimens to be collected, their storage, packaging and transport.
Laboratory Investigations

The procedure to be followed in testing the samples of food or specimens from the human sources upon receipt in the laboratory will depend on the type of food and the information available about the outbreak of food illness. Microscopic examination of smear prepared from sample may give a clue to the causative organism and may indicate the numbers in the original food if sample has been properly refrigerated.

The role of the clinical laboratory in foodborne disease outbreak investigations includes:

- Ensuring that appropriate clinical specimens are collected
- Arranging appropriate laboratory investigations of clinical samples
- To identify and characterize the pathogen involved in the outbreak

The role of the food laboratory in foodborne disease outbreak investigations includes:

- Advising on appropriate samples to be taken from food
- Performing appropriate laboratory investigations of the food to identify the suspect pathogens, toxins or chemicals
- Additional characterization of organisms (e.g. serotyping, phage typing, molecular subtyping)

Molecular Typing

Molecular microbiology technology has contributed substantially to improvements in the detection and investigation of foodborne disease outbreaks.

- **Polymerase Chain Reaction (PCR)** technology is increasingly being used for the rapid identification of pathogens and in many cases allows determination of subtypes that previously required time-consuming and resource-intensive methods.

- **Pulsed-Field Gel Electrophoresis (PFGE)** can provide ‘DNA fingerprints’ of bacterial isolates; if the PFGE patterns of clinical and food specimens are the same. PFGE can also help investigators to include related cases and exclude concurrent cases that are epidemiologically unrelated to an outbreak. Such subtyping can be particularly useful when a pathogen implicated in an outbreak is very common and its presence in related specimens (for example, cases, food and farm animals) may be purely coincidental.

- **Genetic sequencing** technology has been useful for assessing the relatedness of various pathogens involved in outbreaks of foodborne and waterborne disease. For example, sequencing of hepatitis A viruses collected during three large outbreaks associated with green onions demonstrated that similar virus strains caused all three outbreaks and were related to
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hepatitis A strains commonly isolated from patients living in the region where the green onions were grown.

- Sequencing of noroviruses is also becoming increasingly useful in identifying relatedness among potential outbreak-associated viruses.

Chemical Investigations

In acute chemical exposures, most toxins or their metabolites are rapidly cleared from easily accessible specimens such as blood; prompt collection and shipment of specimens is therefore of critical importance. When collecting samples for chemical analyses it is important to make arrangements in advance for chemical samples to be analysed.

12.2.3 Interpretation of Results

The results of the field and laboratory investigations, lead to identification of the guilty food and location and elimination of the ultimate source of the cause of the food borne disease outbreak. When an outbreak is small as within a family; the location of the food responsible for illness is fairly simple. When the numbers of person involved in the outbreak is large, it is difficult to obtain complete and accurate data. Not all the person may be available for interviewing, not all persons are equally susceptible to food illness, and people are forgetful just about what they ate.

Usually local health department officials are responsible for most of the epidemiological investigations and the completion of the initial reports. This information is forwarded to state health departments and sent to the CDC. Consideration of data for confirmation of outbreaks is based on clinical, laboratory and epidemiological criteria.

One of the main purposes of the investigation is to locate the source of the agent causing the food illness so that this source can be eliminated and future outbreaks prevented. The location of the source also usually requires an analysis of both field and laboratory data.

Control Measures

Based on accurate information control measures should be guided such as recalling a food product or closing food premises.

Control of Source

Once investigations have identified an association between a particular food or food premises and transmission of the suspected pathogen, measures should be taken to control the source.

Steps may include:

- Removing implicated foods from the market (food recall, food seizure).
- Modifying a food production or preparation process.
- Closing food premises or prohibiting the sale or use of foods.
Removing Implicated Foods from the Market

The objective of food recall and food seizure is to remove implicated foods as efficiently, rapidly and completely as possible from the market. The extent of recall will depend on the potential risk to the consumer. A recalling business may conduct a recall to the level of the retailer or, if public health is seriously jeopardized, to the level of the individual consumer:

- A food recall is undertaken by any business responsible for the manufacture, wholesale distribution or retailing of the suspect food – from large corporations or partnerships to family-owned businesses – and may be initiated by the business itself or undertaken at the request of an appropriate health authority.
- Food seizure is the process by which an appropriate authority like Government regulatory agencies removes a food product from the market if the business does not comply with the request to recall.

The longer the time that passes between a food appearing on the market and it being identified as a potential source, the less likely is recovery of that food.

The shelf-life of a food product will affect how much of it will be recovered:

- Most establishments ship fresh products (fresh meat, poultry, milk, etc.) to distributors on the day that they produce it, and distributors will quickly pass it on to hotels, institutions, retail stores and restaurants. The product is generally consumed within 3 to 7 days of production and the likelihood of recovery is poor.
- Frozen or shelf-stable food products (for example, cans, dried foods, packaged foods) are more likely to be recovered as there is less urgency to move them through the system.

Thus, if these types of product are recalled, there is a good possibility that they will still be with distribution or retailers or on the consumers shelves.

Once the appropriate authorities have decided to recall a food product, they should:

- Ensure appropriate public notification.
- Monitor the progress and effectiveness of the recall.
- Ensure that corrective actions are taken by the recalling business.

Notification to the Public

Information to the public should include:

- The nature of the problem, the reason for recall of the product, and information about how the problem was discovered.
- Name and brand of the food product (including labelling) being recalled.
- Name and location of the producing establishment and point of contact.
- Locations where the product is likely to be found.
- A description of common symptoms of the illness associated with the suspected pathogen or contaminant.
- Actions that consumers should take to prevent further exposure and illness.
- Actions that consumers should take if illness occurs.

Post-Recall Reporting by the Business

After implementation of a food recall, the business should provide the food safety committee or other appropriate authorities with interim and final reports about the recall, which should contain the following information:
- Circumstances leading to recall.
- Action taken by the business.
- Result of recall (percentage of stock recovered or accounted for).
- Method of disposal or reprocessing of recovered stock.
- Action proposed for the future to prevent a recurrence of the problem.

Modifying a Food Production/Preparation Process

Once food investigations identify faults in production or preparation processes that may have contributed to the outbreak, corrective action must be taken immediately to avoid recurrences. Examples of corrective actions are modification of a recipe or of a process, reorganization of working practices, change in storage temperatures.

Closing Food Premises

If site inspections reveal a situation that poses a continuing health risk to consumers, it may be advisable to close the premises until the problem has been solved.

Control of Transmission

If a contaminated food product cannot be controlled at its source, steps need to be taken to eliminate or minimize the opportunities for further transmission of the pathogen. Depending on the situation, appropriate public advice may be issued during a period of hazard, for example:

Public Advice

**Personal Hygiene:** Advice on personal hygiene should be issued to all individuals with gastrointestinal disease and should include the following:
- Avoid preparing food for other people until free from diarrhea or vomiting.
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Keep clean (thoroughly wash your hands before handling food and often during food preparation, wash and sanitize all surfaces and equipment used for food preparation, wash hands after defecation, urination and before meals. Thorough hand washing with soap in warm running water and drying is the most important factor in preventing the spread of enteric diseases.

- Use your own separate towels to dry hands. Institutions, particularly schools, should use liquid soaps and disposable towels or hand-dryers.
- Protect kitchen areas and food from insects, pests and other animals
- Clean toilet seats, flush handles, hand-basin taps and toilet door handles with distinguished after use. If young children are infected, these cleaning procedures must be undertaken on their behalf. Similar arrangements may also be necessary in schools and residential institutions.

Cook Thoroughly

- Cook food thoroughly, especially meat, poultry, eggs and seafood
- Bring foods like soups and stews to boiling to make sure that they have reached 70 degree C.
- For meat and poultry, make sure that juices are clear, not pink.

Keep Food at Safe Temperatures

- Do not leave cooked food at room temperature for more than 2 hours.
- Refrigerate promptly all cooked and perishable food (preferably below 5 degree C).
- Keep cooked food piping hot (more than 60 degree C) prior to serving.
- Do not store food too long even in the refrigerator.
- Do not thaw frozen food at room temperature.

Use Safe Water and Raw Materials

- Use safe water or treat it to make it safe.
- Select fresh and wholesome foods.
- Choose foods processed for safety, such as pasteurized milk.
- Wash fruits and vegetables, especially if eaten raw.
- Do not use food beyond its expiry date.
- Advice to dispose off foods.
Separate Raw and Cooked

- Separate raw meat, poultry and seafood from other foods
- Use separate equipment and utensils such as knives and cutting boards for handling raw foods
- Store food in containers to avoid contact between raw and prepared foods

Exclusion of Infected Persons from Work and School

The risk of infection being spread by infected individuals depends on their clinical picture and their standards of hygiene. People with diarrhea are far more likely to spread infection than asymptomatic individuals with subclinical illness.

In general, the following groups with diarrhea or vomiting should stay away from work or school until they are no longer infectious:

- Food-handlers whose duties involve touching unwrapped foods to be consumed raw or without further cooking or other forms of treatment.
- People who have direct contact with highly susceptible patient or person in whom gastrointestinal infection would have particularly serious consequences (for example, the young the elderly, the immune compromised).
- Children aged under 5 years.
- Older children and adults with doubtful personal hygiene or with unsatisfactory toilet, hand washing or hand-drying facilities at home, work or school.

Even if clinically well, no person with any of the following conditions should handle unpackaged food:

- Excretor of Salmonella typhi or Salmonella paratyphi.
- Excretor of the etiological agents of cholera, amoebic dysentery or bacillary dysentery.
- Hepatitis A or hepatitis E and all other forms of acute hepatitis until diagnosed as other than hepatitis A or hepatitis E.
- Taenia solium (pork tapeworm) infection.
- Tuberculosis (in the infectious state).

Infection Control Precautions

Infection control precautions for hospitalized and institutionalized persons with infectious diarrhoea include:

- Isolation of patients (for example, in a private room with separate toilet if possible).
NOTES

**Protecting Risk Groups**

Certain groups are at particularly high risk of severe illness after exposure to a foodborne disease. Safe food-handling practices, including strict adherence to thorough hand-washing, should be particularly emphasized to such people. Specific advice for risk groups may be considered in some circumstances. Examples include advice to:

- Pregnant women against consumption of unpasteurized milk, unpasteurized cheeses, and other foods potentially contaminated with Listeria
- Immunocompromised persons, such as those with HIV/AIDS, to avoid eating unpasteurized milk products, raw fish, etc.
- Persons with underlying liver disease to avoid consumption of raw oysters and other food that may transit vibrio bacteria

**Review of Outbreak**

The OCT should formally decide when an outbreak is over and issue a statement to this effect. A structured review should follow all outbreaks for which an OCT is convened and should include a formal debriefing meeting with all parties involved in the investigation. The aims of debriefing are to:

- Ensure that control measures for the outbreak are effective.
- Identify long-term and structural control measures and plan their implementation.
- Assess whether further scientific studies should be conducted.
- Clarify resource needs, structural changes or training needs to optimize future outbreak.
- Identify factors that compromised the investigations and seek solutions.
- Change current guidelines and develop new materials as required.
- Discuss legal issues that may have arisen.
- Arrange for completion of the final outbreak report.

**Outbreak Report**

An interim report should be made available by the OCT 2 to 4 weeks after the end of the investigations, followed by a written final report. The final report should be comprehensive, protect confidentiality and be circulated to appropriate individuals and authorities. The report should follow the usual scientific format of
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an outbreak investigation report and include a statement about the effectiveness of
the investigation, the control measures taken and recommendations for the future.

In addition, a summary report should be completed and forwarded to the
appropriate authorities at national level for collation, analysis and, when appropriate,
reporting to the international level (for example, SalmNet, EnterNet, WHO, etc.)

### Check Your Progress

1. What is foodborne outbreak?
2. Who are the members of an outbreak team?
3. Give the objectives of investigation.
4. What is chemical investigation?
5. What is control of source and what are the steps included?
6. Write a short note on outbreak report.

### 12.3 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. When two or more people get the same illness from the same contaminated
   food or drink, the event is called a foodborne outbreak.

2. Members of an Outbreak Control Team: Outbreak control team comprises
   of a field group, a laboratory group and a person in charge.
   - The field group interviews persons, both ill and healthy, who consumed
     the suspected foods, physicians and nurses who are treating the victims,
     personnel at the place of exposure to the disease; collects samples of
     suspected foods and transmit them to the laboratory; collects specimens
     from patients or foods handlers; inspects the premises where the foods
     were stored, prepared and served; ascertains where suspected foods
     were purchased and the conditions there; and fills out appropriate
     reports on these activities, to be made available to person in charge
     and to the laboratory staff
   - The Laboratory group makes such microbiological and chemical tests
     as are indicated by the reports of the field group and the nature of the
     suspected food and records its findings on appropriate report blanks.
   - A Person in charge is a public health practitioner or epidemiologist
     then can interpret the data from all sources to determine the cause and
     the source of the disease outbreak.
   - At times cooperation may be needed between the epidemiologist, the
     health officer, physicians, nurses, clinician, veterinarian; sanitarians,
     sanitary engineers, laboratory technicians, statisticians in an investigation.
3. Objectives of Investigation

- Control of ongoing outbreaks.
- Detection and removal of implicated foods.
- Identification of specific risk factors related to the host, the agent and the environment.
- Identification of factors that contributed to the contamination, growth, survival and dissemination of the suspected agent.
- Prevention of future outbreaks and strengthening of food safety policies.
- Acquisition of epidemiological data for risk assessment of food borne pathogens.
- Stimulation of research that will help in the prevention of similar outbreaks.

4. Chemical Investigations: In acute chemical exposures, most toxins or their metabolites are rapidly cleared from easily accessible specimens such as blood; prompt collection and shipment of specimens is therefore of critical importance. When collecting samples for chemical analyses it is important to make arrangements in advance for chemical samples to be analysed.

5. Control of Source: Once investigations have identified an association between a particular food or food premises and transmission of the suspected pathogen, measures should be taken to control the source. Steps may include:
   - Removing implicated foods from the market (food recall, food seizure).
   - Modifying a food production or preparation process.
   - Closing food premises or prohibiting the sale or use of foods.

6. Outbreak Report: An interim report should be made available by the OCT 2 to 4 weeks after the end of the investigations, followed by a written final report. The final report should be comprehensive, protect confidentiality and be circulated to appropriate individuals and authorities. The report should follow the usual scientific format of an outbreak investigation report and include a statement about the effectiveness of the investigation, the control measures taken and recommendations for the future.

12.4 SUMMARY

- When two or more people get the same illness from the same contaminated food or drink, the event is called a foodborne outbreak.
- Outbreaks provide important insights into how pathogens (germs, chemicals, or toxins) are spread, which food and pathogen combinations make people sick, and how to prevent food poisoning.
In 2016, 839 foodborne disease outbreaks were reported, resulting in 14,259 illnesses, 875 hospitalizations, 17 deaths, and 18 food recalls.

Norovirus was the most common cause of confirmed, single-etiologic outbreaks, accounting for 145 (36%) outbreaks and 3,794 (42%) illnesses.

Salmonella was the next most common cause, accounting for 132 (33%) outbreaks and 3,047 (33%) illnesses, followed by Shiga toxin-producing Escherichia coli, which caused 25 (6%) confirmed, single-etiologic outbreaks and 366 (4%) illnesses.

The field group interviews persons, both ill and healthy, who consumed the suspected foods, physicians and nurses who are treating the victims, personnel at the place of exposure to the disease; collects samples of suspected foods and transmit them to the laboratory; collects specimens from patients or foods handlers; inspects the premises where the foods were stored, prepared and served; ascertains where suspected foods were purchased and the conditions there; and fills out appropriate reports on these activities, to be made available to person in charge and to the laboratory staff.

The Laboratory group makes such microbiological and chemical tests as are indicated by the reports of the field group and the nature of the suspected food and records its findings on appropriate report blanks.

A Person in charge is a public health practitioner or epidemiologist then can interpret the data from all sources to determine the cause and the source of the disease outbreak.

At times cooperation may be needed between the epidemiologist, the health officer, physicians, nurses, clinician, veterinarian; sanitarians, sanitary engineers, laboratory technicians, statisticians in an investigation.

Diagnosis of most infectious diseases can be confirmed only if the etiologic agent is isolated and identified from ill persons.

Samples of all leftover foods and beverages served at the suspected meal or meals should be taken aseptically into sterile, and samples of perishable foods should be refrigerated immediately and kept cold in transit to the laboratory.

The procedure to be followed in testing the samples of food or specimens from the human sources upon receipt in the laboratory will depend on the type of food and the information available about the outbreak of food illness.

Polymerase Chain Reaction (PCR) technology is increasingly being used for the rapid identification of pathogens and in many cases allows determination of subtypes that previously required time-consuming and resource-intensive methods.
NOTES

Pulsed-Field Gel Electrophoresis (PFGE) can provide ‘DNA fingerprints’ of bacterial isolates; if the PFGE patterns of clinical and food specimens are the same. PFGE can also help investigators to include related cases and exclude concurrent cases that are epidemiologically unrelated to an outbreak.

Genetic sequencing technology has been useful for assessing the relatedness of various pathogens involved in outbreaks of foodborne and waterborne disease.

The objective of food recall and food seizure is to remove implicated foods as efficiently, rapidly and completely as possible from the market.

Once food investigations identify faults in production or preparation processes that may have contributed to the outbreak, corrective action must be taken immediately to avoid recurrences.

If site inspections reveal a situation that poses a continuing health risk to consumers, it may be advisable to close the premises until the problem has been solved.

The risk of infection being spread by infected individuals depends on their clinical picture and their standards of hygiene.

Certain groups are at particularly high risk of severe illness after exposure to a foodborne disease.

Safe food-handling practices, including strict adherence to thorough hand-washing, should be particularly emphasized to such people.

An interim report should be made available by the OCT 2 to 4 weeks after the end of the investigations, followed by a written final report.

The final report should be comprehensive, protect confidentiality and be circulated to appropriate individuals and authorities.

The report should follow the usual scientific format of an outbreak investigation report and include a statement about the effectiveness of the investigation, the control measures taken and recommendations for the future.

12.5 KEY WORDS

- **Foodborne outbreak**: When two or more people get the same illness from the same contaminated food or drink, the event is called a foodborne outbreak.
12.6 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. What is Outbreak Control Team (OCT)?
2. What were the major highlights from annual surveillance report for foodborne disease outbreaks, given by United States in the year 2016?
3. What are the major objectives of investigation?
4. How is field investigation conducted?
5. Write short notes on the following:
   - Gathering Information
   - Collection of Specimens
   - Polymerase Chain Reaction (PCR)
   - Pulsed-Field Gel Electrophoresis (PFGE)
6. How is genetic sequencing done?
7. Why is exclusion of infected persons from work and school important in case if he/she is infected?

Long Answer Questions

1. Write a note on Outbreak Control Team.
2. How is investigation of foodborne disease outbreaks carried out?
3. Give a detailed account on preliminary assessment of the situation.
4. Discuss about the collection of samples from human sources, food samples, and laboratory and chemical investigations.
5. Elaborate a note on interpretation of results, control measures, control of source and removing implicated foods from the market.
6. Discuss about the preventive measure that can be taken to control the transmission of the disease.

12.7 FURTHER READINGS


UNIT 13 FOOD SANITATION

Structure
13.0 Introduction
13.1 Objectives
13.2 Food Sanitation
13.3 Answers to Check Your Progress Questions
13.4 Summary
13.5 Key Words
13.6 Self Assessment Questions and Exercises
13.7 Further Readings

13.0 INTRODUCTION

Food sanitation is the exercise of subsequent measures to avoid the microbial contamination of food processing. The attachment of food pathogenic bacteria to food-contact or food processing surfaces can lead to possible hygienic complications because these food biofilms deliver a basin of contamination to food chain. Microbial species attached to the food surfaces increase the hazard of microbial contamination in food processing and plants. Different cell surface appendages and structures have been involved in biofilm establishment. Cleaning and sanitation in food processing surfaces are significant measures, but disinfectants and other cleaning material alone are not competent to eradicate the anchored biofilm and do not prevent the microbial populations to colonize the surfaces. The prevention of microbial biofilms could be attained through a selection of the constituents to use or through altering them by treatments on surfaces. Of these approaches, surface accustoming by BioSurfactants comes out to be an effective tactic to contest biofilm establishment. Attempts on using BioSurfactants (BS) in food systems increased over the past years; however, for their successful utilization, it is imperative to focus attention in in vivo toxicity. As there are no guidelines indicating the incorporation of BioSurfactants as food additives, the earlier guidelines could be applied for this case, too.

Food safety and hygiene hold great value in hotel and catering industry where food is the prime focus. Food is highly vulnerable to contamination and adulteration due to both human interference and environmental/natural factors. Food is consumed not just for satisfying hunger, but also for taste and enjoyment. All the same, food safety is always a concern for food service provider. It is the first level of defense against food and waterborne diseases. If at any level of food handling, preparation, or storage this aspect is ignored or compromised it can result in major health problems among the customers apart from wastage and losses. The changing food pattern, international trade, public awareness, and expectation have further necessitated the demand for safe and high-quality food in hospitality sector.
In this unit, you will study about food sanitation – microbiology of food plant sanitation, water and milk testing in detail.

13.1 OBJECTIVES

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

- Understand what food sanitation is
- Discuss the microbiology of food plant sanitation
- Analyse the testing of water and milk

13.2 FOOD SANITATION

Food sanitation includes all practices involved in protecting food from risk of contamination, harmful bacteria, toxins, preventing any food spoilage (wherein, the original nutritional value, texture, flavor of the food are damaged), leading to foodborne illness (illness caused by food) of consumers due to food infection (microbial infection resulting from ingestion of contaminated foods), or food intoxication (illness caused by toxins produced by some bacteria); and destroying any harmful bacteria in the food by thorough cooking or processing.

Food sanitation is concern with the following duties:

- Bacteriology of water supply is examined to provide good water for both drinking as well as for plant purposes.
- Microbiology of the food product is examined to prevent contamination of the foods at all stages during processing from equipment, personnel and vermin.
- The general cleanliness and sanitation of plant and premises which includes not only the maintenance of clean and well sanitized surfaces of all equipment touching the foods but also good housekeeping in and about the plant and adequate treatment and disposal of wastes.
- The health of employees which include provision of a potable water supply, supervision of matters of personal hygiene, regulation of sanitary facilities in the plant and in plant operated housing units, and contact with sanitary aspects of plant lighting, heating, and ventilation.

Bacteriology of Water Supplies

Water for Drinking Purposes

The water that the employees drink must meet public health standards when tested by methods recommended in the latest edition of Standard methods for the Examination of Water and Wastewater (American Public health Association, 1985). Coliform bacteria must not be present at levels indicating contamination of the water by sewage.
Water for Plant Use

- Water for plant use must be satisfactory from a bacteriological standpoint for use with the particular food being processed. A water supply may be adjusted potable yet be unsatisfactory for use with a food. For example, water containing appreciable number of psychrophils of the genera *Pseudomonas* or *Alcaligenes* might be unsatisfactory without treatment in a dairy plant making butter or cottage cheese. Therefore, all water that comes into contact with foods should not only meet the bacteriological standards for drinking water, but also be satisfactory from a bacteriological standpoint for use with the particular food being processed.

- Chemical composition of the water must be suited to the use to be made of it. Thus hard water is undesirable in pea canning and brewing; iron and manganese are bad in beet canning and in brewing; excessive organic matter may lead to off-flavors, etc.

- In canning factories if the water in which the cans of processed foods are cooled after their heat treatment contains micro-organisms, then this water can enter defective cans through minute leaks and increase the percentage of cans of food spoiling during storage. Many canneries routinely chlorinate the cooling water to reduce or eliminate this problem.

- The shortage of water in many food plants has necessitated reuse of the part of the water, and micro-organisms may build up in such reused water. Water employed for the final rinse of a food must be fresh and potable, but after use it may be returned for soaking, first wash, or fuming, preferably after with chlorine, chlorine dioxide, or a similar germicide.

- In plant chlorination beyond the break point (the point where chlorine demand has been satisfied) to a residual of 5 to 7 ppm of chlorine is employed for continuous application to area and equipment where slime bacteria may be a problem. The chlorinated water may be applied as a spray, or parts of equipment may be immersed. Contaminated or polluted water lines are held filled with chlorinated water containing 50 to 100 ppm of chlorine for 12 to 48 hours, the strength of chlorine and length of time depending on the extent of pollution.

- Ice used in contact with foods should meet the bacteriological requirements of potable water. Incorporation of bacteriostatic or bactericidal chemicals in water and in ice to aid in food preservation. Antibiotics like chlorotetracycline or oxytetracycline may be incorporated in ice to be applied to fish and other seafood.

Microbiology of the Food Product

Ingredients (the Raw Materials) are examined for quality control and storage: To reduce contamination with micro-organisms to a minimum and obtain good keeping quality of the product, ingredients are examined because:
• Large numbers of spores of aerobes are undesirable in dry milk to be used in bread making because of the increases risk of ropiness developing.
• Heat resistant spores in sugar and starch may add to the difficulty in adequately heat processing canned vegetables to which sugar or starch is added.
• Large number of bacteria in spices may favor the spoilage of summer sausage.
• Excessive mold mycelium in the raw fruit, is indicative of the presence of rotten parts, may lead to condemnation of the canned or frozen product.
• Large number of thermoduric bacteria in raw milk may yield pasteurized milk that will not meet the bacterial standards for numbers as estimated by the standard plate count method.
• Large numbers of bacteria on vegetables or in fruits may indicate inferiority that will carry over into the frozen product.

Packaging and Storage is Supervised
Packaging materials are a possible source of contamination of foods with microorganisms:
• Paper and paperboard used for milk cartons contain mostly bacilli and micrococci and occasionally other rods, actinomycetes and mold spores, but no organism of public health significance.
• Wax paper is practically sterile similar to most plastic packaging materials.
• All packaging materials should be protected from contact with dust or other sources of microorganisms in handling.

The equipment contacting the food is adequately cleaned, sanitized and tested.

Cleaning
To aid in the cleaning action of water, cleaning agents called detergents are employed, which soften or condition the water, improve the wetting ability of the cleaning solution, emulsify or saponify fats, solubilize minerals, deflocculate or disperse suspended materials, and dissolve as much soluble materials as possible. At the same time the detergent should be no corrosive and readily rinsed from the surfaces. Among the detergents used alone or in mixture are the alkaline varieties, such as lye, soda ash, sodium metasilicate, trisodium phosphate, and poly phosphate; acid detergents, usually organic acids, such as hydroxyacetic, gluconic, citric, tartaric, and levulinic acid; and wetting agents. These wetting agents may be anionic such as hydrocarbon sulfonates; non-ionic, for example polyether alcohol; or cationic for example the quaternary ammonium compounds. Cleaning is often made easier by using brushes and water under pressure.
Sanitizing

The sanitizing process is an attempt to reduce the number of microorganisms on equipment surfaces. The kind of sanitizer, the concentration employed, the temperature of the sanitizer, and the method of application vary with the kind of sanitizing agent, the condition during use, the type of equipment to be treated and the microorganisms to be destroyed. Among the sanitizing agents in common use are hot water, flowing steam or steam under pressure, halogens (chlorine or iodine) and halogen derivatives and the quaternary ammonium compounds.

- Steam under pressure is the most effective way of applying heat as a sanitizing agent, but its use is limited to closed systems that can withstand pressure. Steam jets, flowing steam, or hot water may be used. All microorganisms and their spores can be killed by adequate treatment with high pressure steam. Effectively applied flowing steam and boiling water will kill all but some of the more resistant bacterial spores.

- Chlorine, iodine and their compounds (hypochlorites, chloramines, iodophores, etc.) are effective germicide if in proper concentration and if given enough time to act. Chlorine is used to destroy undesirable bacteria in water for drinking, for use in foods, for washing foods or equipment. Hypochlorites are more labile but more effective at acid pH values than at alkaline ones. As stated continuous chlorination beyond the breaking point (where chlorine demand has been satisfied) to a residual of 5 to 7 ppm is employed for continuous application to areas where slime bacteria may be a problem, for example on conveyors, belts, or product washers. Chlorine (50 to 100 ppm) also is used to treat contaminated or polluted waterlines.

- Quatarnary ammonium compounds are in general more effective against gram positive than gram negative bacteria. These compounds have a residual effect, i.e., they adhere to equipment surfaces and decrease bacterial growth-but they rinse off onto foods coming into contact with these surfaces and if they are present in detectable concentrations, might be considered undesirable.

- Detergent sanitizers, which usually are a combination of an alkaline detergent and a quaternary ammonium compounds, sometimes are used to clean and sanitize utensils or equipment in one operation.

Preservation Process is Checked

The sanitarian usually has little to do with preservation of the foods. Laboratory might run keeping quality tests on canned foods and bacterial counts on frozen foods, pasteurized milk, dry milk etc.

Good Manufacturing Practices

Several recent publications promulgated by the Department of health education and welfare, Public health Service, Food and Drug Administration (FDA), especially current Good Manufacturing Practices (GMP) in the manufacturing, processing,
packing or holding of human foods. These can be found in the Code of Federal Regulation, Title 21. Of particular interest are the ‘umbrella GMPs’ since they cover many aspects of the food industry in a general way.

**Vending Machines for Food and Beverages**

During transportation from the commissaries and in the machine, perishable foods should be kept either cold (3.3 to 4.4 degree C) or hot (66 degree C or above). Slow growth of psychrotrophs can take place at the lower temperatures and of thermophiles in the hot foods if these recommendations are barely met, and excessive heat will deteriorate many foods. All parts of vending machines in contact with readily perishable foods should be cleaned and sanitized periodically, daily if the above temperature limitations are not met. Water used in connection with the foods should be potable, and waste disposal should be adequate. Most machines dispensing perishable foods are equipped with safety devices to stop dispensing food when refrigeration or heating fails.

**Food Handling on a Large Scale**

Food handling on large scale is done by caterers, commissaries, restaurants, institutions, airlines, camps etc. General recommendations and a food Service Sanitation Ordinance included in the Food Service Sanitation Manual by the Public Health Service of the US Department of Health, Education and Welfare. The ordinance defines ‘safe’ temperature for storage of foods as 7.2 degree C or below or 60 degree C or above, except during necessary periods of preparation and service. It requires the washing of raw fruits and vegetables and the thorough cooking of stuffing, poultry, stuffed meats and poultry (heating to at least 74 degree C), and pork and pork products (all parts heated to at least 66 degree C) before being served. Ordinance has regulations concerning the health and cleanliness of personnel; the cleanliness, sanitization, and protection of food utensils; and the potability of water.

**Sandwiches**

Sandwiches often are held at ambient temperatures for 18 to 48 hr before being sold, and therefore may be a potential food-poisoning hazard. One survey indicated that wrapped sandwiches can be contaminated during preparation and of growth of bacteria before vending, with higher numbers of bacteria in the moist sandwiches than in the dry. Similarly spiced-ham sandwiches and cheese sandwiches has been found to be more hazardous than sandwiches containing egg salad and chicken-salad sandwiches. A more recent survey by Christian and King (1971) showed that 60% of the sandwiches examined contained coagulase-positive staphylococci.

**The General Cleanliness and Sanitation of Plant and Premises**

The general cleanliness and sanitation of plant and premises are described as below:

**Sewage and Waste Treatment and Disposal**

The food sanitarian is also concerned with the adequate treatment and disposal of wastes from the industry. Solid and concentrated wastes are kept separate from...
Food Sanitation

the watery wastes and may be used directly for food, feed, fertilizer or other purpose; may first be concentrated, dried or fermented or may be carted away to available land as unusable waste. Sewage of human origin should be kept separate from other plant waters because of the possible presence of human intestinal pathogens.

Biological Treatment

The strength of the sewage or food waste containing organic matter is expressed in terms of Biological Oxygen Demand (BOD), which is the quantity of oxygen used by aerobic micro-organisms and reducing compounds in the stabilization of the decomposable organic matter during a selected time at a certain temperature. A period of 5 days at 20 degree C is generally used and results are expressed as 5 days BOD. The BOD is determined by dilution of a measured quantity of a waste with water that has been saturated with oxygen and incubation of the mixture at 20 degree C, along with a control of dilution water alone. After 5 days the residual oxygen in both control and test sample is measured by titration. The difference represents the oxygen-consuming capacity of the waste and is calculated to be expressed as parts per million of oxygen taken up by the waste.

Biological methods for waste disposal and/or treatment include:

- Irrigation, in which waste waters are sprayed onto fields of open-textured soil.
- Lagooning, by running the waste water into shallow artificial ponds.
- Use of trickling filters.
- Use of the activated sludge method, in which waste water is inoculated heavily with sludge from a previous run and is actively aerated in tanks.
- Use of anaerobic tanks like anaerobic digestors, where settling, hydrolysis, putrefaction and fermentation takes place.

Chemical Treatment

In chemical pretreatments, a chemical or mixture of chemicals is added to the sewage or waste to form flocculent precipitate, which, in settling, carries with it much of the suspended and colloidal material, including bacteria. The effluent then is run into a body of water, onto soil or into a biological treatment system. The chemicals commonly used are soluble aluminium or iron salts, such as alums or ferrous sulfate, plus lime, giving a flocculent precipitate of aluminium or ferric hydroxide. Disposal of the sludge (settlings) so obtained may be difficult.

Health of Employees

Duties of sanitarian that affect the health of employees include provision of potable drinking water, supervision of matters of personal hygiene, regulation of sanitary facilities within the plant and of sewage treatment and disposal, and supervision of sanitation in plant eating establishments and in plant-operated housing units. Special places should be designated for eating carried lunches, and such places should be kept neat and clean. If the plant serves meals to employees in a cafeteria, the
sanitarians should be responsible for supervision of sanitation in the preparation, handling, serving and storage of the food to avoid the spread of infectious microorganisms and to prevent outbreaks of food poisoning. To prevent the spread of disease, food equipment and utensils should be handled, washed and sanitized.

Water Quality Testing

The public health importance of clean drinking water requires water quality testing to evaluate the effectiveness of water treatment procedures and to establish drinking water safety standards because a number of viral, bacterial, and protozoan diseases result from water contaminated with human and other animal fecal wastes. Although many of these pathogens can be detected directly, environmental microbiologists have generally used indicator organisms as an index of possible water contamination by human pathogens. The most frequently used indicator organisms for faecal contamination is non-pathogenic *Escherichia coli*. Positive tests for *E. coli* do not confirm the presence of enteropathogenic microorganism but establish the possibility of such a presence. Because *E.coli* is plentiful and easier to grow than the enteropathogens, the test has a built in safety factor for detecting potentially dangerous contamination.

Coliforms are useful indicators of water contamination because many of them inhabit the intestinal tract (colon) of humans and other animals in large numbers. Thus, the presence of coliforms in water may indicate fecal contamination. Coliforms are defined as facultatively aerobic, Gram negative, non-spore-forming, rod-shaped bacteria that ferment lactose with gas formation within 48 hours at 35 degree C.

**Characteristics of an Indicator Organism**

- An indicator organism should be present whenever the pathogens concerned are present.
- Should be present only when there is a real danger of pathogens being present.
- Must occur in greater numbers than the pathogens to provide a safety margin.
- Must survive in the environment as long as the potential pathogens.
- Must be easy to detect with a high reliability of correctly identifying the indicator organism.

**Methods for Testing Water Quality**

Following are some of the methods for testing water quality:

**Multiple Tube Fermentation Technique/Most Probable Number (MPN):**

The three basic tests to detect coliform bacteria in water are presumptive, confirmed, and completed as shown in figure. The tests are performed sequentially on each sample under analysis. They detect the presence of coliform bacteria (indicators of fecal contamination), the Gram-Negative, non–spore-forming bacilli that ferment lactose with the production of acid and gas that is detectable following a 24-hour incubation period at 37°C.
Presumptive Test: The presumptive test is specific for detection of coliform bacteria. Measured aliquots of the water to be tested are added to a lactose fermentation broth containing an inverted gas vial. Because these bacteria are capable of using lactose as a carbon source (the other enteric organisms are not), their detection is facilitated by the use of this medium. Tubes of the lactose medium are inoculated with 10-ml, 1-ml, and 0.1-ml aliquots of the water sample. The series consists of at least three groups, each composed of five tubes of the specified medium. The tubes in each group are then inoculated with the designated volume of the water sample. Development of gas in any of the tubes is presumptive evidence of the presence of coliform bacteria in the sample. The presumptive test also enables the microbiologist to obtain some idea of the number of coliform organisms present by means of the Most Probable Number (MPN) test. The MPN is estimated by determining the number of tubes in each group that show gas following the incubation period.

Confirmed Test: The presence of a positive or doubtful presumptive test immediately suggests that the water sample is non-potable. Confirmation of these results is necessary because gas formation is the characteristic of fecal coliforms such as *Salmonella, Shigella* and *E. coli* but also of the nonfecal coliforms such as *Enterobacter aerogenes* and some *Klebsiella*. The confirmed test requires that selective and differential media (for example, Eosin–Methylene Blue (EMB) or Endo agar) be streaked from a positive lactose broth tube obtained from the presumptive test. Eosin–methylene blue contains the dye methylene blue, which inhibits the growth of Gram-Positive organisms. In the presence of an acid environment, EMB forms a complex that precipitates out onto the coliform colonies, producing dark centers and a green metallic sheen. The reaction is characteristic for *E. coli*, the major indicator of fecal pollution. Endo agar is a nutrient medium containing the dye fuchsin, which is present in the decolorized state. In the presence of acid produced by the coliform bacteria, fuchsin forms a dark pink complex that turns the *E. coli* colonies and the surrounding medium pink or all positive presumptive cultures are used to inoculate tubes of Brilliant Green Lactose Bile Broth (BGLB). After incubation for 48 hours at 35 degree C, gas production in BGLB gives positive confirmed test.

Completed Test: If BGLB is used; it is then subcultured onto EMB plates. The completed test is the final analysis of the water sample. It is used to examine the coliform colonies that appeared on the EMB or Endo agar plates used in the confirmed test. An isolated colony is picked up from the confirmatory test plate and inoculated into a tube of lactose broth and streaked on a nutrient agar slant to perform a Gram stain. Following inoculation and incubation, tubes showing acid and gas in the lactose broth and presence of gram-negative bacilli on microscopic examination are further confirmation of the presence of *E. coli*, and they are indicative of a positive completed test.

Membrane Filtration Technique: Bacteria-tight membrane filters capable of retaining microorganisms larger than 0.45 micrometer (µm) are frequently used for analysis of water. These filters offer several advantages over the conventional,
multiple-tube method of water analysis: (a) results are available in a shorter period of time, (b) larger volumes of sample can be processed, and (c) because of the high accuracy of this method, the results are readily reproducible. A disadvantage involves the processing of turbid specimens that contain large quantities of suspended materials; particulate matter clogs the pores and inhibits passage of the specific volume of water. A water sample is passed through a sterile membrane filter that is housed in a special filter apparatus contained in a suction flask. Following filtration, the filter disc that contains the trapped microorganisms is aseptically placed on the surface of a solid medium (Endo medium or EMB medium, which is selective for gram negative, and differential for lactose-fermenting bacteria including the coliforms) and incubated. Following incubation, coliform colonies of faecal coliform show up with a characteristics metallic sheen and can be counted, and from this value the number of coliforms in the original water sample can be calculated (Refer Figure 13.1).

Fig. 13.1 Development of Colonies on a Membrane Filter Following Incubation

The Presence-Absence Test (P-A Test): These tests can be completed in 24 hours or less. The P-A test is based on the assumption that no coliforms should be present in 100 ml of drinking water. These tests use:

Defined Substrate Technology, in which selective media are used not only to detect total coliforms, but also to specifically identify *E. coli* at the same time. Defined substrate tests are generally faster and usually more accurate than EMB agar tests. These tests are based on the ability of coliforms and *E. coli* to metabolize certain substrates. For example, all coliforms, including *E. coli*, metabolize 4-MethylUmbelliferyl-β-D-Galactopyranoside (MUG) using the enzyme β-galactosidase. If coliforms are present in a sample, MUG is metabolized to produce a fluorescent product visible under UltraViolet (UV) light. To distinguish total coliforms from *E. coli*, another enzyme–substrate reaction is used at the same time. *E. coli*, but not other coliforms, produces the enzyme β-glucuronidase, which metabolizes indoxyl β-D-glucuronide (IBDG) to a blue compound. The blue compound colors only growing *E. coli* colonies. As a result, *E. coli* colonies fluoresce and are also dark blue, and this differentiates them from colonies of other coliforms. The test uses a membrane filter method and media containing...
both MUG and IBDG (called MI media). The filter is overlaid on MI agar, incubated at 35°C for 24 hours, and examined under UV light. *E. coli* colonies appears fluorescing dark blue while, other coliforms that fluoresce and appear white to light blue.

**The IDEXX Colilert Water Quality Test System:** Colilert reagents are added to 100-ml water samples. After incubation for 24 h at 35–37°C, the samples develop yellow color if they contain coliform bacteria. Samples containing *E. coli* develop yellow color and also develop blue fluorescence. Samples negative for coliform bacteria remain clear. This test system relies on the relative ability of β-galactosidase and α-glucuronidase in coliforms and *E. coli*, respectively, to utilize a proprietary substrate mix. Using the same principles as the selective media this method shows colored and fluorescent products for total coliforms and *E. coli*.

Molecular techniques are now used routinely to detect coliforms and *E. coli* in waters.

- PCR used to detect total coliform bacteria based on the *lacZ* gene and *E. coli* based on the *uidA* gene; these genes code for the enzymes detected in the defined substrate tests.
- 16S rRNA gene-targeted primers for coliforms enable the detection of one Colony-Forming Unit (CFU) of *E. coli* per 100 ml of water, if a short enrichment step precedes the use of the PCR amplification. This allows the differentiation of non-pathogenic and enterotoxigenic strains, including the shiga like-toxin producing *E. coli* O157:H7.

**Testing Milk Quality**

During the milking operation, microorganisms can enter the milk from the hands of person drawing, from hides of the animal, from atmosphere, utensils, dust particles in air or from water that is added to dilute the milk. If milk is allowed to stand at room temperature, normal microflora will grow and cause spoilage. Milk quality plays a key role not only for its wide consumption but also its involvement in a large series of derived products, such as yogurt, butter, cheese, and ice cream. Therefore, the growth of these microorganisms has to be monitored and controlled to prevent spoilage and transmission of disease.

**Methods to Determine Quality of Milk**

**Methylene Blue Reduction Time (MBRT) Test**

Methylene blue is an example of a Redox dye, i.e., it shows different colour in oxidized and reduced state. In the oxidized state, it is blue in color, and in the reduced state it becomes colorless. Such dyes which are colorless in reduced state and show color in oxidized State are known as leucoform dyes.

For the milk to have greater shelf life, the number of microorganisms should be low. As the microorganisms enter the milk, they start using it for their nutrition. Most of them are aerobes or facultative anaerobes. As they utilised the dissolved oxygen in milk, the oxidation-reduction potential value of the milk start decreasing.
And when it reaches 10 mv, the methylene blue gets decolorized. The time taken
for methylene blue to get decolorized when added to milk sample and incubated
at 37 degree C in a water bath is called methylene blue reduction time. The length
of time depends on the number of microorganisms present in the milk; holding all
other factors (such as nutrient content, moisture content, and temperature) constant.
This time is inversely proportional to the microbial load in milk. Greater the microbial
load, shorter will be the methylene blue reduction time. And poorer will be the
microbiological quality of the milk.

To perform this test, definite amount of methylene blue is mixed with 10 ml
of milk and incubated at 37 degree C in a water bath and wait for colour change.
The longer it will take for the colour to change, the lower the microbial load. All
the glassware used must be sterile. The interpretation of the results is as follows in
Table 13.1:

<table>
<thead>
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<th>Length of Time (hours)</th>
<th>Quality of Milk</th>
<th>Grade of Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 or More</td>
<td>Excellent</td>
<td>1</td>
</tr>
<tr>
<td>3-5</td>
<td>Good</td>
<td>2</td>
</tr>
<tr>
<td>2-3</td>
<td>Fair</td>
<td>3</td>
</tr>
<tr>
<td>Less Than 2 Hours</td>
<td>Poor</td>
<td>4</td>
</tr>
</tbody>
</table>

Alkaline Phosphatase (ALP) Test

The phosphatase test is used to detect inadequate pasteurisation of milk. Alkaline
Phosphatase (ALP) is a heat sensitive enzyme in raw milk, which is completely
destroyed by the heat treatments applied both for low temperature pasteurisation
and for the High temperature Shorttime Pasteurisation (HTST) processes.

Alkaline phosphatase induces the hydrolysis of p-Nitrophenyl PhosPhate
(pNPP), at alkaline pH (pH 10), and liberates para-nitrophenol, a yellow colored
complex whose intensity is directly proportional to the concentration of ALP in the
sample, and can be measured at 405 nm. The colour intensity is measured by
direct comparison with standard color discs in a Lovibond comparator.

Generally, 5 ml of buffer solution (p-nitrophenyl phosphate) is mixed with 1
ml of milk sample in a test tube. Tube is then inverted and placed in the water bath.
After two hours, the tubes are removed from the water bath, and change in colour
is observed and read using the Lovibond comparator and special disc. The direct
reading is recorded as ppm nitro-phenol in milk. Results of duplicate determination
should not differ by more than 2 ppm of p.nitrophenol over the critical range of
0-14 ppm of p.nitrophenol. Any reading of excess of 10 ppm is recorded as
unsatisfactory.

Resazurin Reduction Tests

Resazurin test is the most widely used test for hygiene and the potential keeping
quality of raw milk. Under specified conditions Resazurin is dissolved in distilled
boiled water. Rasazurin solution must not be exposed to sunlight, and therefore
Food Sanitation

should be stored in dark bottle and can later be used to test the microbial activity in a given milk sample. It should not be used for more than eight hours because it loses strength.

Resazurin is a dye indicator. It is also blue in color in oxidized state. It changes to pink color when in intermediate state (Resorufin) and when the oxidation reduction potential value of milk reaches 100mV, it changes to white color (dihydroresorufin).

Using sterile equipment; 1 ml of the dye is added into 9 ml of milk sample, mixed uniformly and incubated at 37°C in a water bath for 10 minutes for fresh milk and up to one hour for pasteurized milk and then observed for the colour change using a Lovibond comparator.

### Table 13.2 The Results are Interpreted According to the Table

<table>
<thead>
<tr>
<th>Reading</th>
<th>Colour</th>
<th>Quality of Milk</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blue (no color change)</td>
<td>Excellent</td>
<td>Accept</td>
</tr>
<tr>
<td>2</td>
<td>Light blue</td>
<td>Very good</td>
<td>Accept</td>
</tr>
<tr>
<td>3</td>
<td>Purple</td>
<td>Good</td>
<td>Accept (conditionally)</td>
</tr>
<tr>
<td>4</td>
<td>Purple pink</td>
<td>Fair</td>
<td>Separate</td>
</tr>
<tr>
<td>5</td>
<td>Light pink</td>
<td>Poor</td>
<td>Separate</td>
</tr>
<tr>
<td>6</td>
<td>Pink</td>
<td>Bad</td>
<td>Reject</td>
</tr>
<tr>
<td>7</td>
<td>White</td>
<td>Very bad</td>
<td>Reject</td>
</tr>
</tbody>
</table>

**Advantages**

- Test is cheaper and can be measured in a shorter time.
- Microbial activity is measured rather than the number of bacteria.

**Disadvantages**

- Continuous observation is required until reduction takes place.
- Not suitable for milk samples with low bacterial counts (less than 10^5/ml).
- Does not indicate for the type of micro-organisms present in the milk sample

**Litmus Milk Reactions**

Milk being rich in proteins casein, lactalbumin, and lactoglobulin, the sugar lactose, vitamins, minerals and water is an excellent medium for the growth of microorganisms. These milk components can all be metabolized by different types of bacteria based on various metabolic reactions in litmus milk, including reduction, fermentation, clot formation, digestion, and the formation of gas. To distinguish among the metabolic changes produced in milk, a pH indicator, the oxidation-reduction indicator litmus, is incorporated into the medium. This Litmus milk then forms a good differential medium to distinguish between different species of bacteria.

**Lactose Fermentation:** is demonstrated by production of acids like pyruvic and lactic acid which in turn reduces the pH to 4 turning the litmus pink from purple (at neutral pH).
**Litmus Reduction:** Under anaerobic fermentative conditions, the litmus is in the oxidized state and appears to be purple in color which on accepting hydrogen from a substrate get reduced and turn white or milk-colored. The oxidation of lactose present in milk, under anaerobic condition produces lactic acid, butyric acid, CO$_2$, and H$_2$. Excess Hydrogen released during the process reduces Litmus to white.

**Curd Formation:** Under acidic conditions casein is precipitated as calcium caseinate to form an insoluble hard clot which does not retract from the walls of the test tube. This acid curd remains immobile even if the tube is inverted. However, rennin produced by some organism produces paracasein, which on being converted to calcium paracaseinate forms soft semisolid clot also known as rennet curd can flow slowly on tilting the tube.

**Proteolysis (Peptonization):** Degradation of milk proteins primarily casein by proteolytic enzymes, into amino acids is accompanied by the evolution of ammonia which increases the pH of the medium making it alkaline turning the litmus deep purple in the upper portion of the tube, while the medium begins to lose body and appear translucent, brown, wheylike.

**Gas Formation:** Production of end products like CO$_2$ + H$_2$ by lactose fermentation may be seen as cracks or fissures within the curd as gas rises to the surface.

In this test 5 ml of autoclaved litmus milk broth taken in sterile test tubes is inoculated with experimental organism from overnight grown culture by loop inoculation. Inoculated tubes are incubated for 24-48 hours at 37°C and observed for any color and consistency changes taking one uninoculated litmus milk tube as ‘control’ for comparison. Observations are recorded as:

**Positive Test**
- **Acid pH:** Pink to red color
- **Alkaline pH:** Purplish-blue color
- **Reduction:** White
- **Acid curd:** hard curd with clear supernatant (whey)
- **Rennet curd:** soft curd followed by peptonization (alkaline pH, supernatant brown)
- **Gas production:** bubbles in coagulated milk

**Negative Test**
- Color and consistency remain same.

**Standard Plate Count Method**

In this test a known volume of sample is spread played on nutrient agar plates. After incubating at 37 degree C for 24 hours, the bacterial colonies are counted and Colony Forming Units/ml (CFU/ml) is determined.
Check Your Progress

1. What is food sanitation?
2. What are the duties that are concerned with food sanitation?
3. How is sanitizing process done?
4. Give the biological methods for waste disposal and/or treatment.
5. List the characteristics of an indicator organism.

13.3 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Food sanitation includes all practices involved in protecting food from risk of contamination, harmful bacteria, toxins, preventing any food spoilage (wherein, the original nutritional value, texture, flavor of the food are damaged), leading to foodborne illness (illness caused by food) of consumers due to food infection (microbial infection resulting from ingestion of contaminated foods), or food intoxication (illness caused by toxins produced by some bacteria); and destroying any harmful bacteria in the food by thorough cooking or processing.

2. Food sanitation is concern with the following duties:
   - Bacteriology of water supply is examined to provide good water for both drinking as well as for plant purposes.
   - Microbiology of the food product is examined to prevent contamination of the foods at all stages during processing from equipment, personnel and vermin.
   - The general cleanliness and sanitation of plant and premises which includes not only the maintenance of clean and well sanitized surfaces of all equipment touching the foods but also good housekeeping in and about the plant and adequate treatment and disposal of wastes.
   - The health of employees which include provision of a potable water supply, supervision of matters of personal hygiene, regulation of sanitary facilities in the plant and in plant operated housing units, and contact with sanitary aspects of plant lighting, heating, and ventilation.

3. The sanitizing process is an attempt to reduce the number of microorganisms on equipment surfaces. The kind of sanitizer, the concentration employed, the temperature of the sanitizer, and the method of application vary with the kind of sanitizing agent, the condition during use, the type of equipment to be treated and the microorganisms to be destroyed. Among the sanitizing agents in common use are hot water, flowing steam or steam under pressure,
halogens (chlorine or iodine) and halogen derivatives and the quaternary ammonium compounds.

4. Biological methods for waste disposal and/or treatment include:
   - Irrigation, in which waste waters are sprayed onto fields of open-textured soil.
   - Lagooning, by running the waste water into shallow artificial ponds.
   - Use of trickling filters.
   - Use of the activated sludge method, in which waste water is inoculated heavily with sludge from a previous run and is actively aerated in tanks.
   - Use of anaerobic tanks like anaerobic digestors, where settling, hydrolysis, putrefaction and fermentation takes place.

5. Characteristics of an indicator organism are as follow:
   - An indicator organism should be present whenever the pathogens concerned are present.
   - Should be present only when there is a real danger of pathogens being present.
   - Must occur in greater numbers than the pathogens to provide a safety margin.
   - Must survive in the environment as long as the potential pathogens.
   - Must be easy to detect with a high reliability of correctly identifying the indicator organism regardless of what other organisms are present in the sample.

13.4 SUMMARY

- Food sanitation includes all practices involved in protecting food from risk of contamination, harmful bacteria, toxins, preventing any food spoilage, leading to foodborne illness of consumers due to food infection, or food intoxication; and destroying any harmful bacteria in the food by thorough cooking or processing.
- Bacteriology of water supply is examined to provide good water for both drinking as well as for plant purposes.
- Microbiology of the food product is examined to prevent contamination of the foods at all stages during processing from equipment, personnel and vermin.
- The general cleanliness and sanitation of plant and premises which includes not only the maintenance of clean and well sanitized surfaces of all equipment touching the foods but also good housekeeping in and about the plant and adequate treatment and disposal of wastes.
Water for plant use must be satisfactory from a bacteriological standpoint for use with the particular food being processed. A water supply may be adjusted potable yet be unsatisfactory for use with a food.

The shortage of water in many food plants has necessitated reuse of the part of the water, and micro-organisms may build up in such reused water.

Large numbers of spores of aerobes are undesirable in dry milk to be used in bread making because of the increases risk of ropiness developing.

Heat resistant spores in sugar and starch may add to the difficulty in adequately heat processing canned vegetables to which sugar or starch is added.

Paper and paperboard used for milk cartons contain mostly bacilli and micrococci and occasionally other rods, actinomycetes and mold spores, but no organism of public health significance.

The sanitizing process is an attempt to reduce the number of microorganisms on equipment surfaces.

Steam under pressure is the most effective way of applying heat as a sanitizing agent, but its use is limited to closed systems that can withstand pressure.

Chlorine, iodine and their compounds (hypochlorites, chloramines, iodophores, etc.) are effective germicide if in proper concentration and if given enough time to act. Chlorine is used to destroy undesirable bacteria in water for drinking, for use in foods, for washing foods or equipment.

Quaternary ammonium compounds are in general more effective against gram positive than gram negative bacteria.

Several recent publications promulgated by the Department of health education and welfare, Public health Service, Food and Drug Administration (FDA), especially current Good Manufacturing Practices (GMP) in the manufacturing, processing, packing or holding of human foods.

Food handling on large scale is done by caterers, commissaries, restaurants, institutions, airlines, camps, etc.

Sandwiches often are held at ambient temperatures for 18 to 48 hour before being sold, and therefore may be a potential food-poisoning hazard.

The food sanitarian is also concerned with the adequate treatment and disposal of wastes from the industry. Solid and concentrated wastes are kept separate from the watery wastes and may be used directly for food, feed, fertilizer or other purpose; may first be concentrated, dried or fermented or may be carted away to available land as unusable waste.

In chemical pretreatments, a chemical or mixture of chemicals is added to the sewage or waste to form flocculent precipitate, which, in settling, carries with it much of the suspended and colloidal material, including bacteria.
• The public health importance of clean drinking water requires water quality testing to evaluate the effectiveness of water treatment procedures and to establish drinking water safety standards because a number of viral, bacterial, and protozoan diseases result from water contaminated with human and other animal fecal wastes.

• The presence of a positive or doubtful presumptive test immediately suggests that the water sample is non-potable.

• The Presence-Absence Test (P-A Test) can be completed in 24 hours or less. The P-A test is based on the assumption that no coliforms should be present in 100 ml of drinking water.

• During the milking operation, microorganisms can enter the milk from the hands of person drawing, from hides of the animal, from atmosphere, utensils, dust particles in air or from water that is added to dilute the milk.

• Methylene blue is an example of a Redox dye i.e. it shows different colour in oxidized and reduced state.

13.5 KEY WORDS

• Food sanitation: Food sanitation includes all practices involved in protecting food from risk of contamination, harmful bacteria, toxins, preventing any food spoilage.

13.6 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. What are the measures on which water is considered as safe for drinking purposes?
2. Write in brief about microbiology of the food product.
3. How packaging and storage is supervised?
4. How sanitizing is done?
5. Write about the general cleanliness and sanitation of plant and premises.
6. What are the biological methods for waste disposal?
7. Briefly explain the defined substrate technology.

Long Answer Questions

1. Write a note on food sanitation.
2. Discuss the microbiology of food plant sanitation.
3. Elaborate a note on bacteriology of water supplies.
4. How is the testing of water and milk done?
5. Explain membrane filtration technique.

13.7 FURTHER READINGS


UNIT 14 FOOD LAWS AND QUALITY CONTROL

14.0 Introduction
Food law is the collection of laws and regulations that govern food production, distribution and consumption. Food laws aim to protect consumers and provide for the efficient growth and use of food in the United States. Food attorneys focus their careers on helping clients comply with food laws and regulations. In addition, they work on behalf of government agencies making or enforcing food laws and policies. Most people know that food laws govern pesticide use, tariffs on agricultural imports and restaurant cleanliness. Food law also covers other topics that some consumers may not have considered. For example, the Food and Drug Administration regulates the production of bottled water. Food laws regulate what supplement producers can claim about the effectiveness of their products. The U.S. food stamp program is part of food law and run under the U.S. Department of Agriculture. Food laws can even cover constitutional law topics like whether the federal government can lawfully regulate local farm production.

There are federal, state and local food laws. The federal government passes laws like the Food and Drug Administration Revitalization Act. Through the Food and Drug Administration (FDA), the federal government creates additional regulations and takes steps to enforce federal food law. There are also extensive state laws that typically govern topics like packaging, distribution, adulteration and fraud in the food industry.

Food is basic for life. Quality or excellence in our food supply should be an important concern to all food processors. Safety and wholesomeness are the most

14.1 Objectives

14.2 Food Laws and Quality Control
14.2.1 Hazard Analysis and Critical Control Point (HACCP) System
14.2.2 PFA: Prevention of Food Adulteration Act
14.2.3 FPO: Fruit Product Order
14.2.4 BIS: Bureau of Indian Standards
14.2.5 MFPO: Meat Food Products Order
14.2.6 AGMARK
14.2.7 CAC: Codex Alimentarius Commission

14.3 Answers to Check Your Progress Questions

14.4 Summary

14.5 Key Words

14.6 Self Assessment Questions and Exercises

14.7 Further Readings
Food Laws and Quality Control

import important attributes of food quality. The lack of quality as it relates to safety and wholesomeness can result in personal injury, sickness or death. Foodborne illness is an example of sickness or even death when unsafe foods are produced and eaten. Certain foods or food products are defined by regulations or policies called standards of identity. These standards of identity are definitions for a specific food product to avoid confusion or mislabeling of similar processed foods. Milk is a good example. The standard for skim milk is less than 1/2 percent fat, while the standard for whole milk is at least 3-1/4 percent fat. Quality defined by regulations, policies or standards is controlled by federal and state agencies. Failure to meet the degree of excellence defined by the regulations, policies or standards of identity is illegal.

In this unit, you will study about food laws and quality control – HACCP, Codex Alimentarius Commission, PFA, FPO, MFPO, BIS and AGMARK.

14.1 OBJECTIVES

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

- Understand about food laws and quality control
- Discuss about different laws HACCP, Codex Alimentarius Commission, PFA, FPO, MFPO, BIS and AGMARK

14.2 FOOD LAWS AND QUALITY CONTROL

The quality concept is focused on technological attributes and factors that can contribute to product quality performance. A product has physical features that are turned into quality attributes by the perception of a consumer. With respect to agrifood products, quality perception appeared to be affected by different types of attributes. Relevant attributes for consumers involve safety, nutritional value (health aspect), sensory properties (like taste, flavour, texture, and appearance), shelf life, convenience (ready-to-eat meal) and product reliability (correct weight, right composition, etc.). These attributes can be defined as intrinsic attributes and are directly related to the physical product properties.

Extrinsic attributes refer to production system characteristics and other aspects, like environmental impact or marketing influence. They do not necessarily have a direct influence on physical properties but may affect acceptance of products by consumers. For example, the use of pesticides, of antibiotics to improve animal growth, or the application of biotechnologies to modify product properties, can have a significant effect on food acceptance.

Intrinsic attributes characterize the physical product, whereas marketing efforts mainly determine extrinsic attributes. According to this classification, typical intrinsic attributes include appearance, colour, shape and texture. Typical extrinsic attributes are price, brand name, packaging, labelling and product information.
Extrinsic attributes are related to the product but are not part of it, like price, brand or store name. These cues have a predicted value for the consumer and thus influence the quality expectation. Generally, intrinsic cues are more important than extrinsic cues. Production system characteristics do not necessarily affect the physical attributes. The use of pesticides will not directly influence product features but might affect quality expectations but a change in production system characteristics may influence the physical features.

Quality Management Systems

Quality Management Systems (QMS) are used to control the quality and safety of products. The use of QMS ensures that all aspects of a business are working efficiently and cost effectively. A system will provide a competitive advantage, which can increase marketing and sales opportunities. This will help a company gain new customers as well as retaining existing business. By working within a QMS the whole workforce will be involved which thereby improves communication, morale and job satisfaction.

Food Quality Management Systems

Food quality management, which assures the health and safety of food, has become increasingly important during the past few years. This is primarily due to changing consumer requirements, increased competition, environmental issues and governmental interests.

The food market faces new challenges everyday. The situation is further complicated by the complex characteristics of food and food ingredients, which include such factors as variability, restricted shelf life, potential safety hazards, and many chemical, biochemical, physical, and microbial processes. To face this challenge, continuous improvement in food quality management methods is required, where knowledge of modern technologies and management methods play a crucial role.

Moreover, human handling plays a crucial role in quality management and is rather unpredictable and changeable. As a consequence, the result of agribusiness and food industry, is much more uncertain than is often assumed.

The food sector utilises various quality assurance systems, such as HACCP (Hazard Analysis Critical Control Point), ISO (International Organisation for Standardisation), Codex Alimentarius Commission guidelines and other food standards. These systems, and combinations of these systems, are applied in order to assure food quality.

Need for Quality Control

Quality control is the sum of all those controllable factors that ultimately influence positively or negatively the quality of the finished product e.g. selection of raw materials, processing methods, packaging, methods of storage, distribution, etc. Quality is defined as any of the features that make something what it is or the degree of excellence or superiority. The word ‘quality’ is used in various ways as
applied to food. Quality product to the salesman means a product which is of high quality and of expensive nature.

In food processing, the general rule is that the effective methods must be carefully applied to conserve the original qualities of the raw materials. Processing cannot improve the raw material.

The aim of quality control is to achieve as good and as consistent a standard of quality in the product being produced as is compatible with the market for which the product is designed.

**Principles of Quality Control**

The principles of quality control are considered under the following:

- Raw Material Control
- Process Control
- Finished Product Inspection

Invariably, once a food product has been through a manufacturing process, little can be done to alter its quality. Thus examination of finished products only permits acceptance of material reaching the desired standard and rejection of material which fails to reach this standard. Such a process is therefore one of inspection and not one of control. It can be claimed that if control of raw materials and control of process is perfect, the final product will not require inspection. However, in practice it is seldom possible to guarantee complete control over raw materials and processing conditions.

In a food processing industry the stores and warehouses often contain a large range of raw materials. Raw materials such as artificial colouring, spices and essences may be used slowly and may deteriorate on long storage. Others such as wheat or milk powder may be rapidly replaced in the flour and milk industry respectively. Fresh meat is highly perishable and requires good refrigeration whereas sugar, salt, etc. may be very stable and only requires a cool dry condition to remain in excellent condition for a long period. Those used in insignificant amounts can be omitted from frequent inspection since the cost of inspection will often far outweigh the advantages gained.

In any product, there is a dominant raw material (sometimes it could be several of these) upon which the quality of the finished product is mainly dependent for example, in bread production, flour is the essential raw material, malt for beer, wheat for flour and the like.

**Integrated Food Law in India**

According to apex industrial bodies there should be only one national food safety code, which should cover all aspects of Indian food safety under a unified system. Realizing the importance of introducing an integrated, contemporary and comprehensive law, the Food Safety and Standards Act (FSSA), 2006, that overrides all earlier food laws.
This Act covers all articles of food or drink for human consumption except drugs, tobacco, alcoholic beverages and natural agricultural/horticulture/marine produce, which need to be notified separately. It will specifically repeal eight food laws:

- The Prevention of Food Adulteration Act (Ministry of Health), 1954.
- The Fruit Products Order (FPO), 1955.
- The Vegetable Oil Products (Control) Order, 1947.
- The Solvent Extracted Oil, De-oiled Meal, and Edible Flour (Control) Order, 1967.
- Essential Commodities Act, 1955 relating to food.

This integrated law provides safe and wholesome food to the consumer, creates an enabling-environment for value addition to primary agricultural produce and encourage innovation and creativity for rapid development of food processing industries in an integrated manner thus ensuring a high degree of objectivity and transparency. The law intends to ensure better consumer safety through the introduction of food safety management systems based on science and transparency.

Main Features of the Law Include

- Single reference point for all issues related to food safety and standards
- Harmonization with international standards such as CODEX and, hence, responsive to international trade requirements.
- Responsive to dynamic issues such as genetically modified food.
- Clear procedures for food recall.
- Shift from a regulatory regime to self-compliance through food safety management systems.

General Principles of Food Law

The regulation (official order) establishes several basic principles for food to be marketed. Food regulation is principally composed of three distinct areas: general principles of food law; the creation of food safety and the rapid alert system, crisis management and emergencies. The basic principles are outlined in the text below:

Food Safety Authority

Food is not allowed to be placed on the market if it is unsafe. Food is considered unsafe if it is injurious to health or unfit for human consumption. This general food safety requirement implies that although a product that complies with all specific
requirements of food legislation; it is not allowed on the market if a new hazard is found for which no requirements yet exist.

The following assumptions should be checked in order to establish food safety:

- **Unsafe**: The normal conditions of use of the food by the consumer at each stage of production, processing and distribution are taken into account as well as the information provided to the consumer.

- **Injurious to Health**: Probable immediate, short term and long term effects (including future generations) of the food to health, probable cumulative toxic effects and particular health sensitivities of a specific category of consumers should be considered.

- **Unfit for Human Consumption**: If the food is unacceptable for human consumption according to its intended use, for reasons of contamination, whether by extraneous matter or otherwise, or through putrefaction, deterioration or decay.

If food that is found to be unsafe is part of a batch, lot or consignment, it is presumed that all the food is unsafe, unless a detailed assessment proves that there is no evidence that the rest of the batch is unsafe. Food that complies with specific requirements on food safety is considered safe in so far as it concerns specific issues for which the requirements are set. If however, despite compliance to these requirements, the competent authorities have reasons to suspect that the food is not safe, they can still require its withdrawal from the market.

**Responsibilities**

Food business operators at all stages of production, processing and distribution of food are primarily responsible for safe food. They have to ensure that food under their control meets all the safety requirements of the law. If the food is unsafe, they cannot place it on the market. Further, they are obliged to withdraw and report to the competent authority if they have suspicion that their food products do not meet the standards.

**Traceability**

The Regulation includes provisions on the traceability of food in the food chain. At all times the origin of food products must be retrieved. Strictly speaking the requirements apply to food and feed businesses (including importers). These people are obliged to:

- Know and document from whom they have bought their food (ingredients).
- Know and document to whom they supply their products.
- Label at length their products so that they can establish traceability in case of a food safety problem.
However, importers are likely to be requested to trace faults in the food chain. Importers are legally responsible for marketing the food and therefore must be able to guarantee that the food brought onto the market meets all requirements.

Precautionary Principle

The General Food Law establishes that food legislation is based on scientific risk analysis. This analysis consists of risk assessment, risk management and risk communication.

- Risk assessments are based on the available scientific evidence and must be undertaken in an independent, objective and transparent manner.
- Risk management takes the results of the risk assessment into account, but also the opinions of the Food Safety Authority. If after an assessment of available information the possibility of harmful effects on health is identified, but scientific uncertainty persists, provisional measures may be necessary. The findings need to be communicated to the retailer, manufacturer and notified to the consumer. This whole exercise is called the precautionary principle. Such measures need to be proportionate and no more restrictive of trade than is required to achieve the high level of health protection. The measures are reviewed within a reasonable period of time. This period depends on the nature of the risk to health and the type of scientific information needed to clarify uncertainty.
- Presentation: Food law aims at the protection of the interests of consumers in relation to the foods they consume. Provision of food law about the food labelling, advertising and presentation of food including their shape, appearance, packaging and packaging materials used cannot mislead consumers.

14.2.1 Hazard Analysis and Critical Control Point (HACCP) System

The Committee for Food Safety of FAO (Food and Agriculture Organization) and WHO (World Health Organization) contended in 1984 that one of the biggest problems today is the diseases caused by food. Therefore, the safety of consumers, i.e., food safety, shall be increased in order to prevent health damages caused by food. This goal can be achieved by creating a production system that specifies, evaluates and checks hazards besides observing and applying the food hygiene and public health regulations.

HACCP has become synonymous with food safety. It is a worldwide-recognized systematic and preventive approach that addresses biological, chemical and physical hazards through anticipation and prevention, rather than through end-product inspection and testing. The HACCP system for managing food safety concerns grew from two major developments. The first breakthrough was associated with W.E. Deming, whose theories of quality management are widely regarded as a major factor in turning around the quality of Japanese products in the 1950s. Deming and others developed Total Quality Management (TQM)
systems which emphasized a total systems approach to manufacturing that could improve quality while lowering costs.

The HACCP is a systematic approach that identifies specific hazards and establishes measures for their control to ensure the safety of food. It focuses on prevention rather than relying mainly on end-product testing. Hazard analysis involves the identification of ingredients and products which might have a pronounced effect on food safety. Once the sensitivity of the ingredients is known, various critical control points can be identified. This involves the identification and control over those processing parameters whose loss of control would result in an unacceptable risk to consumers. Microbiological critical control points have been summarised for frozen and canned foods. HACCP utilizes education and training, inspection of processing facilities, food handling operations, warehouses, etc. and microbiological surveys and testing of product. HACCP can be applied throughout from food processing industry (primary production) to food service establishments and to the consumer level. In addition, the application of HACCP systems can aid inspection by regulatory authorities and promote international trade by increasing confidence in food safety.

The main elements of the HACCP system are:
- Identify potential hazards and assess the risk (likelihood) of occurrence.
- Determine the Critical Control Points (CCPs) and the steps that can be controlled to eliminate or minimise the hazard.
- Establish the criteria (tolerances, target level) that must be met to ensure that CCP is under control.
- Establish a monitoring system.
- Establish the corrective action when CCP is not under control.
- Establish procedures for verification.
- Establish documentation and record keeping.

**Principles of HACCP System**

The HACCP system consists of the following seven principles:
- **Conduct a Hazard Analysis:** The HACCP team conducts a hazard analysis and lists all the biological, chemical and physical hazards that may be reasonably expected to occur at each step from primary production, processing, manufacture and distribution until the point of consumption.
- **Identify the Critical Control Points (CCPs):** A critical control point is a point/procedure where a food safety hazard can be prevented, eliminated or reduced to acceptable levels. For the identification of CCP’s, a decision tree can be used.
- **Establish Critical Limit(s) for Each CCP:** Critical limits must be specified for each preventive measure. In some cases more than one critical limit will be elaborated at a particular step. Criteria often used include measurements...
of temperature, time, moisture level, pH, and available chlorine and sensory parameters such as visual appearance and texture.

- **Establish a Monitoring System for Each CCP:** The monitoring procedures must be able to detect loss of control at the CCP and provide information in time for corrective action to regain control of the process. Most monitoring procedures for CCPs will need to be done rapidly because they relate to on-line processes and there will not be time for lengthy analytical testing. Physical and chemical measurements are often preferred to microbiological testing because they may be done rapidly and can often indicate the microbiological control of the product.

- **Establish the Corrective Action:** Specific corrective actions must be taken when monitoring indicates that a particular CCP is not under control. Deviation and product disposition procedures must be documented in the HACCP record keeping. Corrective action should also be taken when monitoring results indicate a trend towards loss of control at a CCP. Action should be taken to bring the process back into control before the deviation leads to a safety hazard.

- **Establish Verification Procedures:** The HACCP system should include verification procedures to provide assurance that the HACCP system is being complied with on a day-to-day basis. Monitoring and auditing methods can be used to determine if the HACCP system is working correctly. Verification activities include:
  - Review of the HACCP system and its records.
  - Review of deviations and product dispositions.
  - Confirmation if CCPs are under control.
  - Validation of established critical limits.

- **Establish Record Keeping and Documentation:** Efficient and accurate record keeping is essential to the application of a HACCP system. Records need to be kept of all areas which are critical to product safety to demonstrate that the HACCP system is in compliance with the documented system. Documentation of HACCP operation at all steps should be included and assembled in a HACCP plan. Records are useful in providing a basis for analysis of trends as well as for internal investigation of any food safety incidents which may occur. It is extremely useful to allocate a unique reference number to each HACCP record. The types of records that might be retained are as follows:
  - HACCP Plan
  - Modification to HACCP Plan
  - CCP Monitoring Records
  - Deviations and Associated Corrective Action
  - Training Records
14.2.2 PFA: Prevention of Food Adulteration Act

Prevention of food adulteration act of 1954 has been in force since 1 June 1955. According to PFA act, an article of food shall be deemed to be adulterated:

- If the article sold by a vendor is not of the nature, substance or quality demanded by purchaser and is to his prejudice, or is not of the nature, substance of quality which it purports or is represented to be.
- If the article contains any other substance which affects, or if the article is so processed as to affect injuriously the nature, substance or quality thereof.
- If any inferior or cheaper substance has been substituted wholly or in part for the article so as to affect injuriously the nature as substance or quality thereof.
- If any constituent of the article has been wholly or in part abstracted so as to affect injuriously the nature, substance or quality thereof.
- If the article had been prepared, packed or kept under unsanitary conditions whereby it has become contaminated or injurious to health.
- If the article consists wholly or in part of any filthy, putrid disgusting, rotten, decomposed, or diseased animal or vegetable substance or is insect-infested or otherwise unfit for human consumption.
- If the article is obtained from a diseased animal.
- If the article contains any poisonous or any ingredient which renders its contents injurious to health.
- If the container of the article is composed, whether wholly or in part of any poisonous or deleterious substance which renders its contents injurious to health.
- If any coloring matter other than that prescribed in respect thereof and in amounts not within the prescribed limits of variability is present in the article.
- If the article contains any prohibited preservative or permitted preservative in excess of the prescribed limits.
- If the quality or purity of the article falls below the prescribed standards or its constituents are present in quantities which are in excess of the prescribed limits of variability.

Objective

- To protect the public from poisonous and harmful foods.
- To prevent the sale of substandard foods.
- To protect the interests of the consumers by eliminating fraudulent practices.
**Meaning of Adulterant:** Any material which is or could be employed for the purposes of adulteration.

**Definition of Food:** Any article used as food or drink for human consumption other than drugs and water and includes:
- Any article which ordinarily enters into or is used in the composition or preparation of human food.
- Any flavouring matter or condiments.
- Any other article which the Central Government may having regard to its use, nature, substance or quality, declare, by notification in the official gazette as food for the purpose of this Act.

**Concept of Adulteration**
An article of food shall be deemed to be adulterated:
- If the article sold by vendor is not of the nature, substance or quality demanded by the purchaser.
- If the article contains any other substance which affects the substance or quality thereof. If any inferior or cheaper substance has been substituted wholly or in part for the article so as to affect the nature, substance or quality of the product.
- If any constituent of the article has been wholly or in part extracted to affect the quality thereof.
- If the article has been prepared, packed or kept under unsanitary conditions whereby it has become contaminated or injurious to health.
- If the article consists wholly or in part of any filthy, putrefied, rotten decomposed or diseased animal or vegetable substance or is insect-infested or is otherwise unfit for human consumption.
- If the article is obtained from a diseased animal.
- If the article contains any poisonous or other ingredient which renders it injurious to health.
- If the container of the article is composed, whether, wholly or in part of any poisonous or deleterious substance which renders its contents injurious to health.
- If any colouring matter other than that prescribed in respect thereof is present in the article or if the amounts of the prescribed colouring matter which is present in the article are not within the prescribed limits.
- If the article contains any prohibited preservative or permitted preservative in excess of the prescribed limits.
- If the quality or purity of the Article falls below the prescribed limits of variability which renders it injurious to health.
If the quality or purity of the article falls below the prescribed standard or its constituents are present in quantities not within the prescribed limits of variability which renders it injurious to health.

Sale of Certain Admixtures Prohibited
Sale by himself or by his servant or agent is prohibited in case of:
- Cream which has not been prepared exclusively from milk or which contains less than 25 per cent of milk fat.
- Milk which contains added water.
- Ghee which contains any added matter not exclusively derived from milk fat.
- Selling skimmed milk as whole milk.
- Mixture of two or more edible oils as an edible oil.
- Vanaspati to which ghee or any other substance has been added.
- Any article of food which contains any artificial sweetener beyond the prescribed limit.
- Turmeric containing any foreign substance.
- Mixture of coffee and other substance except chicory.
- Dahi or curd not made out of milk.
- Milk or milk products containing constituents other than of milk.

Procedure for Sampling and Analysis
Any food inspector can enter and inspect any place where any article of food is manufactured or stored for sale or stored for the manufacture of any other article of food for sale or exposed or exhibited for sale or where any adulterant is manufactured or kept and take samples of such article of food or adulterant for analysis.
- Notice will be issued by the inspector in writing then and there to the seller indicating his intention.
- Three samples are taken and the signature of the seller is affixed to them.
- One sample is sent for analysis to public analyst under intimation to the local health authority.
- The other two samples are sent to the local health authority for further reference.

Penalties: Guilty will be punished with imprisonment for a term which shall not be less than six months and up to 3 years and with fine up to one thousand rupees.

Important Miscellaneous Provisions
- If any extraneous additions of colouring matter is added, the same should be indicated on the labels.
Food Laws and Quality Control

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- From the labels the blending composition of ingredients should be clear to the customer.
- Sale of kesari gram individually or as an admixture is prohibited.
- Prohibition of use of carbide (acetylene) gas in ripening is prohibited.
- Sale of ghee with Reichert value less than the permitted level.
- Sale of admixture of ghee or butter is prohibited.
- Addition of artificial sweetener should be mentioned on the label.
- Sale of food colours without license prohibited.
- Sale of insect damaged dry fruits and nuts prohibited.
- Food prepared in rusted containers, chipped enamel containers and untinned copper/brass utensils are treated as unfit for human consumption.
- Containers not made of plastic material which is not according to the standards are not to be used.
- Selling salseed fat or any other purpose except for bakery and confectionery is prohibited.
- Store of insecticides in the same premises where food articles are stored is prohibited.
- Milk powder or condensed milk can be sold only with ISI mark.
- Use of more than one type of preservative is prohibited.
- Crop contaminants beyond certain specified level is treated as adulterant.
- Naturally occurring toxic substances in the food material beyond certain level is considered as unfit for human consumption.
- No anti-oxidant, emulsifiers and stabilising agent is permitted beyond the prescribed level.
- No insecticides should be sprayed on the food items.
- Oils can be manufactured only in factories licensed for such purpose.

14.2.3 FPO: Fruit Product Order

The Government of India promulgated a fruit products order in 1946. In 1955, the order was revised under the Essential Commodities Act. This order is operated by the Food and Nutrition Board of the Ministry of Food Processing Industries.

The Fruit Product Order (FPO) lays down statutory minimum standards in respect of the quality of various fruits and vegetable products and processing facilities at manufacture, storage and sale. The FPO is enforced by the Department of Health. The Agricultural marketing Advisor is authorized by law to issue a license for manufacturing fruits and vegetable products, after due inspection of the factory for hygiene, sanitation and quality of formulation. Periodic inspection by government inspectors in registered establishments is carried out to ensure
conformity of standards by processors. Packaging fruits and vegetables of a standard below the minimum prescribed standards is an offence punishable by law.

Manufacture of labeling of fruit and vegetable products can be carried out only after a valid license is issued by the licensing officer after himself satisfying with regard to the quality of product, sanitation, personnel, machinery and equipment, work area as required in the order. Licensee is empowered to put the FPO specification mark on the product. The labels on the final product are required by law to display the FPO license number. Fruit and vegetable products, not conforming to the FPO specifications are considered adulterated. The FPO specifications cover list of constituents, a method of presentation permissible colours in the preparation and also minimum quality requirement of the product. An expert committee known as the Central Food Product Advisory Committee deals with all matters relating to the FPO. The main objective of the PFA and FPO standard is to determine the minimum level of quality that can be attained, under the farming, manufacturing and retailing conditions in India. When qualities above the minimum are attained they are identified by other standards set up by the government, through the Directorate of Marketing Inspection. These cover definitions of quality for various agricultural products such as cereals, oils, butter, ghee, legumes etc. Depending on their quality the products are grade in four categories as ordinary, fair, good and special (4-1).

The order lays down specifications and quality control requirements on production and marketing of the following food products:

- Fruit juice, pulp concentrate, squashes, cordials, crush, fruit syrups, nectar, aerated water containing fruit juice or pulp and read to serve beverages.
- Fruit nectar, canned mango pulp (natural and sweetened), and sweetened aerated beverages with no fruit juice or pulp or containing less than 10 per cent fruit juice or pulp.
- Sweetened aerated water with 10 per cent or more fruit juice or pulp.
- Barley waters (lemon, orange, grape fruits).
- Synthetic syrups, ginger cocktails, ginger beer, ginger ale and sharbats.
- Bottled and canned fruits and vegetables.
- Jams and fruit cheese.
- Fruit jellies and marmalades.
- Candied and crystallized or glazed fruit and peel.
- Preserves.
- Fruit chutneys.
- Tomato juices and soups.
- Vegetable soups.
- Tomato puree and paste.
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- Tomato ketchup and sauce.
- Sauces other than soya bean sauce and tomato sauce.
- Soya bean sauce.
- Tamarind concentrate.
- Brewed and synthetic vinegar.
- Pickles in vinegar.
- Pickles in citric juice or in brine.
- Oil pickles.
- Sun dried and dehydrated fruits.
- Mango cereal flakes.
- Sun dried and dehydrated vegetables.
- Dehydrated onions.

Other than these specifications, the order also lays down specific requirements in regard to the following:

- Containers and labeling requirement.
- Limits of poisonous metals in fruit products.
- List of permissible harmless food colours.
- Limits for permitted preservatives in fruit products.
- Other permitted additives.

12.2.4 BIS: Bureau of Indian Standards

ISI is now known as Bureau of Indian standards. Safety, performance and reliability are assured when the product is ISI marked. BIS operates a certification mark scheme under the BIS Act, 1986. Standards covering more than 450 different food products have been published.

The organization runs a voluntary certification scheme known as ISI mark for certification of processed food items standard are laid for vegetable and fruit product, spices and condiments, animal products and processed foods. Once these standards are accepted, manufacturers whose products conform to these standards to use BIS label on each unit of their product. The products are checked for quality by the BIS in their own network of testing laboratories.

The certification scheme is basically voluntary in character but some of the items require compulsory BIS certification under PFA. They are food colors, food additives, infant, milk food, infant formula, milk and cereal based meaning food, milk powder.

BIS has on record standards for most of processed foods. In general, these standards cover raw materials permitted and their quality parameters, hygienic conditions under which products are manufactured and packing and labeling requirements.
Functions of BIS

These are the following functions of BIS:

- Standard Formulation
- Certification
- Laboratory Testing, Management
- Consumer Affairs
- Awareness and Training Programme

14.2.5 MFPO: Meat Food Products Order

Ministry of Food Processing Industries administers Meat Food Products Order (MFPO), 1973 which ensure quality and hygienic production of meat food products including fish products.

Meat food products are regulated through licensing under MFPO, 1973. The directorate of marketing and inspection at the Ministry of Agriculture is the regulatory for the order, which is equally applicable to domestic processors and importers of meat products. The order:

- Species sanitation and hygiene requirement for slaughter houses and manufacture of meat products.
- It also contains packing, marking and labeling provisions for containers of meat products.
- Define the permissible quantity of heavy metals, preservatives and insecticides residues in meat products.

Provision of MFPO requires four stages of inspection by qualified veterinary doctors for hygienic production of meat products.

- Anti-mortem inspection of animals before slaughter.
- Post mortem inspection of car cases after slaughter.
- The process physical inspection of meat, sanitary and hygienic processing licensed unit.
- Sampling and analysis of meat products in meat testing laboratories for various parameters.

Powers of MFPO Officers

- The Veterinarian inspecting officers of the Directorate are issued necessary authority cards to seek compliance of MFPO.
- These officers are authorized:
  - To enter and inspect the premises of meat food products manufacturers with a view to satisfy themselves that the requirements of this Order are being complied with.
To seize or detain on giving proper receipt of raw materials, documents, account books or evidence connected with the manufacture of meat food products in respect of which they have reasons to believe that contravention of the Order has taken place.

To dispose of all meat food products and raw materials so seized or detained as they deem fit.

Categories

MFPO, 1973 initially categorises the meat food manufacturers into the following three broad heads on the basis of source of raw meat:

- Category A: Includes those manufacturers or licencees of meat food products who possess their own slaughterhouse.
- Category B: Includes those manufacturers of meat food products who purchase meat from approved slaughterhouse.
- Category C: Includes those manufacturers of meat food products, who purchase raw meat from any other source.

The licence fee for each category differs and is collected every year at the time of renewal of licence.

Schedules

Meat Food Products Order, 1973 contains four schedules:

The First Schedule

- Deals with application for licence or renewal of licence under MFPO.
- The information related to applicant, address of factory, source of raw material, description of meat food products which the applicants proposes to manufacture, installed capacity, a plan of factory and a list of equipments has to be provided.
- Application for renewal of licence should invariably contain the statements pertaining to the quality and value of meat food products manufactured in the previous year.

The Second Schedule

- Deals with the minimum sanitary requirements to be complied with by a licencee.
- It contains detailed instructions regarding factory premises, construction, doors, windows and ceiling, plumbing and drainage system, equipment and manufacturing area, cold storage facilities, precautions against flies, rats and mice, water supply, personnel hygiene and vaccination of factory workers, provision of proper aprons and head gears, etc.
The Third Schedule

- Deals with hygienic requirements to be complied with by a licencee who also slaughters animals in his factory.
- It contains detailed instructions regarding separation between clean and dirty sections within the slaughterhouse, provision of lairage, slaughter hall and refrigeration facilities, ante-mortem examination, humane slaughter, post-mortem inspection and disposal of condemned carcasses or organs, etc.

The Fourth Schedule

- Deals with the requirements to be complied with as regards to packaging, marking and labeling the containers of meat food products.
- It contains detailed instructions with respect to proper packing and sealing of flexible containers, use of internal lacquers and hermetic sealing in tin plate cans, use of internal lacquers and hermetic sealing in tin plate cans, use of bottles and jars.

As per MFPO standards, canned meat food products should not contain poisonous elements viz. lead, copper, arsenic, tin, zinc in excess of 2.5, 20, 2, 250 and 50 ppm respectively by weight.

14.2.6 AGMARK

The word ‘AGMARK’ is derived from agricultural marketing. AGMARK was set up in the directorate of marketing and inspection of the government of India by introducing an Agricultural Products Act in 1937.

The word ‘AGMARK’ seal ensures quality and purity of food products. The quality of the product is determined with reference to the size, variety, weight, color, moisture, fat, content, etc. It core quality assurances for unprocessed, semi-processed and processed agricultural commodities.

Salient Features of AGMARK Standard

The following are the salient features of AGMARK:
- Quality standards for agricultural commodities are formed based on their intrinsic quality.
- Food safety factor are incorporated in the standards.
- Standards are being harmonized with international standard keeping in view the WTO requirements.
- Check is kept on the quality of certified products through 23 laboratories and 43 offices.

The grades incorporated are 1, 2, 3 and 4 or special, good, fair ordinary. There are four stages before affixing the ‘AGMARK’ label:
- Preliminary testing.
Food Laws and 
Quality Control

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- From the product, inspecting officers take representative samples.
- Technically qualified and experienced officers test the samples and assign AGMARK quality grades.
- Afterwards the commodity is packed using AGMARK label or AGMARK replica on pouches/containers.

The act defines quality of cereals, spices, oil seeds, oil, butter, ghee, legumes and provides for the categorization of commodities into various grades depending on the degree of purity. The standards also specify the types of packaging to be used for different products. The physical and chemical characteristics of products are kept in mind while formulating the AGMARK standards.

The certificate of ‘authorization’ is granted only to those in the trade having adequate experience and standing in the market. The staff of state government is generally present at the time of selection of goods, their processing, grading and packing before applying the ‘AGMARK’ labels.

Products available under AGMARK are pulses, wheat products, vegetables oils, spices, rice and milk products.

‘AGMARK’ seal:

Grading and marketing rules required for pulses, wheat, rice, vegetable oils, spices, milk products are as follows:

List of commodities for which grade standards have been prescribed under the agricultural produce (Grading and Marking) Act, 1937.

I. Food Grains and Allied Products

1. Paddy
2. Wheat
3. Jowar
4. Maize
5. Barley
6. Bajra
7. Ragi
8. Rice
9. Basmati Rice (export)
10. Padmini Rice
11. Pusa Basmati Rice
12. Pusa Rice
13. Pusa Broken Rice
14. Pusa Broken Rice
15. Pusa Broken Rice
16. Peas
17. Wheat Atta
18. Suji
19. Maida
20. Bread Wheat Flour
21. Besan (Gram Flour)
22. Wheat Porridge (Dalia)
23. Roasted Bengal Gram
24. Vermicelli
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<tr>
<th>I. Food Laws and Quality Control</th>
<th>II. Fruits and Vegetables</th>
<th>III. Spices and Condiments</th>
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<td>14. Seed Potato 40. Sapota</td>
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<td>15. Onions 41. Custard apple</td>
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<td>16. Dried edible mushrooms 42. Gherkins</td>
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<td>17. Canned/Bottled fruits and fruit products</td>
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4. Pepper
5. Celery seed
6. Cumin
7. Poppy Seed
8. Ajowan
9. Saffron
10. Garlic
11. Ginger
12. Coriander
13. Fennel
14. Ajwain
15. Saffron
16. Garlic
17. Seedless Tamarind
18. Sundried raw mango slices
19. Sundried raw mango powder
20. Nutmeg
21. Tejpat
22. Clove
23. Mace
24. Large Cardamom
25. Mixed Masala
26. Caraway and Black Caraway
27. Cumin Black (Kalonji)

IV. Edible Nuts
1. Areecanuts
2. Walnuts
3. Cashew Kernels
4. Raw Cashew nuts
5. HPS Ground nuts
6. Water chestnuts
7. Coconuts
8. Ball Copra and Cup Copra

V. Oilseeds
1. Groundnuts
2. Rapeseeds
3. Mustard Seeds
4. Taramira Seeds
5. Sesamum Seeds
6. Niger Seed
7. Linseed
8. Safflower Seed
9. Sunflower Seed
10. Cotton Seed
11. Mahua Seed
12. Salseed
13. Ambadi seed
14. Castor seed
15. Soybeans
16. Karanja seeds
17. Jatropha seeds

VI. Vegetable Oils and Fats
1. Groundnut Oil
2. Mustard Oil
3. Sesame Oil
4. Coconut Oil
5. Niger Seed Oil
6. Sal Seed Oil (Fat)
7. Linseed Oil
8. Safflower seed oil
9. Cotton Seed Oil
10. Rice bran oil
11. Castor oil
12. Vanaspati
13. Blended Edible Vegetable oil
14. Sun Flower Seed Oil
15. Maize Corn Oii
16. Mahua oil (Mowrah oil)
17. Soyabean Oil
18. Fat Spread.
19. Palm Oil and Palmolein
VII. Oil Cakes
1. Groundnut oil cake
2. Cotton seed oil cake
3. Linseed oil cake
4. Sheekakai pods
5. Mahua Flower
6. Amla dried and Powder
7. Puwad Seeds
8. Sesamum oil cake
9. Coconut oil cake
10. Safflower oil cake
11. Guar Seeds

Groupwise list of the commodities for which Agmark grade standards have been formulated under the agricultural produce (grading and marking) act, 1937.

Name of the Group: No. of Commodities Notified
1. Food Grain and Allied Products 30
2. Fruits and Vegetables 51
3. Spices and Condiments 27
4. Edible Nuts 8
5. OilSeeds 17
6. Vegetable Oils and Fats 19
7. Oilcakes 8
8. Essential Oils 8
9. Fibre Crops 5
10. Livestock, Dairy and Poultry Products 10
11. Other Products 30
TOTAL 213

VIII. Essential Oils
1. Sandalwood oil
2. Lemon grass oil
3. Palmarosa oil
4. Gingergrass oil
5. Oil of Vetiver (Khus)
6. Oil of vetiver roots (cultivated)
7. Eucalyptus oil
8. Himalayan cedarwood oil

IX. Fibre Crops
1. Cotton
2. Jute (raw)
3. Sannhemp
4. Palmyra fibre
5. Aloe fibre

X. Livestock Dairy and Poultry Products
1. Wool
2. Bristles
3. Goat hair
4. Animal casings
5. Raw meat (Chilled/Frozen)
6. Table eggs
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XI. Other Products

1. Honey 13. Myrobalan
2. Bura 14. Tendu leaves
3. Sugarcane gur (Jaggery) 15. Lac
5. Kangra Tea 17. Tamarind (with seed)
6. Isubgol husk 18. Tamarind Seed and powder
7. Papain 19. Tapioca sago (sabudana)
8. Catechu 20. Tapioca products (Animal feed)
10. Senna pods 22. Cocoa Powder
12. Gum Karaya 24. Makhana

Grade Designation Mark: The grade designation mark shall consist of ‘AGMARK insignia’ consisting of a design incorporating the certificate of authorization number, the word ‘AGMARK’, name of commodity and grade designation resembling the design as set out in Schedule-1.

Grade designation and quality: The grade designation and quality of Fruits and vegetables shall be as set out in Schedules II to XIX.

Method of Packing

- Fruits and vegetables shall be packed in such a way as to protect the produce properly.
- The materials used inside the package must be new, clean and of such a quality as to avoid causing any external or internal damage to the produce.
- The use of materials particularly of paper or stamps bearing trade specifications is permitted provided the printing or labeling has been done with non toxic ink or glue.
- Fruits and Vegetables shall be packed in each container in compliance with the recommended international code of practice for Packaging and
- The containers shall meet the quality, hygiene, ventilation and resistance characteristics to ensure suitable handling, shipping and preserving of the Fruits and Vegetables. Packages must be free of harmful foreign matter and obnoxious smell.
- Contents of each package or lot must be uniform and contain only Fruits and Vegetables of same origin, variety and grade designation.
- The visible part of the contents of the package (if present) must be representative of the entire content.
Contents of package may have different fruits and vegetables of different grades as per buyer’s requirements with proper labeling.

**Method of Marking and Labeling**

- The grade designation mark shall be securely affixed to or printed on each package in a manner approved by the Agricultural Marketing Adviser or an officer authorized by him on this behalf.
- The following particulars shall be clearly and indelibly marked on each package, namely:
  - Name of the Commodity
  - Variety
  - Grade Designation
  - Size Code (if prescribed)
  - Lot/Batch/Code Number
  - Country of Origin
  - Net Weight/No. of Units
  - Name and Address of the Packer/Exporter
  - Best Before Date (where Applicable)
  - Storage Condition
  - Date of Packing
- Such other particulars as may be specified by the Agricultural Marketing Adviser.
- The ink used for marking on packages shall be of such quality which may not contaminate the product.
- The authorized packer may, after obtaining the prior approval of the Agricultural Marketing Adviser, mark his private trade mark or trade brand on the graded packages provided that the same do not indicate quality other than that indicated by the grade designation mark affixed to the graded packages in accordance with these rules.
- Fruits and Vegetables may be graded and marked as per buyer’s requirements for exports provided the minimum requirements specified in the relevant schedule are met.

**Grading and Marketing Rules for Spices**

They shall apply to the following spices (whole and powder) namely:

- Turmeric
- Chillies
- Black Pepper
- Cardamom
- Large Cardamom
- Coriander
14.2.7 CAC: Codex Alimentarius Commission

Codex Alimentarius Commission (CAC) implements joint FAO/WHO Food Standards Programme. The purpose of the CAC programme is to protect the health of consumers and ensure fair practices in the food trade. The CAC is a collection of internationally adopted food standards presented in a uniform manner. Sanitary and Phyto-Sanitary Agreement and Technical Barriers to Trade Agreement of World Trade Organization recognize standards framed by CAC with respect to safety and quality aspects of food items. Thus for international trade standards framed by CAC are recognized.

The codex alimentarius is a code for food, which not includes a series of general and specific requirements for food safety.

Codex alimentarius commission was established in 1962. The codex alimentarius which means ‘food can’ or ‘food code’ in Latin. The dual objectives of codex alimentarius commission are to protect the health of consumers and to facilitate the international. The codex contract point in India is the Directorate General of Health Services (DGHS) in the ministry of health; however the ministry of food processing industries is closely association with the activities of codex alimentarius.

Salient features of codex alimentarius are as follows:

- Protecting health of the consumers and ensuring fair trade practices.
- Finalizing the standards.
- Determining priorities and initiating and guiding the preparation of draft standards.
- Amending published standards.
- Submission of proposal for a standard.
- A decision by the commission the executive committee.
- Adoption of standard by the commission.
- Addition of CODEX STANDARD in the codex alimentarius.

The codex secretariat is located at home and is financed jointly by the FAO and the WHO. At present there are 170 countries, including India.

Codex can be divided into three main groups:

- The commodity standards committee work vertically dealing with food products such as processed points and vegetables, fats and oil, mineral
water, coco products and chocolates, sugar, milk and products cereals and meat products.

- The general subject committee work horizontally on standards such as food additives and contaminants, pesticide residues, hygiene, labeling, inspection and certification systems, analysis and sampling, nutrition and foods for special dietary uses.
- The six regional coordinating committees are based in Africa, Asia, Europe, Latin America and Caribbean, North America and South West Pacific and Near East.

Criteria Applicable to Commodities

- Consumer protection from the point of view of health and fraudulent practices.
- Volume of production and consumption in individual countries and volume and pattern of trade between countries.
- International or regional market potential.
- Amenability of the commodity to standardization.
- Coverage of the main consumer protection and trade issues by existing or proposed general standards.
- Number of commodities which would need separate standards indicating whether raw, semi-processed or processed.

Check Your Progress

1. Write about quality concept in brief.
2. Distinguish between extrinsic attributes and intrinsic attributes.
3. What is Quality Management Systems?
4. List the principles of quality control.
5. What are the assumptions that need to be checked in order to establish food safety?
6. Give the main elements of the HACCP system.
7. What are the functions of BIS?

14.3 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. The quality concept is focused on technological attributes and factors that can contribute to product quality performance. A product has physical features that are turned into quality attributes by the perception of a consumer. With respect to agrifood products, quality perception appeared to be...
affected by different types of attributes. Relevant attributes for consumers involve safety, nutritional value (health aspect), sensory properties (like taste, flavour, texture, and appearance), shelf life, convenience (ready-to-eat meal) and product reliability (correct weight, right composition, etc). These attributes can be defined as intrinsic attributes and are directly related to the physical product properties.

2. Extrinsic attributes refer to production system characteristics and other aspects, like environmental impact or marketing influence. They do not necessarily have a direct influence on physical properties but may affect acceptance of products by consumers. For example, the use of pesticides, of antibiotics to improve animal growth, or the application of biotechnologies to modify product properties, can have a significant effect on food acceptance.

Intrinsic attributes characterize the physical product, whereas marketing efforts mainly determine extrinsic attributes. According to this classification, typical intrinsic attributes include appearance, colour, shape and texture. Typical extrinsic attributes are price, brand name, packaging, labelling and product information. Extrinsic attributes are related to the product but are not part of it, like price, brand or store name. These cues have a predicted value for the consumer and thus influence the quality expectation. Generally, intrinsic cues are more important than extrinsic cues. Production system characteristics do not necessarily affect the physical attributes. The use of pesticides will not directly influence product features but might affect quality expectations but a change in production system characteristics may influence the physical features.

3. Quality Management Systems (QMS) are used to control the quality and safety of products. The use of QMS ensures that all aspects of a business are working efficiently and cost effectively. A system will provide a competitive advantage, which can increase marketing and sales opportunities. This will help a company gain new customers as well as retaining existing business. By working within a QMS the whole workforce will be involved which thereby improves communication, morale and job satisfaction.

4. The principles of quality control are considered under the following:
   - Raw material control
   - Process control
   - Finished product inspection

5. The following assumptions should be checked in order to establish food safety:
   - Unsafe: The normal conditions of use of the food by the consumer at each stage of production, processing and distribution are taken into account as well as the information provided to the consumer.
• Injurious to Health: Probable immediate, short term and long term effects (including future generations) of the food to health, probable cumulative toxic effects and particular health sensitivities of a specific category of consumers should be considered.

• Unfit for Human Consumption: If the food is unacceptable for human consumption according to its intended use, for reasons of contamination, whether by extraneous matter or otherwise, or through putrefaction, deterioration or decay.

6. The main elements of the HACCP system are:
   • Identify potential hazards and assess the risk (likelihood) of occurrence
   • Determine the Critical Control Points (CCPs) and the steps that can be controlled to eliminate or minimise the hazard
   • Establish the criteria (tolerances, target level) that must be met to ensure that CCP is under control
   • Establish a monitoring system
   • Establish the corrective action when CCP is not under control
   • Establish procedures for verification
   • Establish documentation and record keeping

7. These are the following functions of BIS:
   • Standard formulation
   • Certification
   • Laboratory testing, management
   • Consumer affairs
   • Awareness and training programme

14.4 SUMMARY

• The quality concept is focused on technological attributes and factors that can contribute to product quality performance.

• A product has physical features that are turned into quality attributes by the perception of a consumer.

• Extrinsic attributes refer to production system characteristics and other aspects, like environmental impact or marketing influence.

• Intrinsic attributes characterize the physical product, whereas marketing efforts mainly determine extrinsic attributes.

• Quality Management Systems (QMS) are used to control the quality and safety of products. The use of QMS ensures that all aspects of a business are working efficiently and cost effectively.
Food quality management, which assures the health and safety of food, has become increasingly important during the past few years.

Quality is defined as any of the features that make something what it is or the degree of excellence or superiority.

The word ‘quality’ is used in various ways as applied to food. Quality product to the salesman means a product which is of high quality and of expensive nature.

In food processing, the general rule is that the effective methods must be carefully applied to conserve the original qualities of the raw materials. Processing cannot improve the raw material.

The aim of quality control is to achieve as good and as consistent a standard of quality in the product being produced as is compatible with the market for which the product is designed.

Raw materials such as artificial colouring, spices and essences may be used slowly and may deteriorate on long storage.

According to apex industrial bodies there should be only one national food safety code, which should cover all aspects of Indian food safety under a unified system.

Food is not allowed to be placed on the market if it is unsafe. Food is considered unsafe if it is injurious to health or unfit for human consumption.

Food business operators at all stages of production, processing and distribution of food are primarily responsible for safe food.

The Committee for Food Safety of FAO (Food and Agriculture Organization) and WHO (World Health Organization) contended in 1984 that one of the biggest problems today is the diseases caused by food.

HACCP has become synonymous with food safety. It is a worldwide-recognized systematic and preventive approach that addresses biological, chemical and physical hazards through anticipation and prevention, rather than through end-product inspection and testing.

The HACCP is a systematic approach that identifies specific hazards and establishes measures for their control to ensure the safety of food.

Specific corrective actions must be taken when monitoring indicates that a particular CCP is not under control.

Any food inspector can enter and inspect any place where any article of food is manufactured or stored for sale or stored for the manufacture of any other article of food for sale or exposed or exhibited for sale or where any adulterant is manufactured or kept and take samples of such article of food or adulterant for analysis.

The Government of India promulgated a fruit products order in 1946. In 1955, the order was revised under the Essential Commodities Act.
The FPO specifications cover list of constituents, a method of presentation permissible colours in the preparation and also minimum quality requirement of the product.

ISI is now known as Bureau of Indian standards. Safety, performance and reliability are assured when the product is ISI marked. BIS operates a certification operates a certification mark scheme under the BIS Act, 1986. Standards covering more than 450 different food products have been published.

Ministry of Food Processing Industries administers Meat Food Products Order (MFPO), 1973 which ensure quality and hygienic production of meat food products including fish products.

The word ‘AGMARK’ is derived from agricultural marketing. AGMARK was set up in the directorate of marketing and inspection of the government of India by introducing an Agricultural Products Act in 1937.

Codex Alimentarius Commission (CAC) implements joint FAO/WHO Food Standards Programme. The purpose of the CAC programme is to protect the health of consumers and ensure fair practices in the food trade.

The codex alimentarius is a code for food, which not includes a series of general and specific requirements for food safety.

### 14.5 KEY WORDS

- **Quality**: Quality is defined as any of the features that make something what it is or the degree of excellence or superiority.

- **Adulterant**: Any material which is or could be employed for the purposes of adulteration.

- **Food**: Any article used as food or drink for human consumption other than drugs and water

- **Extrinsic attributes**: Extrinsic attributes refer to production system characteristics and other aspects, like environmental impact or marketing influence.

- **Intrinsic attributes**: Intrinsic attributes characterize the physical product, whereas marketing efforts mainly determine extrinsic attributes.

### 14.6 SELF ASSESSMENT QUESTIONS AND EXERCISES

**Short Answer Questions**

1. What are the general principles of quality control?
2. Write in brief about food quality management systems.
3. List the principles of HACCP system.
NOTES

5. What are the four schedules of Meat Food Products Order (MFOP)?

Long Answer Questions

1. What is food laws and quality control all about? Explain.
2. Explain the integrated food law in India.
3. Write about the HACCP system in detail.
4. What are the national food laws and standards?
5. Discuss about Fruit Product Order (FPO).
6. Write a detailed note on MFPO.
7. What is AGMARK? Explain how it is useful.

14.7 FURTHER READINGS