Programme: M.Sc. Food and Nutrition

Course Title: ADVANCES IN FOOD MICROBIOLOGY

Course Code: MFN 103

Course Study Material

UNIT-I INTRODUCTION TO HISTORICAL DEVELOPMENTS

Structure

1.1 INTRODUCTION

The first unit of this course gives you an introduction to fundamentals of microbiology specially food microbiology. Through this unit, we will try to examine the definition of microbiology and food microbiology. Historic milestones of Microbiology covered in the unit will expose the reader to the different contributors to the field of microbiology. We will also get an idea ofsignificance of microorganisms in foods. Further, we will also understand the scope of microbiology and its relation with other fields. The students will also get an insight into advance developments in microbiology.

Objectives

After learning this unit, you will be able to:

- comprehend the concept of microbiology and food microbiology
- explain the historical development of microbiology

- discuss the significance of microorganisms in food
- describe the scope of microbiology
- understand the developments occurring in the field of microbiology

1.2 FUNDAMENTALS OF MICROBIOLOGY

The word "microbiology" is derived from three Greek words: micros (meaning "small"), bios (meaning "life") and logos (meaning "science"). Microbiology is defined as the study of microbes that are not visible to the naked eye. Micro-organisms, also called microbes, apply to all of the microscopic organisms that are visible under a microscope. This group includes bacteria, fungi (yeasts and moulds), protozoa and microalgae. It also includes viruses, non-cellular entities that are sometimes considered to be the boundary between living and non-living. It is the study of microbial cells, their functioning and their effects on humans, plants, animals and the environment. Microbiology studies the diversity and evolution of microbial cells and how different forms of microbial life (including bacteria, viruses, algae, archaea, protozoa, and fungi) affect other life forms. This includes the impact microbes have on the world, including their beneficial and harmful activities in soil, water, human, animal bodies, plants and even the food we eat.

Microbiology is the basis of all biological sciences because microorganisms affect (directly or indirectly) all life forms on Earth in one way or another. The study in the field of microbiology would not have been possible without the successful development of the instrument - "Microscope" - which seperated microbiology from the rest of the biological sciences. The discovery of the microscope laid the foundation for the development and study of a microbial life form too small to be seen with the naked eye. The existence of microorganisms was unknown until the discovery and development of the microscope. A microscope is an optical instrument that can magnify small objects or entities. The microscope was invented at the beginning of the 17^{th} century, with mainly two types of microscopes developed, the simple microscope and the compound microscope. The simple microscope has a single lens with a short focal length, while the compound microscope has two double-convex lens systems, with the objective lens and eyepieces having higher magnification than the simple microscope. Electron microscopes are another class of microscopes with an extremely higher magnification than simple and compound microscopes. Electron microscopes can be used to view and observe smaller microbial life forms, like viruses, which was not possible to be seen with ordinary light microscopes.

1.3 HISTORY OF MICROBIOLOGY

Microbiology is a very old discipline of biological sciences, discovered due to innovative research and experiments by pioneers in the field. The discoveries, scientific inventions and breakthroughs of renowned microbiologists and scientists have contributed immensely to developments in microbiology discipline. The contributions and experiences made by these researchers are highlighted in Table 1. Although global research is now trending towards molecules and high technology, it would be unfair to move forward without acknowledging the accidental and innovative works and inventions of these legendary microbiologists. Microbiology is both applied biology and fundamental biology, and it demonstrates in all respects fundamental principles consistent with the discipline of biology.

The first person to postulate the existence of microorganisms was Aristotle in 4 BC. He postulated that organisms are composed of cells. But it wasn't until the 13^{th} century that people realized that ground glass plates could provide greater magnification and they can see small objects that the naked eye cannot see with these plates. Along with these developments, Roger Bacon hypothesized that invisible creatures could cause disease. In 1530, Fracastoro of Verona coined the term syphilis to describe an epidemic that swept through Europe when French soldiers returning in the 1400s spread the disease. He named the pathogen "seminaria morbi" (live bacteria), which spreads "infectious viruses" through contact with individuals carrying the bacteria. In 1658, Athanasius Kircher, defined Worms as invisible creatures found in rotting corpses, flesh, milk and secretions. Even before the discovery of germs, some researchers suspected their existence and linked them to disease. The Roman philosopher Lucretius (c. 98-55 BC) and the physician Girolamo Fracastoro (1478-1553) were among others that suggested that the disease is being caused by invisible beings. The first microscopic observations were apparently made by the Italian Francesco Stelluti between 1625 and 1630 on bees and weevils using a microscope possibly provided by Galileo.

Robert Hooke (1635-1703) was actually the first person to use a microscope for observing invisible life forms (notably the fruiting bodies of 7 fungi), and became the first person to describe microbes. Hooke reported that he visualized plant and fungal structures under his rudimentary compound microscope, the lens of which could not see bacteria. His observations were charted and recorded in his famous book titled “Micrographia”. In 1665, Robert Hooke's Micrographia published the first map of microorganisms. However, Antonie van Leeuwenhoek (1632-1723), widely considered as the father of the field of microbiology,

was the first microbiologist to observe and describe bacteria. He was the first person to publish detailed and accurate observations of microbes (1632–1723) in Delft, the Netherlands. He spent most of his time constructing simple microscopes consisting of a biconvex glass lens between two silver plates. His microscope could magnify about 50 to 300 times, and illuminated liquid samples by placing them between two glasses and illuminating them at a 45° angle to the plane of the sample. This provided with a form of darkfield lighting, making organisms appear as glowing objects against dark backgrounds and making bacteria clearly visible. Beginning in 1673, Leeuwenhoek sent detailed letters to the Royal Society of London describing his findings. It is clear from his description that he saw both bacteria and protozoa.

Thus, the development of microbiology as a biological science depended on the availability of microscopes, in addition to the ability of the microbiologist to isolate and cultivate pure forms or cultures of microorganisms in vitro and in vivo. Today, there is a variety of microscopes, from simple light microscopes to complex electron microscopes, that provide a better view of the microbial world. These devices have enabled microbiologists to better understand microbial cells at the cellular and molecular levels. Despite the importance of Leeuwenhoek's observations, the development of microbiology largely stagnated for the next 200 years. Little progress has been made, largely because microscopic observations of microbes have not provided enough information to understand their biology. For the discipline to develop, techniques for isolating and culturing microorganisms in the laboratory are necessary.

During the 18th century, there were debates about the origin of microorganisms. Some scientists believed in "spontaneous generation," the idea that microorganisms could arise spontaneously from non-living matter, while others argued in favor of "biogenesis," the concept that living organisms can only arise from pre-existing living organisms. This controversy continued for several decades and was finally resolved in the 19th century through experiments conducted by scientists such as Louis Pasteur. He made groundbreaking contributions to microbiology, establishing the germ theory of disease. He conducted experiments that disproved spontaneous generation and showed that microorganisms are responsible for causing diseases. Pasteur's work on fermentation also led to the development of pasteurization, a process used to prevent spoilage and contamination of food and beverages by microorganisms. The German physician Robert Koch made significant contributions to microbiology in the late 19th century. He developed a set of criteria known as Koch's

postulates, which are still used today to establish the causative agent of infectious diseases. Koch also discovered the causative agents of several diseases, including tuberculosis and cholera, and developed techniques for isolating and cultivating bacteria in pure culture, laying the foundation for modern bacteriology. In the late 19th and early 20th centuries, microbiologists made significant advances in vaccination and immunology. The development of vaccines against diseases such as smallpox, rabies, and diphtheria revolutionized public health and contributed to the eradication of deadly diseases. Scientists such as Edward Jenner, Emil von Behring, and Paul Ehrlich made groundbreaking discoveries in the field of immunology, leading to the development of vaccines and the understanding of the immune response.

The mid-20th century was marked by the discovery of antibiotics, which revolutionized the field of microbiology and transformed medical practices. In 1928, Scottish biologist Alexander Fleming discovered the first antibiotic, penicillin, which was later developed into a life-saving drug by Howard Florey and Ernest Chain. This marked the beginning of the golden age of microbiology, as scientists continued to discover and develop antibiotics to combat various bacterial infections. The post-World War II era saw rapid advancements in molecular biology and genetic engineering, leading to a deeper understanding of the genetic makeup and functioning of microorganisms. In 1953, James Watson and Francis Crick elucidated the structure of DNA, providing a foundation for understanding genetics and molecular biology. This laid the groundwork for the development of genetic engineering techniques, such as recombinant DNA technology, which allowed for the manipulation of genes in microorganisms, leading to the production of important medical and industrial products.

The history of microbiology has been a fascinating journey of scientific discoveries, innovations, and applications that have transformed our understanding of the microbial world and had a profound impact on various fields of science, medicine, and industry. From the discovery of microorganisms with the advent of microscopes, to the establishment of germ theory of disease, the development of antibiotics, and the recent advancements in molecular biology and genetic engineering, microbiology continues to play a critical role in improving human health, agriculture, food safety, and environmental sustainability.

Table 1: Contribution of Famous Microbiologists

Name of Microbiologist	Period	Contribution
Antonie van Leeuwenhoek	1632–1723	The First Microbiologist
Robert Hooke	1635–1703	The First to Observe the Existence of Microorganisms
Edward Jenner	1749–1823	The First Success of Immunization
Louis Pasteur	1822–1895	The Master of Microbiology
Ferdinand Julius Cohn	1828–1898	Pioneer of Bacteriology
Joseph Lister	1827–1912	Pioneer of Antisepsis
Robert Koch	1843–1910	The Great Medical Microbiologist
Emil von Behring	1854–1917	Pioneer of Serology
Erwin F Smith	1854–1927	Father of Plant Pathology
Albert Leon Charles Calmette	1863–1933	Antituberculosis and BCG Vaccination
Chaim A Weizmann	1874–1952	Pioneer of Industrial Microbiology
Oswald Theodore Avery	1877–1955	Microbiological Genetic Transmission and DNA
Alexander Fleming	1881–1955	Discovery of Penicillin
William C Frazier	1894–1991	Pioneer of Dairy and Food Microbiologist

1.4 FOOD MICROBIOLOGY

Although the process of food spoilage and the methods of preserving and fermenting food have been recognized since ancient times, the relationship between food and microbes was not established until the 1800s. In 1837, Schwann suggested that the yeast that is observed while fermentation appeared like a small microscopic plant. Later Pasteur showed that microorganisms were causing the chemical changes that were observed in food and beverages. Their findings initiated the progress of food microbiology. Shortly after these early discoveries, emphasis on the role of microbes in preservation of food, its spoilage, food borne illness increased steadily till today where, food microbiology has become a distinct discipline. So, let’s understand Food Microbiology.

Food microbiology is a branch of microbiology that focuses on the study of microorganisms that can affect the safety, quality, and shelf-life of food. It involves the study of various microorganisms, including bacteria, yeasts, molds, viruses, and parasites, that can be present in food and can have both positive and negative effects. Food microbiologists study the microbiological aspects of food production, processing, preservation, and storage. They investigate the sources, growth, survival, and control of microorganisms in food, and how these microorganisms can impact food safety and quality. They also study the interactions between microorganisms and the chemical, physical, and sensory properties of food. Additionally, microbes are required for the development of fermented food products like fermented milks, sauerkraut, cheese, yogurt, curd, beer, wine etc. On the other hand, food safety is a major concern in food microbiology. Toxins produced by pathogenic bacteria, viruses and microorganisms can contaminate food. On the other hand, microorganisms and their metabolites can also be utilized to fight against the pathogenic microorganisms. Probiotics, includes those strains that have the ability to produce bacteriocins, inhibit and kill pathogenic microbes. The isolated and purified bacteriocins for eg. nisin can be added directly to foods. Moreover, bacteriophages and viruses that can infect bacteria, may be used to kill bacterial pathogens. Preparing the food carefully, with proper cooking, can remove most of the bacteria and viruses. But the toxins produced by pollutants cannot be reversed into non-toxic forms by any heating or cooking method in contaminated food due to other safety conditions.

1.5 SIGNIFICANCE OF MICROORGANISM IN FOODS

Microorganisms play an important role in the production, storage and use of food. The food chains of organisms capable of photosynthesis have the ability to use light energy and carbon dioxide to produce macromolecules, water and mineral salts to produce carbohydrates, fats and proteins that form all other organisms and also used to provide energy. The food we eat contains a mixture of microbes whose composition depends on the organisms that come into contact with the food and their growth rate, survival and food interaction over time. The microbes come from the general flora present in the raw material and transmission route of microorganisms after harvesting or slaughtering. Food preservation methods have been employed to prevent or delay decomposition or spoilage of food. It is recognized that the dangers of food spoilage and food poisoning have existed since pre-scientific times. In ancient eras, dead sea salts were used to preserve different types of food.

The Chinese, Greeks followed by Romans consumed salted fish as part of their diet. Olive and sesame oils have also been used since ages as a method of preserving food.

Microorganisms have been used to produce types of fermented food across various civilizations. Microbes were used for pickling, brewing, baking and wine making. The food industry uses *Lactobacillus* and *Bifidobacterium* hugely in its production technology. Buttermilk is acidified from low-fat milk with its unique flavor due to production of compunds like diacetyl and acetaldehyde during fermentation. They are produced by different species of *Streptococcus, Leuconostoc* and *Lactobacillus*. Fungi also play an important role in food production. Molds are used in the food industry for food manufacturing. The carbohydrates present in the dough are fermented by the yeast to produce carbon dioxide and water, which further produces small quantities of alcohol on continous growth. The produced carbon dioxide causes the dough to swell as air bubbles get trapped in the dough and devlop a soft and elastic texture. Various kinds of microorganisms are also used in cheese making. Yeast is an integral part of production of beer and alcoholic beverages. *Botrytis cinerea* is used to rot grapes to make wine. *Saccharomyces carlsbergensis* is the most common yeast used in the fermentation of beers. Important yeast strains in the production of include *Brettanomyces, Candida, Debaria* and *Saccharomyce*s sp. Some other fermented foods developed from microorganisms are coffee, soy saucem olives, meat products like sausages, and even some food additives eg. monosodium glutamate and citric acid.

1.6 SCOPE OF MICROBIOLOGY

Microbiology is one of the most important and complex biological sciences. In addition to studying the natural history of microbes, it addresses aspects of microbe-human and microbe-environment interactions. These interactions include genetics, metabolism, infection, disease, drug therapy, immunology, genetic engineering, industry, agriculture, and ecology. Microbiology has a wide scope as it is both a fundamental science and an applied science. Microbiology is associated with many fields, including but not limited to medicine, agriculture, biochemistry, food science, ecology, genetics and molecular biology. Since the different aspects or areas of microbiology are interrelated, applied microbiologists should be familiar with basic microbiology. However, microbiologists may be interested in specific types of organisms that represent what they are ultimately called, including

- virologist - who studies viruses
- bacteriologist - who studies bacteria

- mycologist - who studies species of fungi
- protozoa - which studies protozoa
- phycologist or phycologist - who studies species of algae

In addition to organisms that may be of interest to them, microbiologists may also be interested in the activities or characteristics of these microorganisms, including

- Microbial cytology - those that study the cellular components of microorganisms
- Microbiology Physiology - study of the life processes of microorganisms
- Microbial ecology - study of activities of microorganisms in their habitat/niche
- Microbial genetics and molecular biology - study of microorganisms at the molecular level
- Microbial taxonomy - those concerned with the identification, classification and nomenclature of microorganisms

Microbiologists may also have a more applied orientation. This field of microbiology is known as applied microbiology. Applied microbiology is the application of microbiological principles and techniques for the production of economically useful products and solutions to social problems. The use of microorganisms is done to produce certain products, including pharmaceuticals, foods, fuels and beverages etc. The different areas of applied microbiology include:

- Industrial microbiology - the field that uses microorganisms for industrial processes such as wastewater treatment, bioremediation, and fermentation processes
- Medical microbiology - the study of pathogenic microorganisms and their role in human disease
- Pharmaceutical microbiology - the field that studies microorganisms associated with the production of antibiotics, enzymes, vitamins, vaccines and other pharmaceuticals
- Agricultural Microbiology - the field that studies microorganisms relevant to agriculture this includes Soil Microbiology and Plant Microbiology/Pathology
- Food and Dairy Microbiology - the microorganisms that cause food spoilage and the function and diversity of food sources in the natural environment Fields.

Other fields of microbiology such as airborne microbiology and soil microbiology also exist. Microbiology as a career can be very fulfilling and rewarding as the field is now rising with numerous opportunities for pursuing a career in this field.

1. Microbiologists are now at the forefront of almost all biomedical sciences, including medicine, where the assistance of microbiologists is needed in the development of methods for prevention, detection, diagnosis and treatment of infectious diseases.
2. The past and present scientific achievements of microbiologists, have encouraged research and development in the fields of biotechnology, pharmaceuticals, biochemistry, medical treatment and the chemical industry.
3. Microbiologists play an important role in the public health sector, university education and their research centers, government and the food and beverage industry, areas where the principles of microbiology are applied to various aspects of human, animal life and environment.
4. Microbiologists also provide teaching and training assistance in almost all of the biological sciences in educational institutions.

Microbiological research plays an important role in promoting human health and well-being, particularly through the production of drugs and vaccines for the treatment and prevention of human infectious diseases. Each major discipline of microbiology contains a number of subsections or specializations, which in turn deal with specific fields or areas. In fact, many areas of this science have become so specialized that it is not uncommon for a microbiologist to devote its entire research to a single group or type of microorganisms, biochemical processes, or diseases. For example, there are Bacterial Physiologists who study industrial processes, Molecular Biologists who specialize in viral genetics, Mycologists who study fungi and Epidemiologists who are public health workers who investigate patterns and causes of disease and injury.

Research in microbiology advances the understanding of many theoretical biological principles. The study of microbes has established general concepts about the chemistry of life, genetic systems, and the global circulation of nutrients, minerals, and gases. Microbiology has many practical applications in industry and medicine. Microbiology has shown wide-ranging applications in various fields. In medicine, microbiology plays a critical role in the diagnosis, treatment, and prevention of infectious diseases. Microorganisms are used in the production of vaccines, antibiotics, and other pharmaceuticals. In agriculture, microbiology is used in the development of microbial fertilizers, biocontrol agents, and biotechnology for crop improvement. In food industry, microbiology is used in food safety, fermentation processes, and production of dairy products, beverages, and baked goods. Microorganisms are also used in environmental applications such as bioremediation, wastewater treatment, and bioenergy production (Fig. 1).

Some important application areas of based on microbiology are discussed below:

- Immunology studies the body's defense system against infections.
- Serology, a discipline that looks for the products of immune reactions in blood and tissues and thus aids in the diagnosis of infectious diseases, and allergy, the study of allergic reactions to common materials and harmless.
- Public health microbiology and epidemiology are designed to monitor and control the spread of disease in the community. The main US and global agencies involved in this case are the US Public Health Service (USPHS) and its lead agency, the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, and the World Health Organization (WHO).
- Food Microbiology, Dairy Microbiology, and Aquatic Microbiology: The study of the ecological and practical role of microorganisms in food, dairy and water.
- Agricultural Microbiology focuses on the relationship between microorganisms and crops, and emphasizes improving yields and controlling plant diseases.
- Biotechnology includes any process in which people use the metabolism of living things to obtain desired products, from bread-making to gene therapy.
- Industrial microbiology involves the use of microorganisms to produce or harvest large quantities of substances such as beer, vitamins, amino acids, pharmaceuticals, and enzymes.
- Genetic engineering and recombinant DNA technology involve techniques for deliberately altering the genetic makeup of an organism in order to mass-produce human hormones and other drugs, create entirely new substances, and develop organisms with unique methods of synthesis and adaptation. It is the strongest and most dynamic field of modern microbiology.

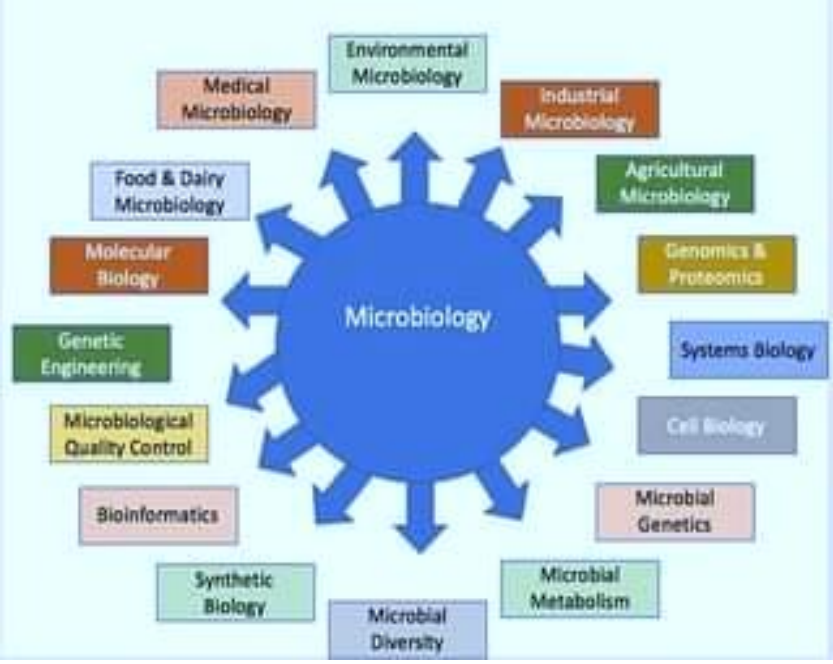

Fig 1: Various application areas of Microbiology

1.7 DEVELOPMENTS IN MICROBIOLOGY

Microbiology has had a great impact on society. This is necessary both in the face of new and re-emerging threats from human infectious diseases, and in the development of more efficient and greener industrial technologies. Perhaps the biggest challenge faced by microbiologists is assessing the impact of new discoveries and technological developments? Microbiologists need to convey a balanced view of the long-term positive and negative impacts of these developments on society. For some time now, a revolution has been taking place discreetly in the microbiology laboratories of hospitals. Molecular techniques based on the detection of DNA, RNA or microbial proteins have moved from research laboratories into the realm of routine diagnostic testing. These technologies promise rapid detection, more accurate pathogen identification and increased sensitivity, facilitating confident clinical decision-making. Combined with automation, Modern Microbiology offers a high throughput option for large sample numbers at a reasonable cost.

In 1952, Alfred Hershey and Martha Chase, with the help of bacteriophages, discovered that DNA as heredity and genetic material has become so important that bacteriophages have become standard laboratory materials. These findings led to the double helix model of Watson and Crick in 1953, who used X-ray diffraction patterns to clarify their model. For this exceptional model of DNA symmetry, they received a Nobel Prize. The work

of Nirenberg, Holley and Khorana laid the groundwork for deciphering the genetic code. The study of viral and bacterial DNA has provided an insight into genetic engineering knowledge. Bacteria have additional DNA in the form of a plasmid which can encode many important functions such as antibiotic resistance or toxin production or host range which has become a tool for genetic engineering. These plasmids are used as vectors to carry an external DNA and transfer it to another DNA. This DNA is also used to develop gene banks. The discovery of restriction endonucleases revolutionized the field of biotechnology. This technique provides the basis for the cloning of many useful genes that cause many chronic diseases in humans and are currently being studied.

Microbiology has continued to evolve with the emergence of new fields and technological advancements. Fields such as environmental microbiology, astrobiology, and microbial ecology gained prominence, exploring the role of microorganisms in various ecosystems and their potential applications in bioremediation and biotechnology. Technological advances such as DNA sequencing, genomics, metagenomics, and high-throughput screening have revolutionized the study of microorganisms, enabling the identification, characterization, and manipulation of microorganisms with unprecedented precision and speed.

1.8 SUM UP

This unit outlines briefly the definition and concept of microbiology. It explains the historical developments occurred in the field of microbiology. The contents of the unit emphasize on significance of microorganisms in foods. It also highlights the scope of food microbiology. The advance developments in the field of microbiology have also been discussed.

1.9 CHECK YOUR PROGRESS

1. Define microbiology and food microbiology.

.....

.....

.....

3. What are the different areas of applied microbiology?

.....

.....

.....

4. Write about the contribution of following scientists in the field of microbiology.

(a) Robert Hooke

.....

.....

(b) Antonie Van Leuwenhoek

.....

.....

(c) Aristotle

.....

.....

(d) Louis Pasteur

.....

.....

(e) Watson and Crick

.....

.....

5. Explain in brief:

(a) Mycologist

.....

.....

(b) Phycologist

.....

.....

(c) Microbial taxonomy

.....

.....

(d) Virologist

.....

.....

(e) Microbial physiology

.....

.....

(f) Microscope

.....

.....

(g) Agriculture Microbiology

.....

.....

Unit-II MICROORGANISMS OF IMPORTANCE IN FOOD

Structure

2.1 INTRODUCTION

This unit will give you a detailed insight into different types of microorganisms associated with food. Through this unit, we will try to understand the structural, morphological and cultural characteristics of microorganisms. We will also study about different asexual and sexual reproduction methods of molds, yeast and bacteria. Further, we will also examine the important groups of mold, yeast and bacteria associated with food.

Objectives

After studying this unit, you will be able to:

- comprehend the different microorganisms present in food
- understand the structural, morphological and cultural characteristics of microorganisms
- explain the different asexual and sexual reproduction methods of molds, yeast and bacteria
- describe the the important groups of mold, yeast and bacteria associated with food

2.2 MOLDS

Molds are a type of fungi that belong to the group known as filamentous fungi. They are ubiquitous in nature and can be found in various environments, including soil, air, water, and food. Molds play important roles in the ecosystem, as they are involved in the decomposition of organic materials, nutrient recycling, and production of certain foods and beverages (such as cheese, soy sauce, and tempeh). However, molds can also pose risks to human health and food safety. Some molds can produce toxic secondary metabolites called mycotoxins, which can contaminate food and cause health problems when consumed. Mycotoxins are heat-stable and can withstand processing, so they can persist in food products even after cooking or processing.

2.2.1 Morphological Characteristics:

The morphological characteristics of molds refer to the physical and structural features of mold colonies and structures that can be used for their identification and classification. Molds grow as multicellular structures called mycelium, which are composed of thread-like structures called hyphae. The characteristics of hyphae, such as their size,

branching pattern, and color, can also be used for mold identification. For example, some molds have septate hyphae, which are divided into compartments by cross-walls (septa), while others have non-septate or coenocytic hyphae, which lack cross-walls. Molds reproduce by forming spores, which are reproductive structures that can be dispersed in the air and can lead to mold contamination in food and other environments. The size, shape, color, and arrangement of spores can be used for mold identification. For example, some molds produce spores in structures called conidia, which can be spherical, oval, or elongated, while others produce spores in specialized structures, such as sporangia or fruiting bodies. Some molds may produce specialized structures, such as sclerotia (dense, compact masses of mycelium) or rhizoids (root-like structures), which can be used for identification. Additionally, microscopy techniques, such as staining and microscopic examination of mold structures, can provide valuable information for mold identification, such as the presence of reproductive structures, cell walls, or other cellular features.

2.2.2 Cultural Characteristics

The cultural characteristics of molds refer to the observable macroscopic and microscopic features of mold colonies that can be used for their identification and classification. These characteristics are typically studied in a laboratory setting and can provide valuable information for identifying different types of molds. Molds can form colonies on agar plates or other growth media, and their colonies can exhibit different appearances, such as color, texture, size, and shape. Colony color can vary widely, ranging from white, gray, green, yellow, brown, to black, depending on the species. Colony texture can be fluffy, powdery, cottony, or velvety, and can provide clues about the type of mold. Other cultural characteristics of molds that can be used for identification include their odor, which can range from musty to foul.

2.2.3 Physiological characteristics:

Moisture Conditions

Molds usually require less water activity than yeasts and bacteria. It has been claimed through various studies that moisture content below 14 to 15 percent in flour or other dried products, dried fruits can prevent or greatly delay mold growth. The ideal moisture conditions for mold growth typically fall within the range of 60% to 100% relative humidity (RH), although some molds may be able to grow at lower humidity levels. Mold growth can be inhibited or prevented by keeping indoor humidity levels below 60%. Mold spores are

ubiquitous in the environment and can easily settle on surfaces. When these spores encounter a conducive environment with sufficient moisture, along with a suitable organic food source, such as wood, paper, carpet, or other organic materials, they can germinate and grow into visible mold colonies.

Temperature Conditions

The temperature requirements for mold growth can vary depending on the species of mold. However, most molds thrive in a relatively narrow temperature range, typically between 68°F (20°C) and 86°F (30°C). This temperature range is commonly referred to as the "mold growth temperature range." Some molds may be able to grow at temperatures outside of this range, with some species able to tolerate temperatures as low as freezing or as high as 122°F (50°C). However, the optimal temperature range for most common indoor molds is within the range of 68°F to 86°F.

Oxygen and pH Conditions

Molds are generally aerobic in nature which means oxygen is required for their growth on foods. Most molds can easily grow over a broad range of pH 2 to 8.5 (hydrogen-ion concentration), but mostly prefer an acidic pH.

Nutrition Required

Molds can utilize many types of simple as well as complex foods. Common molds have a variety of hydrolytic enzymes, amylases, pectinases, proteinases, and lipases which cause hydrolysis of complex foods for their metabolism.

Presence of Inhibitors

Certain compounds produced by molds show inhibitory action against other organisms, for example penicillin from *Penicillium chrysogenum* and clavacin from *Aspergillus clavatus*. Some of these compounds are mycostatic in nature which means they can inhibit the growth of molds like sorbic acid, propionates, and acetates are examples or may be specifically fungicidal i.e. ability of killing molds.

2.2.4 Reproduction

Reproduction in molds mainly occurs by means of asexual spores. Such molds are called "imperfect" molds or the Fungi Imperfecti. These molds are typically septate and produce only asexual spores. Some molds *form* sexual spores also known as “perfect" molds

and are differentiated as *Oomycetes* or *Zygomycetes* if they are nonseptate, and *Ascomycetes* or *Basidiomycetes* if they are septate.

Reproduction by Asexual Spores

Asexual spores produced by molds are small, light, large in number and are resistant to drying. They usually spread through air being light and can start new mold thallus where ever they find conditions to be favorable. The three principal types of asexual spores are:

1. **Conidia** (singular is known as conidium): In this type conidia cut off, or bud off, from a fertile hypha known as conidiophores.
2. **Arthrospores or oidia** (singular is known as oidium): The arthrospores are developed by fragmentation of a hypha, and the seperated cells of the hypha become arthrospores.
3. **Sporangiospores**: These are found in sporangium, or sac present at the tip of a fertile hypha called the sporangiophore.
4. **Chlamydospore**: This is formed by many mold species. In this type, a cell randomly stores up reserve food, swells, and forms a thick wall around it in the mycelium. The chlamydospore, is more capable to withstand unfavourable conditions than common mold mycelium and can later can grow into a new mold, under favorable conditions.

Reproduction by Sexual Spores

Sexual reproduction in molds usually occurs under specific environmental conditions, such as when nutrient availability is low, or other factors trigger the formation of sexual structures. Different molds have different modes of sexual reproduction, some of which are explained below:

1. **Ascospores**: Ascospores are sexual spores produced by molds belonging to the group Ascomycetes. Ascomycetes are a diverse group of fungi that includes many common molds, such as Penicillium, Aspergillus, and Neurospora. Ascospores are formed within specialized structures called asci, which are sac-like structures. Ascus is the reproductive structure that contains typically eight ascospores, although the number may vary depending on the species. Ascospores are typically forcibly discharged from the ascus, and they can be dispersed by air currents, water, or other means to find new habitats for germination and growth.
2. **Zygospores**: Zygospores are sexual spores produced by molds belonging to the group Zygomycetes. Zygomycetes are a group of fungi that includes molds such as Rhizopus and Mucor. Sexual reproduction in Zygomycetes involves the fusion of specialized

structures called gametangia, which are formed by (+) and (-) mating types. The fusion of gametangia results in the formation of a zygosporangium, which contains the zygospore. The zygosporangium has a thick, protective wall that helps it survive adverse conditions. When conditions are favorable, the zygospore undergoes germination to produce new mold colonies.

3. **Basidiospores**: Basidiospores are sexual spores produced by molds belonging to the group Basidiomycetes. Basidiomycetes are a group of fungi that includes many familiar mushrooms, such as Agaricus and Coprinus. Basidiospores are formed on the surface of specialized structures called basidia, which are club-shaped structures that are typically found in fruiting bodies called basidiocarps. Basidiocarps are often the visible part of mushrooms. Basidiospores are usually forcibly discharged from the basidia and are dispersed by air currents to find new habitats for germination and growth.
4. **Oospores**: Oospores are sexual spores produced by molds belonging to the group Oomycetes. Oomycetes are a distinct group of fungi-like microorganisms that also includes water molds, downy mildews, and white rusts. Oomycetes reproduce sexually by producing specialized structures called oogonia and antheridia. The oogonia produce the oospores, which are thick-walled sexual spores capable of surviving in adverse conditions. Oospores are typically released into the environment and can be dispersed by water or other means to initiate new infections or colonize new habitats.

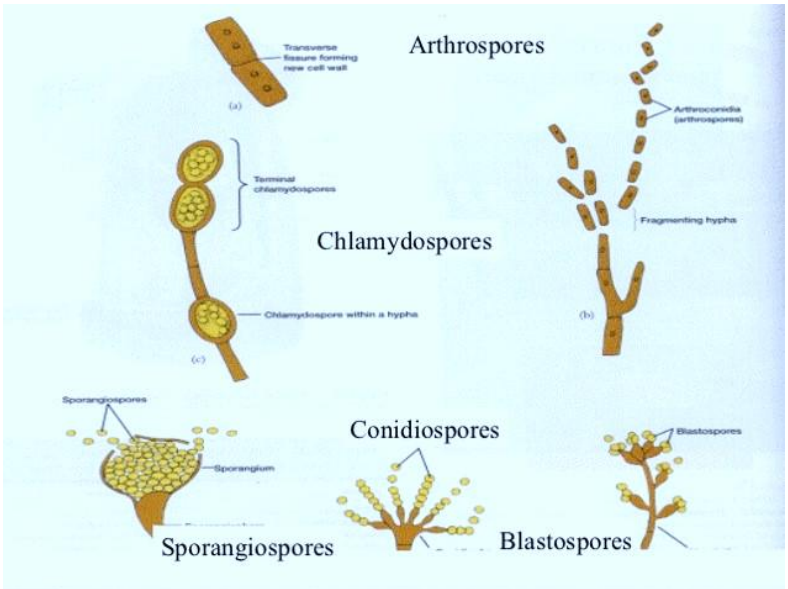

Fig 1: Asexual and Sexual Reproduction in Molds

2.2.5 Molds of Industrial Importance

- ***Aspergillus*:**

  Aspergillus is a genus of molds that is widely used in various industrial processes. Some species of Aspergillus are used for the production of enzymes, organic acids, and other valuable metabolites. For example, *Aspergillus oryzae* is used in the production of soy sauce, sake, and other fermented food products. *Aspergillus niger* is used for the production of citric acid, an important industrial acid used in food and pharmaceutical industries. *Aspergillus terreus* is used for the production of lovastatin, a statin drug used for lowering cholesterol.

- ***Penicillium:***

  Penicillium is a genus of molds that has significant industrial importance. Some species of Penicillium are used in the production of antibiotics, such as penicillin. *Penicillium chrysogenum* is commonly used for the commercial production of penicillin, which is an important antibiotic used in the treatment of bacterial infections. *Penicillium roqueforti* is used in the production of blue cheese, while *Penicillium camemberti* and Penicillium candidum are used in the production of Camembert and Brie cheeses, respectively.

- ***Trichoderma***:

  Trichoderma is a genus of molds that is widely used in agriculture and industry. Some species of Trichoderma are used as biological control agents to combat plant diseases caused by other fungi. They are used as biofungicides to protect crops from pathogenic fungi, reducing the reliance on chemical pesticides. Trichoderma species are also used in the production of cellulase enzymes, which have various industrial applications, including in biofuel production and textile industries.

- ***Rhizopus*:**

  Rhizopus is a genus of molds that has industrial importance in the production of fermented foods and enzymes. *Rhizopus oryzae* is used in the production of tempeh, a traditional Indonesian fermented food made from soybeans. Another species, *Rhizopus stolonifer* is commonly known as bread mold and is responsible for spoilage of bread and other foods. Rhizopus species are also used in the production of enzymes, such as lipases and proteases, which have applications in food, feed, and detergent industries.

- ***Saccharomyces:***

  Saccharomyces is a genus of yeasts that has significant industrial importance in the production of alcoholic beverages, bioethanol, and various biotechnological products. Saccharomyces cerevisiae, also known as baker's yeast or brewer's yeast, is used in the fermentation of bread, beer, wine, and other alcoholic beverages. Saccharomyces species are also used in the production of bioethanol, a renewable fuel, and in the production of enzymes, vaccines, and other biotechnological products.

- ***Fusarium:***

  Fusarium is a genus of molds that has industrial importance in the production of enzymes, mycotoxins, and other bioactive compounds. Some species of Fusarium are used in the production of enzymes, such as amylases and proteases, which have applications in food, feed, and textile industries. Fusarium species are also known for producing mycotoxins, which are toxic compounds that can contaminate food and feed, and are of significant concern in food safety and animal health.

- ***Mucor*:**

  These are involved in the spoilage of some foods as well as in the manufacture of food. One of the common species is *M. racemosus*; *M. rouxii* is used in the saccharification of starch, mucors also help ripen cheese like Gammelost and also used in preparation of certain Oriental foods.

- ***Thamnidium:***

  The most common variety is *Thamnidium elegans* which is found on meat during chilled storage, causing a defect called "whiskers" on the surface of meat.

- ***Trichothecium:***

  The most common species found is *T. roseum*, which is a pink mold that grows mostly on surface of wood, paper, fruits such as apples and peaches, and even vegetables like cucumbers and cantaloupes.

- ***Neurospora (Monilia)***:

  It is classified among the perfect molds i.e. producing sexual spores. *Neurospora (Monilia) sitophila*, is one of the most important species in foods. It is termed as the "red bread mold" because of its pink, loose-textured growth usually found on bread surface.

- ***Sporotrichum:***

  S. carnis is generally found growing on chilled meats, where it causes “white spot."

- ***Botrytis:***

  An important species of this genus in foods is *B. cinerea*. It causes a spoilage of grapes but may also grow saprophytically on many foods.

- ***Cladosporium:***

  C. herbarum is the most commonly found species. These are dark coloured molds and cause formation of "black spots" on a number of foods. Colonies of *C. herbarum* are thick, velvety, and olive to gray green; whereas the reverse side of the plant has a sharp opalescent blue-black to greenish-black colour.

- ***Alternaria:***

  A. citri causes rotting of citrus fruits, alongwith *A. tenuis*, and *A. brassicae* which are common species causing spoilage of foods.

- ***Monascus:***

  M. purpureus colonies are thin and reddish or purple in color. Usually found on dairy products and used for production of Chinese red rice.

- ***Sclerotinia:***

  Some species of this genius are responsible for rots of vegetables and fruits, where they are present in the conidial stage. The conidia are lemon-shaped and found in chains, with a “plug” separating conidia from each other.

2.3 Yeast

Yeasts are eukaryotic, single-celled microorganisms classified as members of the fungus kingdom. The yeasts are proposed to be originated hundreds of millions of years ago, and 1,500 species are currently identified. They are estimated to constitute 1% of all described fungal species. Yeasts are unicellular organisms which evolved from multicellular ancestors, with some species having the ability to develop multicellular characteristics by forming strings of connected budding cells known as pseudohyphae or false hyphae. Yeast sizes vary greatly, depending on species and environment, mostly measuring 3–4 μm in diameter, although some yeast can even grow to 40 μm in size. Most yeasts reproduce asexually by mitosis, while others do so by the asymmetric division process known as budding. Yeasts, with their single-celled growth habit, can be contrasted with molds, which grow hyphae. Fungal species that can take both forms, depending on temperature or other conditions are called dimorphic fungi (“dimorphic” means “having two forms”).

Yeast have remained important for mankind through ages. Through the process of fermentation, the yeast species *Saccharomyces cerevisiae* converts carbohydrates to carbon dioxide and alcohols. For thousands of years the produced carbon dioxide has been used in baking and the alcohol in alcoholic beverages. It is also a centrally important model organism in modern cell biology research, and is one of the most thoroughly researched eukaryotic microorganisms. Researchers have used it to gather information about the biology of the eukaryotic cell and ultimately human biology. Other species of yeasts, such as *Candida albicans*, are opportunistic pathogens and can cause infections in humans. Yeasts have recently been used to generate electricity in microbial fuel cells, and produce ethanol also for the biofuel industry.

Yeasts do not form a single taxonomic or phylogenetic grouping. The phylogenetic diversity of yeasts is shown by their placement in two separate phyla: the Ascomycota and the Basidiomycota. The budding yeasts ("true yeasts") are classified in the order Saccharomycetales, within the phylum Ascomycota. Yeast microbes are probably one of the earliest domesticated organisms. In 1680, Dutch naturalist, Anton van Leeuwenhoek first microscopically observed yeast, but did not consider them to be living organisms, but rather globular structures. Researchers were doubtful whether yeasts were to be placed as algae or fungi, but in 1837, Theodor Schwann recognized them as fungi. In 1857, French microbiologist Louis Pasteur proved that alcoholic fermentation was conducted by living yeasts and not by a chemical catalyst. Pasteur showed that by bubbling oxygen into the yeast broth, cell growth could be increased, but fermentation was inhibited; an observation later called the "Pasteur effect". By the late 18th century, two yeast strains used in brewing had been identified as *Saccharomyces cerevisiae* (top-fermenting yeast) and *S. carlsbergensis* (bottom-fermenting yeast).

2.3.1 Nutrition and Growth

Yeasts are classified as chemoorganotrophs because they use organic compounds as a source of energy and do not require sunlight to grow. Carbon is obtained mostly from hexose sugars, like glucose and fructose, or from disaccharides i.e. sucrose and maltose. Some species can even metabolize pentose sugars such as ribose, alcohols, and organic acids. Yeast species either require oxygen for aerobic cellular respiration (obligate aerobes) or are anaerobic, but may also have aerobic methods of energy production (facultative anaerobes). Unlike bacteria, no known yeast species grow only anaerobically (obligate anaerobes). Yeasts grow best in a neutral or slightly acidic pH environment.

Yeasts vary in regard to the temperature range in which they grow best. The cells can even survive freezing under certain conditions, with viability decreasing over time. In general, yeasts are grown in the laboratory on solid growth media or in liquid broths. Common media used for the cultivation of yeasts include potato dextrose agar or potato dextrose broth, Wallerstein Laboratories nutrient agar, yeast peptone dextrose agar, and yeast mould agar or broth. Home brewers who cultivate yeast frequently use dried malt extract and agar as a solid growth medium.

2.3.2 Ecology

Yeasts are very common in the environment, and are often isolated from sugar-rich materials. There are naturally occurring yeasts on the skins of fruits and berries (such as grapes, apples, or peaches), and exudates from plants (such as plant saps or cacti). Some yeast are found in association with soil and insects also. The ecological function and biodiversity of yeasts are relatively unknown compared to those of other microorganisms. Yeasts, including *Candida albicans, Rhodotorula rubra, Torulopsis* and *Trichosporoncutaneum*, have been found as part of human skin flora. Yeasts are also present in the gut flora of mammals and some insects and even deep-sea environments host a plethora of yeasts. Certain strains of some species of yeasts produce proteins called yeast killer toxins that allow them to eliminate competing strains. This can cause problems for winemaking but could potentially also be used to advantage by using killer toxin-producing strains to make the wine. Yeast killer toxins may also have medical applications in treating yeast infections.

2.3.3 Morphological Characteristics

The microscopic examination is crucial in determining the morphological characteristics of yeasts. Yeasts can exhibit various forms, such as spherical to ovoid, lemon-shaped, pear-shaped, cylindrical, triangular, as well as elongated into false or true mycelium. Additionally, yeasts differ in size. The visible components of yeast structure under microscopic examination include the cell wall, cytoplasm, water vacuoles, fat globules, and granules, which may exhibit metachromatic, albuminous, or starchy properties.

2.3.4 Cultural Characteristics

Yeasts exhibit oxidative, fermentative, or mixed metabolic characteristics. Oxidative yeasts can form films, pellicles, or scums on the surface of liquids, commonly referred to as film yeasts. Fermentative yeasts typically grow uniformly throughout the liquid and produce carbon dioxide. The appearance of yeast growth is significant as it can cause colored spots on

food. Distinguishing yeast colonies from bacterial colonies on agar plates can be challenging, and microscopic examination of the organisms is the most accurate method. Young yeast colonies are usually moist and somewhat slimy, sometimes appearing mealy. Most colonies are whitish, but some can be cream-colored or pink. Colony appearance may change with age, with some colonies becoming dry and wrinkled while others remain relatively unchanged.

2.3.5 Physiological Characteristics

Most common yeasts thrive in environments with ample moisture. Unlike bacteria, yeast can tolerate higher concentrations of solutes, such as sugar or salt. Yeasts can be classified based on water activity (aw), with ordinary yeasts unable to grow in high solute concentrations or low aw, while osmophilic yeasts can grow in such conditions. The lower limits of aw for ordinary yeasts range from 0.88 to 0.94, while osmophilic yeasts have been found to grow slowly in media with aw as low as 0.62 to 0.65 in syrups, although some may be inhibited at around 0.78 in salt brine or sugar syrup. Aw values are influenced by substrate nutritive properties, pH, temperature, oxygen availability, and presence of inhibitory substances.

Yeasts generally thrive in a temperature range of 25 to 30°C, with a maximum temperature of 35 to 47°C. Some yeast are capable of growing at 0°C or lower. Yeasts prefer slightly acidic conditions with a pH of around 4 to 4.5, and do not grow well in alkaline environments. While yeasts typically grow best in aerobic conditions, fermentative yeasts can also grow anaerobically, albeit at a slower pace. Sugars are the preferred energy source for most yeast, although oxidative yeasts, such as film yeasts, can oxidize organic acids and alcohol. Carbon dioxide produced by bread yeasts is responsible for leavening bread, and alcohol produced by fermentative yeasts is a key component in the production of wines, beers, industrial alcohol, and other products. Yeasts also contribute to the development of flavors or "bouquet" in wines. Yeasts utilize a variety of nitrogenous foods ranging from simple compounds like ammonia and urea to amino acids and polypeptides. Additionally, yeasts require accessory growth factors. Most yeast possess the ability to adapt to suboptimal growth conditions.

2.3.6 Reproduction

Yeasts, like all fungi, may have asexual and sexual reproductive cycles. The most common mode of vegetative growth in yeast is asexual reproduction by budding. Here, a

small bud (also known as a bleb), or daughter cell, is formed on the parent cell. The nucleus of the parent cell splits into a daughter nucleus and migrates into the daughter cell (Fig. 2). The bud continues to grow until it separates from the parent cell, forming a new cell. The daughter cell produced during the budding process is generally smaller than the mother cell.

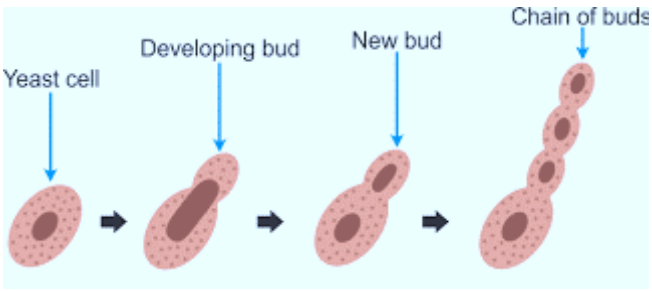

Fig 2: Reproduction in Yeast by budding

As the bud grows, it eventually becomes similar in size to the parent cell. The nucleus of the parent cell undergoes mitosis, resulting in the formation of a nucleus in the bud as well. The bud continues to enlarge and may eventually develop its own bud, which can repeat the process and result in the formation of multiple generations of cells attached to each other in a chain-like structure. Once the bud has fully matured and reached a size similar to the parent cell, it becomes physically separated from the parent cell. This can occur through constriction of the neck between the parent cell and the bud, and eventually, the bud breaks off from the parent cell to become an independent yeast cell. The separated bud grows into a fully mature yeast cell capable of carrying out its own metabolic functions and reproducing. It can also initiate its own budding process, thereby repeating the cycle and producing more yeast cells. The daughter cell produced through budding is genetically identical to the parent cell, as it is a result of asexual reproduction and does not involve genetic recombination or exchange.

Some yeast, including *Schizosaccharomyces pombe*, reproduces by fission instead of budding, thereby creating two identically sized daughter cells. In general, under high-stress conditions such as nutrient starvation, haploid cells will die; under the same conditions, however, diploid cells can undergo sporulation, initiating sexual reproduction (meiosis) and producing a variety of haploid spores, which can go on to mate (conjugate), reforming the diploid.

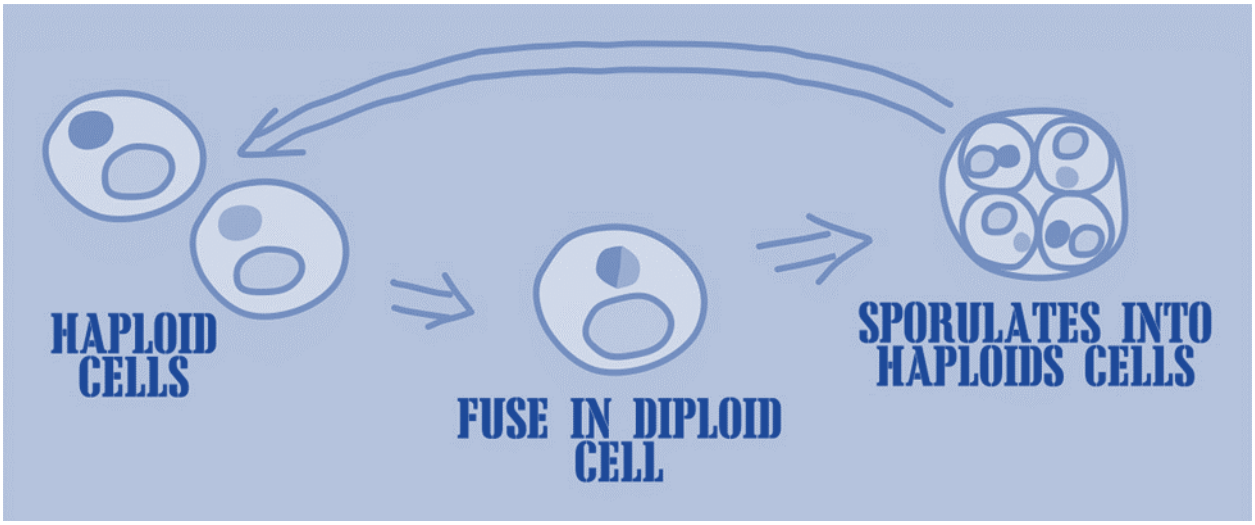

Fig 3: Sexual reproduction in Yeast

2.3.7 Yeasts of industrial importance

Most of the yeasts used in industrial applications belong to the genus Saccharomyces. Yeasts other than the intended species are referred to as "wild yeasts", which could cause problems in certain processes. Asporogenous or false yeasts are usually the troublesome ones.

- **Schizosaccharomyces:**

  It is a genus that reproduces asexually through fission and forms four or eight ascospores per ascus after isogamic conjugation. They are commonly found in tropical fruits, molasses, soil, and honey, among other places. *S. pombe* is a common species.

- **Saccharomyces:**

  It is a genus whose cells may be round, ovate, or elongated, and may form a pseudo-mycelium. Reproduction can be by multipolar budding or ascospore formation. The ascospores are usually round or ovate, with one to four per ascus. *S. cerevisiae*, the most well-known species, is used in numerous food industries, with specific strains for bread leavening, top yeasts for ale, wine production, and the manufacture of alcohol, glycerol, and invertase.

  Top yeasts are highly active fermenters that grow rapidly at 20°C. Their clumping and fast CO_2 evolution, sweeps the cells to the surface, earning them their name. Bottom yeasts, on the other hand, do not clump, grow more slowly, and are better fermenters at lower temperatures (10-15°C). The absence of clumping and the slower growth and evolution of CO_2 enable the yeast to settle to the bottom, hence the term bottom yeast. *S. cerevisiae* var. *ellipsoideus* is a high alcohol-yielding variety utilized to manufacture

industrial alcohol, wines, and distilled liquors. *S. uvarum*, a bottom yeast, is used in beer production. *S. fragilis* and *S. lactis*, due to their lactose fermentation ability, may be important in milk and dairy products. *S. rouxii* and *S. mellis* are osmophilic.

- **Kluyveromyces:**

  It reproduces through multilateral budding, with ascopores released when mature. Zygosaccharomyces, known for its ability to grow in high sugar concentrations (thus, osmophilic), is involved in honey, sirup, and molasses spoilage, as well as soy sauce and some wine fermentation. *Zygosaccharomyces nussbaumeri* thrives in honey.

- **Pichia:**

  It comprises oval to cylindrical yeasts that may form pseudomycelia, with round or hat-shaped ascospores and one to four per ascus. A pellicle forms on liquids, with *P. membranaefaciens* creating a pellicle on beers or wines. *Hansenula*, is a yeast that resembles Pichia but is typically more fermentative, with some species forming pellicles.

- **False yeasts:**

  Also known as 'Fungi Imperfect' include Torulopsis, which are round to oval, fermentative yeasts with multilateral budding that can cause issues in breweries and spoil various foods. *T. sphaerica* ferments lactose and may spoil dairy products. Other species can spoil sweetened condensed milk, fruit juice concentrates, and acid foods. Candida forms pseudohyphae or true hyphae, with abundant budding cells or blastospores, and can form chlamydospores. Many form films and can spoil acidic and salty foods. *C. utilis* is grown for food and feed. *C. krusei* has been grown with dairy starter cultures to maintain the activity and increase the longevity of the lactic acid bacteria. *C. lipolytica* can spoil butter and margarine.

- **Candida:**

  These yeasts form pseudohyphae or true hyphae, with large number of budding cells or blastospores, and may form chlamydospores. Some sp form films and can spoil even foods rich in acid and salt. *C. utilis* is grown for food and feed. *C. krusei* has been grown associated with starter cultures in dairy industry to maintain the activity and viability of the lactic acid bacteria. *C. lipolytica* causes spoilage of fat rich products like butter and margarine.

- **Brettanomyces:**

  These are ogive or arch shaped yeasts that produce high amounts of acid and are responsible for late fermentation of Belgian lambic beer and English beers. They are also commonly found in French wines. *B. bruxellansis* and *B. lambicus* are important species of this genus.

- **Trichosporon:**

  They devlop bud and form arthrospores. They grow best at lower temperatures and are found mostly in breweries and on chilled beef. *T. pullulans* is a common species.

- **Rhodotorula:**

  These are red, pink, or yellow yeasts that may cause discolorations on foods, like development of colored spots on meats or pink pigmentation in sauerkraut.

2.4 BACTERIA

Bacteria constitute a large domain of prokaryotic microorganisms. Generally, a few micrometres in length, bacteria have a number of shapes, ranging from spheres to rods and spirals. Bacteria were among the first life forms to appear on Earth, and are present in most of its habitats. Bacteria inhabit soil, water, acidic hot springs, radioactive waste, and the deep portions of Earth's crust. Bacteria also live in symbiotic and parasitic relationships with plants and animals. There are typically 40 million bacterial cells in a gram of soil and a million bacterial cells in a millilitre of fresh water. Bacteria are important in recycling nutrients, as many of the stages in nutrient cycles are dependent on these organisms, like the fixation of nitrogen from the atmosphere and putrefaction. According to the researchers, "You can find microbes everywhere — they're extremely adaptable to conditions, and survive wherever they are." Most bacteria have not been characterised, and only about half of the bacterial phyla have species that can be grown in the laboratory. The study of bacteria is known as bacteriology, a branch of microbiology.

2.4.1 Morphology

Bacteria display a wide diversity of shapes and sizes, called morphologies. Cells of bacteria are very small about 1/10 of the size of eukaryotic cells and are typically 0.5–5.0 micrometres in length. However, a few species are visible to the unaided eye for example, *Thiomargarita namibiensis* is about half a millimetre long and *Epulopiscium fishelsoni*

reaches to 0.7 mm. Among the smallest bacteria are members of the genus Mycoplasma, which measure only 0.3 micrometres. Some bacteria may be even smaller, but these ultramicrobacteria are not well-studied. Mostly bacterial species are either spherical in shape, known as cocci, or having rod-shaped structure, thus called bacilli. Some bacteria, also called vibrio, as they are shaped like slightly curved rods or comma-shaped; others can be spiral-shaped, called spirilla, or tightly coiled, called spirochaetes. A small number of species even have tetrahedral or cuboidal shapes. More recently, some bacteria were discovered deep under Earth's crust that grow as branching filamentous types with a star-shaped cross-section. The large surface area to volume ratio of this morphology may give these bacteria an advantage in nutrient-poor environments. This wide variety of shapes is determined by the bacterial cell wall and cytoskeleton, and is important because it can influence the ability of bacteria to acquire nutrients, attach to surfaces, swim through liquids and escape predators.

2.4.2 Cultural characteristics

Bacterial growth in and on foods can be significant, leading to various effects such as discoloration, sliminess, films, cloudiness, and sediment formation. Pigmented bacteria are known to cause discolorations on the surfaces of foods. Films can develop on the surfaces of liquids, and the growth of bacteria can make food surfaces slimy. In addition, bacterial growth throughout liquids can result in undesirable cloudiness or sedimentation.

2.4.3 Physiological characteristics

Bacterial growth in food can result in specific changes, including the hydrolysis of complex carbohydrates into simpler forms, such as simple sugars. Proteins can also be hydrolyzed by bacteria into polypeptides, amino acids, ammonia, or amines. Fats can undergo hydrolysis by bacteria into glycerol and fatty acids. Bacteria utilize oxidation-reduction reactions to obtain energy from food sources, such as carbohydrates, carbon compounds, and nitrogen-carbon compounds. These reactions can produce organic acids, alcohols, aldehydes, ketones, and various gases as byproducts.

2.4.4 Reproduction

Bacteria grow to a fixed size and then reproduce through binary fission, a form of asexual reproduction. Under optimal conditions, bacteria can grow and divide extremely rapidly, and bacterial populations can double as quickly as every 9.8 minutes. In cell division, two identical clone daughter cells are produced. Some bacteria, while still reproducing asexually, form more complex reproductive structures that help disperse the newly formed

daughter cells. Examples include fruiting body formation by Myxobacteria and aerial hyphae formation by Streptomyces, or budding. Budding involves a cell forming a protrusion that breaks away and produces a daughter cell.

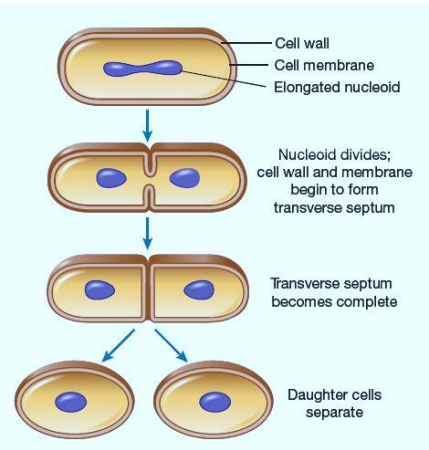

Fig 4: Reproduction in bacteria

In the laboratory, bacteria are usually grown using solid or liquid media. Solid growth media, such as agar plates, are used to isolate pure cultures of a bacterial strain. However, liquid growth media are used when measurement of growth or large volumes of cells are required. The use of selective media (media with specific nutrients added or deficient or with antibiotics added) can help identify specific microorganisms. Most laboratory techniques for growing bacteria use high levels of nutrients to produce large amounts of cells cheaply and quickly. However, in natural environments, nutrients are limited, meaning that bacteria cannot continue to reproduce indefinitely. This nutrient limitation has led the evolution of different growth strategies. Some organisms can grow extremely rapidly when nutrients become available, such as the formation of algal (and cyanobacterial) blooms that often occur in lakes during the summer. Other organisms have adaptations to harsh environments, such as the production of multiple antibiotics by Streptomyces that inhibit the growth of competing microorganisms.

In nature, many organisms live in communities forming biofilms that may allow for increased supply of nutrients and protection from environmental stresses. These relationships can be essential for growth of a particular organism or group of organisms (syntrophy). When a population of bacteria first enters a high-nutrient environment that allows growth, the cells need to adapt to their new environment. The growth of bacteria occurs through following four phases:

- The first phase of growth is the lag phase, a period of slow growth when the cells are adapting to the high-nutrient environment and preparing for fast growth. The lag phase has high biosynthesis rates, as proteins necessary for rapid growth are produced.
- The second phase of growth is the log phase, also known as the logarithmic or exponential phase. The log phase is marked by rapid exponential growth. The rate at which cells grow during this phase is known as the growth rate (k), and the time it takes the cells to double is known as the generation time (g). During log phase, nutrients are metabolised at maximum speed until one of the nutrients is depleted and starts limiting growth.
- The third phase of growth is the stationary phase and is caused by depleted nutrients. The cells reduce their metabolic activity and consume non-essential cellular proteins. The stationary phase is a transition from rapid growth to a stress response state and there is increased expression of genes involved in DNA repair, antioxidant metabolism and nutrient transport.
- The final phase is the death phase where the bacteria run out of nutrients and die.

2.4.5 Bacteria of Industrial Importance

- ***Acinetobacter***:

  These Gram-negative rods exhibit similarities to the Neisseriaceae family, and some strains that were previously classified as Achromobacters and Moraxellae have been reclassified as Acinetobacter. They are strict aerobes, initially appearing as rod-shaped cells in fresh cultures, but mature cultures often contain coccoid-shaped cells. Acinetobacter is widely distributed in soil and water, and can also establish on various foods, particularly refrigerated fresh products.

- ***Bacillus*:**

  These are Gram-positive rods that are capable of forming spores and are aerobes, in contrast to the anaerobic nature of clostridia. While most Bacillus species are mesophiles, there are also psychrotrophs and thermophiles within the genus. Bacillus comprises of only two pathogens, *B. anthracis* (the causative agent of anthrax) and *B. cereus*. Although the majority of strains of *B. cereus* are nonpathogenic, some can cause foodborne gastroenteritis.

- ***Lactobacillus***:

Lactobacilli are rod-shaped bacteria, typically long and slender, that form chains in most species. They are microaerophilic, although some strict anaerobes are also known, and are catalase-negative and gram-positive. Lactobacilli ferment sugars to produce lactic acid as the main product. Homo-fermentative lactobacilli, which produce mainly lactic acid along with small amounts of acetic acid, carbon dioxide, and trace products, have optimal temperatures of 37°C or above, and include species such as *L. bulgaricus, L. helveticus, L. lactis, L. acidophilus* and *L. thermophilus.* Homo-fermentative lactobacilli with lower optimal temperatures include *L. casei, L. plantarum*, and *L. leichmannii*.

Some lactobacilli are hetero-fermentative, producing appreciable amounts of volatile products, including alcohol, in addition to lactic acid. *L. delbrueckii* is an example of a hetero-fermentative lactobacillus that grows well at higher temperatures. Hetero-fermentative species that grow at lower temperatures include *L. brevis, L. buchneri, L. pastorianus, L. hilgardii*, and *L. trichodes*. Certain strains of *L. brevis* are capable of fermenting lactose with the production of lactic acid, which may be of importance in the dairy industry. Lactobacilli are commonly found on plant surfaces, in manure, and in dairy products.

- ***Brochothrix*:**

These are nonspore forming rod shaped Gram-positive bacteria that are considered to be closely related to the genera *Lactobacillus* and *Listeria*. Specifically, the exponential phase cells are rods in shape, and mature cells are coccoids. They are found generally on preserved meats that are packed in gas impermeable packaging with frozen storage.

- ***Clostridium perfringens**:*

Clostridium perfringens is a widespread bacterium that can reproduce rapidly under optimal conditions. Infants, young children, and older adults are particularly vulnerable to illness caused by *C. perfringens*. Infection often occurs through consumption of food contaminated with high numbers of the bacterium, which produces toxins that cause abdominal cramping and diarrhea. *C. perfringens* is sometimes referred to as the "buffet germ" because it tends to grow in bulk portions of food, such as gravies, casseroles, and stews, especially when kept at room temperature in the danger zone.

Contamination of food can occur from inadequately cooked food, leading to illness. It is important to cook food thoroughly and maintain it at temperatures above 140°F or below 40°F to prevent the growth of *C. perfringens*. Leftovers should be properly handled by dividing roasts and stews into smaller quantities for faster cooling and refrigerating promptly. When serving food, ensure it is reheated to 165°F or higher to ensure food safety.

- ***Campylobacter***:

  Campylobacter is a common cause of diarrhea, with most cases of campylobacteriosis, the illness caused by Campylobacter bacteria, being associated with consumption of undercooked poultry and meat, or cross-contamination of other foods by these substances. Proper cooking of food is crucial, as freezing only reduces the quantity of Campylobacter bacteria on raw meat. Campylobacteriosis is more common in the summer and is most frequently seen in infants and young children. Sources of infection include consumption of undercooked poultry and other meats, unpasteurized dairy products, untreated water, or contaminated products. Thoroughly cooking all foods to their appropriate internal temperatures and preventing cross-contamination by using separate cutting boards for raw and cooked foods, as well as proper washing of food materials, are important preventive measures.

- ***E. coli O157:H7:***

  Escherichia coli is a large group of bacteria, and while most strains are harmless, some can cause severe illness. One particular strain, *E. coli* O157:H7 (STEC), is commonly associated with food poisoning outbreaks, as its effects can be very severe. This strain is often linked to consumption of raw or undercooked food, and unpasteurized dairy products or juices. Proper washing of foods, maintaining appropriate internal temperatures, and avoiding unpasteurized dairy products and juices are important preventive measures.

- ***Flavobacterium***:

  These Gram-negative rods are characterized by their production of yellow to red pigments on agar. Some species are mesotrophs, while others are psychrotrophs, and they can contribute to the spoilage of refrigerated meats and vegetables.

- ***Micrococcus***:

  These Gram-positive and catalase-positive cocci are inhabitants of mammalian skin and can grow in the presence of high levels of NaCl. This genus includes species such as Dermacoccus, Kytococcus, and Stomatococcus. Currently, *M. luteus* and *M. lylae* are the only two common species of Micrococcus.

- ***Pseudomonas***:

  These bacteria are commonly found in soil and water, and are widespread in fresh foods, particularly in vegetables, meats, poultry, and seafood products.

- ***Salmonella***:

  Salmonella is a group of bacteria that can cause the infection salmonellosis. It is one of the most common bacterial causes of diarrhea and the most common source of foodborne-related hospitalizations and deaths. Salmonella bacteria can survive in the intestinal tract of humans and animals, and can spread easily if proper sanitation and cooking methods are not followed.

- ***Staphylococcus aureus****:*

  Staphylococcus aureus (Staph) is typically found on the skin, throats, and nostrils of healthy people and animals. It generally does not cause illness unless it is transmitted to food products where it can grow and produce harmful toxins. Symptoms of staphylococcal infection include nausea, stomach cramps, and diarrhea. Staphylococcal bacteria are heat resistant, but cooking can damage them in some cases. Staph infection can affect anyone, but certain groups of people, such as those with chronic conditions like diabetes, vascular disease, cancer, and lung disease, may be at greater risk.

- ***Propionibacterium***:

  Some species of this genus may be found in foods. For example, in Swiss cheese, certain species like *Propionibacterium freudenreichii* ferment lactates to produce gas, which helps form the holes or "eyes" in the cheese and contributes to its flavor. Pigmented propionic bacteria can also cause color defects in cheese.

- ***Proteus***:

  Bacteria of this genus have been implicated in the spoilage of meats, seafood, and eggs. The presence of these bacteria in sufficient numbers in foods stored at room temperatures has made them concern for food poisoning cases.

2.5 SUM UP

This unit has informed you about different types of microorganisms associated with food. Through this unit, we understood the structural, morphological and cultural characteristics of microorganisms. We also studied about different asexual and sexual reproduction methods of molds, yeast and bacteria. Further, the important groups of mold, yeast and bacteria of industrial importance were also discussed.

2.6 CHECK YOUR PROGRESS

1. What are molds? Write about their physiological requirements.

.....

.....

.....

2. Explain sexual spores of molds.

.....

.....

.....

3. Why are Aspergillus and Penicillium sp. considered to be industrially important?

.....

.....

.....

4. What are yeasts? What are their morphological characteristics?

.....

.....

.....

5. Write a short note on yeasts of industrial importance.

.....

.....

.....

6. Explain reproduction in bacteria.

.....

.....

.....

7. Illustrate the importance of lactobacillus genus.

.....

.....

.....

8. Write in detail about bacterial species of industrial importance.

.....

.....

.....

.....

.....

Unit-III FACTORS AFFECTING THE GROWTH OF MICROORGANISMS IN FOOD

Structure

3.1 INTRODUCTION

The unit three of this course gives you an understanding about the growth of a microorganism and the phases involved. The unit will cover various extrinsic, and intrinsic that affects the growth of a microorganism. Further, we will also understand the effect of some implicit factors also on the microbial growth.

Objectives

Learning this unit will make you:

- apprehend the concept of microbial growth curve
- explain the intrinsic factors and their importance in growth of microorganism
- discuss the extrinsic factors affecting microbial growth
- describe the importance of implicit factors

3.2 BACTERIAL GROWTH

The term growth generally refers to an increase in size; for example, growing from a tiny newborn baby to a large adult. Although bacteria do increase in size before cell division, bacterial growth refers to an increase in the number of organisms rather than an increase in their size. So how do we define Bacterial growth? Bacterial growth can be defined as an "orderly increase of all the chemical components of the cell". Growth of bacterial cultures can also be defined as "enhancement in the number of bacterial cells in a population and not the change in the size of bacterial cells". Cell multiplication is a consequence of growth that leads to an increase in the number of bacteria making up a population or culture. Bacterial growth is a complex process that involves numerous anabolic and catabolic reactions, which result in cell division. Most bacteria divide by binary fission in which the bacteria undergo cell division to produce two daughter cells identical to the parent cell. The increase in numbers or bacterial mass can be measured as a function of time under pure culture conditions, where the nutrients and environmental conditions are controlled. There are four distinct phases during growth of bacteria i.e. lag phase, log phase, stationary phase and death phase (Fig. 1). Expalantion of these phases is as follows:

- **Lag phase**

The first phase of bacterial growth is called the lag phase. This phase occurs when bacteria first enter a nutrient-rich environment. During this phase, the growth rate is quite slow, as bacteria begin adapting to their new environment. In the lag phase, bacteria produce the enzymes they break down the substrate or food source. If the environment is nutrient-rich and conditions are favourable for growth, the lag phase tends to be quite short. However, the length of the lag phase is the most variable of the four phases and the one most susceptible to change according to conditions. For example, the lag phase can be much longer if the temperature isn't ideal for bacterial growth. There are other environmental factors that can also impact the length of the lag phase including pH, water activity, and competition with other microbial species for nutrients.

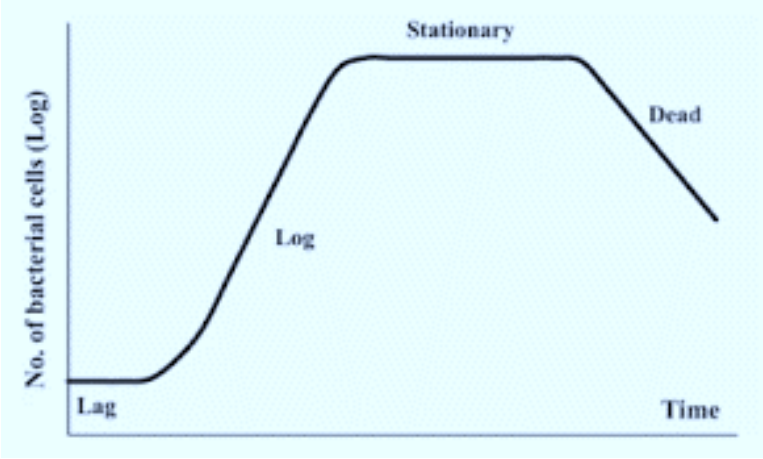

Fig. 1: Typical growth curve of bacteria

- **Log phase**

As bacteria begin to multiply more quickly under favourable conditions, they enter the actual growth phase known as the log phase, in which the bacteria experience very fast growth characterized by doubling of the population after each generation. Doubling means, eight bacteria divide into sixteen bacteria, sixteen bacteria into thirty-two bacteria, and so on. Logarithmic values (log values) are used to count the rapidly increasing numbers of bacteria in the log phase. The mathematical expression for this is log10. Each time a log10 value increases by 1, the number of bacteria increases by a multiple of 10. So, a log10 value of 1 means 10 bacteria, a log10 value of 2 means $10 \times 10 = 100$ bacteria, a log10 value of 3 means $10 \times 10 \times 10 = 1000$, and so on. The log phase is also called the exponential growth phase.

During the log phase, bacterial numbers increase drastically. If it takes twenty minutes for the population to double, after twenty-four hours there will be hundreds of billions of bacteria. The length and rapidity of bacterial growth during the log phase depends on a variety of factors, including nutrient availability, the build-up of bacterial waste products, and temperature. In optimal conditions, most bacteria can double in only ten to thirty minutes. This time period for binary fission to complete is called the generation phase. Bacteria may even continue to grow under refrigeration temperature; however the rate of growth will generally be slower than at temperatures in the danger zone (between 4°C and 60°C). The log phase comes to an end when resources required for growth are exhausted.

- **Stationary phase**

As growth slows, the bacteria enter the stationary phase. During this period, the bacteria are still alive, but the environment will not support more growth and the rate of bacterial growth will be equal to the rate of bacterial death. A steady state is reached between the availability of nutrients and the increase in bacterial waste products. As food becomes scarce and waste products increase, the environment will become toxic for the remaining bacteria. It is during this phase that bacteria may produce chemicals such as antibiotics or toxins to inhibit other types of bacteria from growing.

- **Death phase**

Once nutrients are exhausted, bacteria will enter the death phase and begin to die off in large numbers. Some bacteria enter into a viable but non-culturable form. This is important to the food microbiologist, because it means that bacteria may not grow in the laboratory, but are still alive. It is unlikely that 100 % of the bacteria die during the death phase. The bacteria that do survive, however, are adaptable and able to begin growing again once more favourable conditions occur.

Some types of bacteria have the ability to enter the endospore phase, during which they change their shape to form spores for longer term survival. This form of the bacteria is tougher and more resistant to heat and ultraviolet radiation. These bacteria spores will remain viable but dormant until conditions improve, and so serve as a way for bacteria to ensure the survival of their species. For the food industry, spore-forming bacteria are of concern because bacterial spores can withstand common preservation and sanitation methods such as heating, freezing, chemicals, and other processes that are used to ensure our food is safe to eat. Take an example of cooked rice. We know that once rice has been boiled, there should be no

bacteria present? If you agreed with this statement, you would be overlooking that there might be spores of bacteria present in rice that are highly resistant to boiling. Rice, dried beans, and other grains are grown in or near soils and prone to contamination with spore-forming bacteria such as *Bacillus* and *Clostridium* spp. Boiling these foods will destroy the vegetative or normal forms of these bacteria, but not the spore forms. That is why it is important to store cooked rice and other foods out of the temperature danger zone (4°C to 60°C).

3.3 FACTORS AFFECTING MICROBIAL GROWTH

The growth of a microorganism depends on various factors, which include temperature, pH, nutrients, oxygen availability and other environmental conditions. After understanding the growth pattern of a microorganism, microbiologists can optimize conditions for the production of beneficial microorganisms or control the growth of harmful microorganisms. Factors affecting the microbial growth include., physico-chemical attributes of the food known as intrinsic factors; conditions of the storage called extrinsic factors; relationship between various microorganisms present in the food sample called as implicit factors; and changes occurring due to processing steps known as processing factors.

3.3.1 Intrinsic Factors

a) Water Activity

Water is essential for life. Aqueous solutions have different amounts of water available, depending on how many solutes are dissolved in it. Consider a very simple example, considering two glasses, one full of pure water, the other containing the same amount of water plus a sponge. Which one would be easier to drink? The one with pure water only, because in the second glass, sponge will absorb all the water making it unavailable. Dissolved solutes act just like a sponge, making water less available for microorganisms. It is well known that the moisture requirement of microbes is stated in terms of the *water activity* (*a*w). It is determined as the ratio of the water vapor pressure of food to the water vapor pressure of pure water at the same temperature (i.e. under standard conditions) as follows:

$$a\text{w} = p/po,$$

where p is the vapor pressure of the solution and po is the vapor pressure of the solvent.

This is related to relative humidity (RH) as: RH = 100 × *a*w. Water activity (aw) can be decreased by the addition of any soluble molecule although salt (NaCl) and sugars are probably the most common. Generally, requirement of bacteria for *a*w for growth is higher than fungi. Among bacteria, gram-negative bacteria require higher levels of water activity than Gram positive bacteria. Spoilage molds may require *a*w as low as 0.80 whereas spoilage bacteria will not grow preferably below *a*w 0.91. The lowest reported *a*w for foodborne bacteria is 0.75 for halophiles ("salt-loving"), Microbes that requires a high-water activity (near or at 1) are termed nonhalophiles. A nonhalophile that can grow best with almost no salt but can still grow in presence of low levels of salt is called halotolerant.

The reason of reducing *a*w below optimum level is to enhance the time of the lag phase of growth of bacteria and to slow down the growth rate as well as amount of final population. This will adversely influence all metabolic activities and chemical reactions of cells, as they need an aqueous environment for proper functioning. Lowering of *a*w definitely has negative effects on the cell membrane function. Microorganisms that can survive and grow under unfavourable conditions of low *a*w can do so by their ability of withstanding concentrated salts, polyols and amino acids etc. These highly concentrated levels are sufficient not only to avoid the cells from losing water, but may also allow the cell to extract water from the water-depressed external environment.

b) Nutrient content

We all require nutrition for our growth and so do the microbes. Every organism must find in its environment all of the substances required for energy generation and cellular biosynthesis. The chemicals and elements from the environment that are utilized for bacterial growth are referred to as nutrients or nutritional requirements (Fig. 2). Many bacteria can be grown in the laboratory on culture media that are prepared to provide all the essential nutrients in solution for bacterial growth. At an elementary level, the nutritional requirements of a bacterium are revealed by the cell's elemental composition, which consists of C, H, O, N, S. P, K, Mg, Fe, Ca, Mn, and traces of Zn, Co, Cu, and Mo. These elements are found in the form of water, inorganic ions, small molecules, and macromolecules which serve either a structural or functional role in the cells.

Bacteria require a source of carbon for their growth and metabolism. Carbon is a key element used in the synthesis of organic molecules, such as proteins, nucleic acids, and lipids. Bacteria can be classified into different groups based on their carbon source requirements, such as autotrophic bacteria that can synthesize their own organic molecules from inorganic

sources of carbon (e.g., carbon dioxide), and heterotrophic bacteria that require pre-formed organic molecules as a source of carbon (e.g., carbohydrates, amino acids). Bacteria need a source of energy to carry out their metabolic processes. Energy is used to drive cellular reactions, maintain cellular structure, and support growth and reproduction. Bacteria can use different sources of energy, such as sunlight (in the case of photosynthetic bacteria), organic compounds (e.g., carbohydrates, lipids), or inorganic compounds (e.g., sulfur, hydrogen) through various metabolic pathways, such as fermentation, respiration, or photosynthesis.

Nitrogen is an essential element that is required for the synthesis of proteins, nucleic acids, and other essential molecules. Bacteria need a source of nitrogen to meet their nitrogen requirements. Bacteria can use various nitrogen sources, such as inorganic nitrogen compounds (e.g., ammonia, nitrate), organic nitrogen compounds (e.g., amino acids, proteins), or even atmospheric nitrogen (in the case of nitrogen-fixing bacteria). Bacteria also require various mineral elements, such as phosphorus, sulfur, magnesium, potassium, and trace elements, as essential nutrients for their growth and metabolism. These elements are involved in many cellular processes, including enzyme activity, cell structure, and regulation of cellular functions. Vitamins and cofactors as essential organic compounds that are necessary for their growth and metabolism. These include vitamins, coenzymes, and other organic molecules that act as cofactors for enzymes and are involved in various metabolic pathways.

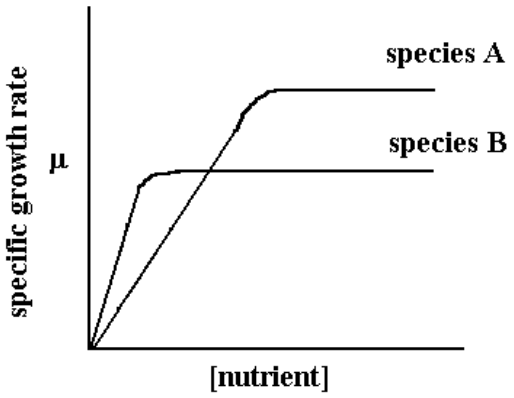

Fig. 2: Nutrients affecting the Growth Rate of Microorganism

c) pH

The optimum growth pH is the most favorable pH for the growth of an organism. The lowest pH value that an organism can tolerate is called the minimum growth pH and the highest pH is the maximum growth pH**.** These values can cover a wide range, which is important for the preservation of food and to microorganisms' survival in the stomach. For example, the optimum growth pH of *Salmonella* spp. is 7.0–7.5, but the minimum growth pH is closer to 4.2. Most bacteria are neutrophiles**,** meaning they grow optimally around neutral pH of 7 (Figure 2). Most familiar bacteria, like *Escherichia coli*, *Staphylococci*, and *Salmonella* spp. are neutrophiles and do not grow well in the acidic pH of the stomach. However, there are pathogenic strains of *E. coli, S. typhi,* and other species of intestinal pathogens that are much more resistant to stomach acid. In comparison, fungi thrive at slightly acidic pH values of 5.0–6.0.

Microorganisms that grow optimally at pH less than 5.55 are called acidophiles. For example, the sulfur-oxidizing *Sulfolobus* spp. isolated from sulfur mud fields and hot springs in Yellowstone National Park are extreme acidophiles. These archaea survive at pH values of 2.5–3.5. Species of the Archaean genus *Ferroplasma* live in acid mine drainage at pH values of 0–2.9. Acidophilic microorganisms display a number of adaptations to survive in strong acidic environments. For example, proteins show increased negative surface charge that stabilizes them at low pH. Pumps actively eject H+ ions out of the cells. The changes in the composition of membrane phospholipids probably reflect the need to maintain membrane fluidity at low pH.

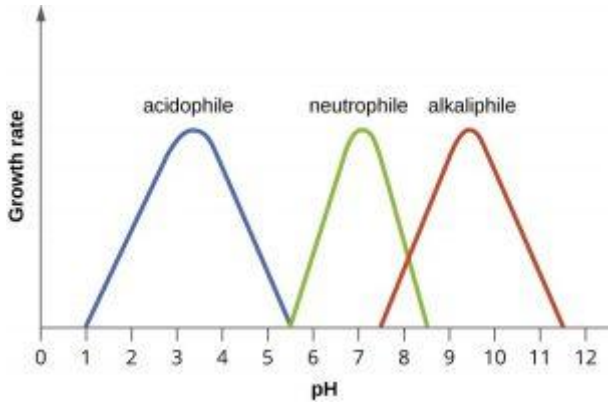

Fig. 3: pH dependent microbial growth

Fig. 3 shows how the pH influences growth of different types of microorganisms. On the basis of pH we can group the bacteria as following:

a) Neutrophiles grow best around neutral pH (7)
b) Acidophiles grow best at pH < 7
c) Alkophiles grow best at pH > 7
d) Acidotolerant grow best at pH 7 but can also grow at lower pH
e) Alkotolerant grow best at pH 7 but can also grow at higher pH.

The acidity or alkalinity of the environment has a significant impact on the activity of macromolecules, such as enzymes, and consequently affects the growth and metabolism of microorganisms. Bacteria generally grow best in a pH range of 6.0 to 8.0, yeasts prefer a slightly more acidic range of 4.5 to 6.0, and filamentous fungi thrive in an even lower pH range of 3.5 to 4.0. However, there may be exceptions, particularly among bacteria that produce large amounts of acids as byproducts of their energy-yielding metabolism.

Several essential cell functions, including ATP synthesis in bacteria, active nutrient transport, and cytoplasmic regulation, occur at the cell membrane and rely on the proton motive force, which is the potential energy stored in the membrane due to the presence of protons. At higher pH, the equilibrium shifts towards dissociation of acids, resulting in the ionization of protons and acidification of the cytoplasm, which can disrupt the proton motive force. In response, the microorganism may attempt to maintain its internal pH by neutralizing or expelling protons, but this can divert energy from growth-related functions and slow down development.

d) Oxidation reduction Potential

Redox potential (Eh**)** refers to the likelihood that chemical components of a food (for e.g., vitamins and proteins that are part of the food) will accept or lose (transfer) electrons, and is determined based on a chemical assessment of a food. Electron transfers take place during oxidation-reduction reactions (or redox reactions). Transfer of electrons drives many chemical activities in microbes, such as the actions of enzymes and various living functions known as metabolic activities. The redox potential of a food can indicate which microbes can survive in a food and what microbial activity has already occurred in a food.

Aerobic microorganisms require positive Eh values (oxidized) for growth, whereas anaerobes need negative Eh values (reduced). Microorganisms have varying requirements for the redox potential (Eh) of their environment. Some bacteria require reduced conditions with

an Eh of about -200 mV for growth initiation, such as anaerobic bacteria like Clostridium. On the other hand, some aerobic bacteria, like certain species of Bacillus, require a positive Eh for growth. Examples of microaerophilic bacteria include lactobacilli and campylobacters. There are also bacteria that can grow under both aerobic and anaerobic conditions, and they are referred to as facultative anaerobes. The Eh of foods, particularly plant foods and juices, tend to have Eh values ranging from 300 to 400. Microorganisms can influence the Eh of their environment during growth, similar to pH. This is particularly true for aerobes, which can modify the Eh of their surroundings, while anaerobes cannot do so.

As aerobes grow, O_2 in the medium is exhausted, resulting in the lowering of Eh. The consequence is that the medium becomes poorer in oxidizing and richer in reducing substances. Generally, Eh tends to be highly negative under increasing alkaline conditions. Mostly the growth of anaerobes usually occurs at lower values of Eh, the removal of O_2 may be necessary for growth of some anaerobes.

e) Antimicrobial Substances

All foods at some stage have been a part of living organisms and through the course of development have dealt with possible ways of preventing or limiting damage through microbial infections. The first of these is the presence of a physical barrier to infection in the form of skin, shell, husk or rind of the product. Destruction of these physical structures allows microbial attack on the nutrient-rich tissues and it is a generally observed that fruits and vegetables which are damaged, cut or bruised decline faster than whole products. Secondly, the presence of certain chemical compounds in food which act as natural antimicrobial compounds like benzoic and sorbic acids found in cranberries. Considerable attention has also been given to the antimicrobial properties of herbs and spices which are primarily used to flavour food. Organic compounds such as eugenol, allicin, thymol, cinnamic aldehyde etc. have been found to show substantial antimicrobial activity.

Animal products also contain various antimicrobial components. For example, the albumen or white of a hen's egg is known to have inhibitory properties. It contains lysozyme, an enzyme that breaks down the glycosidic linkages in peptidoglycan, a structural polymer that gives bacterial cell walls their strength and rigidity. When the peptidoglycan layer is destroyed or weakened, it can cause the bacterial cell to rupture or lyse due to osmotic pressure. Lysozyme is particularly effective against Gram-positive bacteria, as their

peptidoglycan is more accessible, but it can also have an effect on Gram-negative bacteria if their protective outer membrane is compromised.

In addition to lysozyme, animal products like egg white and milk contain other proteins with antimicrobial properties. Ovotransferrin in egg white and lactoferrin in milk are proteins that scavenge iron from the environment, limiting its availability to bacteria for growth. Moreover, egg white contains cofactor-binding proteins such as avidin and ovoflavoprotein, which bind to biotin and riboflavin, essential nutrients for bacteria, and thereby restrict their availability, inhibiting bacterial growth. These antimicrobial constituents in animal products contribute to their natural defense mechanisms against microbial contamination.

Milk also possesses the ability to produce antimicrobial substances in the presence of hydrogen peroxide. The enzyme lactoperoxidase, which makes up approximately 0.5% of whey proteins, catalyzes the oxidation of thiocyanate by hydrogen peroxide. Hydrogen peroxide can be generated through endogenous enzyme activity or through the aerobic metabolism of lactic acid bacteria. The reaction results in the formation of short-lived oxidation products, such as hypothiocyanite, which have been shown to have antimicrobial effects against Gram-negative bacteria and can inhibit Gram-positive bacteria, possibly by disrupting the bacterial cytoplasmic membrane. This antimicrobial mechanism is one of the ways in which milk can naturally defend against microbial contamination.

3.2 EXTRINSIC FACTORS

Factors extrinsic to the food are essentially the environmental surroundings of the food. They include the temperature of the food during preparation and storage, the gaseous atmosphere around the food and relative humidity which is related to the level of moisture around the food. Let’s study each one of these in detail.

a) Relative humidity

The amount of moisture in air around the food or packaging is signified as relative humidity. It is affected by temperature and pressure. For the food microbiologist, relative humidity is important as it can affect the water activity of foods. A high relative humidity will allow water to be absorbed by food, while a low relative humidity may cause the food to lose water and shrivel (desiccate). High humidity can also cause food spoilage, as the excess moisture on the surface of foods allows growth of moulds, other fungi and microbes.

Relative humidity can affect microbial metabolism, including enzymatic activities and nutrient utilization. Changes in relative humidity can alter the metabolic activity of microorganisms, which can impact their growth rates and overall physiology. For example, some microorganisms may exhibit reduced metabolic activity at low relative humidity levels, leading to slower growth rates. Mold, a type of fungi, is particularly sensitive to relative humidity levels. High relative humidity above 70% can promote mold growth on various surfaces, including building materials, food, and other organic substrates. Mold can release spores into the air, which can cause respiratory issues and allergies in humans. On the other hand, low relative humidity below 30% can inhibit mold growth as it reduces the availability of water necessary for mold to thrive. relative humidity is an important environmental factor that can affect microbial growth, metabolism, survival, and dissemination. Optimal relative humidity ranges for microbial growth vary depending on the type of microorganism, and both high and low relative humidity levels can impact microbial growth and survival differently. Proper control of relative humidity is crucial in various settings, including indoor environments, food processing, and healthcare facilities, to manage microbial growth and mitigate potential health risks.

b) Temperature

Temperature affects the rate of microbial growth. All bacteria have an optimum, maximum, and minimum temperature for growth (Fig. 4). Temperature variations of only a few degrees may favour the growth of a completely different species of bacteria, so not only can a change in temperature alter the rate of growth of bacterial colonies, it can impact which species that thrive at a particular temperature. The “danger zone” is a term used to describe the temperatures between 4°C and 60°C. This is the optimal temperature range in which most food pathogens and spoilage bacteria grow. Bacteria and other microbes can be classified into four groups, depending on the temperature zone in which they grow.

- Psychrophiles grow in cold temperatures between −20°C and 20°C, preferring temperatures in the range of 0°C to 10°C. These microbes will grow in refrigerators and even on frozen foods.
- Mesophiles grow in moderate temperature between 10°C and 50°C, preferring temperatures between 20°C and 40°C. Most human pathogens are mesophiles and prefer body temperatures, which range from 35°C to 37°C.

- Thermophiles grow in hot temperatures between 20°C up to 120°C. Thermophiles prefer temperatures between 40°C and 80°C.
- Hyperthermophiles (a subset of the thermophiles group) have an optimum temperature greater than 75°C. This class of microorganisms tolerates the highest temperatures of any known organisms (some living at temperatures greater than 100°C and up to 120°C).

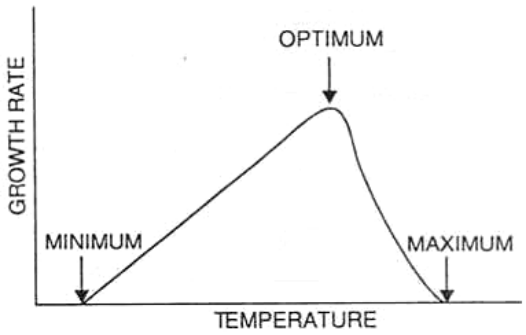

Fig. 4: Effect of temperature on microbial growth

With the decrease in the temperature from the optimum, the rate of microbial growth slows down due in part to the decreased enzymatic reactions within the cell. Another important factor contributing to the slow or eventual cessation of microbial growth at low temperatures is changes in membrane structure, which can affect the uptake and delivery of nutrients to enzyme systems within the cell. On the other hand, as the temperature increases above the optimum, the growth rate decreases more sharply due to irreversible denaturation of proteins and thermal breakdown of the cell's plasma membrane. At temperatures above the maximum for growth, these changes are sufficient to kill the organism. Temperature plays a critical role in microbial growth and survival, with both low and high temperatures having detrimental effects on microbial cells.

c) Gaseous atmosphere

Oxygen constitutes approximately 21% of the Earth's atmosphere and is the most abundant gas in contact with food under normal conditions. Its presence and control of redox potential play significant roles in determining microbial associations and their growth rates. The effect of carbon dioxide (CO_2) on microorganisms is not consistent. Molds and oxidative Gram-negative bacteria are generally more susceptible, while Gram-positive bacteria,

particularly lactobacilli, tend to be more tolerant. Some yeasts, such as Brettanomyces spp., also exhibit substantial tolerance to high levels of CO_2, and can influence the spoilage microflora of carbonated beverages.

The mechanism of CO_2 inhibition is a combination of several processes, including the effect of CO_2 on pH. Carbon dioxide dissolves in water to form carbonic acid, which partially dissociates into bicarbonate ions and protons. CO_2 can act as a weak organic acid, disrupting the plasma membrane and acidifying the cell's interior. Other contributing factors may include changes in the physical properties of the plasma membrane, which can disrupt solute transport; inhibition of key enzymes, particularly those involving carboxylation/ decarboxylation reactions where CO_2 is a reactant; and reaction with protein amino groups, leading to modifications in their properties and functions. CO_2 can have multiple effects on microorganisms, and its impact can vary depending on the specific microorganism and environmental conditions.

Oxygen is used by aerobic bacteria during the process of cellular respiration as a final electron acceptor. For aerobic organisms, oxygen is an absolute requirement for their energy-yielding properties. Certain microorganisms grow in oxygen-free environments and are described as anaerobic. Pathogenic species, such as *Clostridium* species, are anaerobic. Certain species of microorganisms are said to be facultative. These species grow in either the presence or absence of oxygen. Some bacteria species are microaerophilic, meaning that they can grow in low concentrations of oxygen. In some cases, these organisms must have an environment rich in carbon dioxide. Organisms such as these are said to be capnophilic.

3.3.3 Implicit Factors

Bacteria, yeasts, moulds, and all other microbes require energy to grow and survive. They get energy from nutrients and water found in foods and other substrates. But microbes do not live alone as single species; they live in a mixed community. For example, a tree has microbes living in its roots, leaves and other structures, and a human body has many different microbes living on its skin and lung tissues and in the digestive tract.

Microbes can compete for the same resources. If one microbe species is better than another at utilizing nutrients and grows faster, we describe this as a competition, where one microbe species wins over the other. The successful microbe species outgrows or outcompetes the other species for the food and energy. Competition for essential nutrients impacts the rate at which different microbial species grow and reproduce. A rapid increase in

one species may well be at the cost of another species. Changes in the substrate or food environment can also change the growth rate of microbes. This might occur when one microbe consumes all the food, so there is nothing left for a competing microbe to eat, which dies off. Or, this might occur when one microbe secretes a chemical into the food, which changes the environmental conditions, making it more acidic and beneficial for the growth of other microbe species as occurs in fermentation. Table 1 depicts the different types of interactions between microbes.

Table 1: Types of Microbial interaction

Type of Interaction	Effect of Interaction	Example
Competition and antagonism	One microbial community harms another	The growth of lactic acid bacteria (LAB), *Leuconostoc,* produces acids that inhibit the growth of *E. coli.* *Penicillium,* the black bread mould, secretes chemicals that kill other bacteria.
Mutualism	Both microorganisms communities benefit each other.	The growth of one strain of LAB, *Leuconostoc*, promotes the growth of another type of LAB, *Lactobacillus.*
Commensalism	One microbe community benefits from growth but doesn't affect the other community.	During fermentation of apples into vinegar, yeast will break down sugars into alcohol. *Acetobacter* bacteria benefit from this and break down the alcohol into acetic acid. This end-product is not harmful to yeasts.

3.3.4 Processing factors

Food processing is often not given importance when foods are evaluated, but it is essential to understand associated risks. Activities during processing can increase contamination risk. For example, the slicing of a tomato increases the risk of *Salmonella* growth. When a fruit is cut, such as a tomato or a melon, the outer skin and protective covering of the fruit is damaged. If the interior flesh of the fruit can support bacterial growth, when its outer skin (tomato) or rind (melon) is damaged, there is a risk that bacteria can enter

the fruit and grow. This may occur if the skin is contaminated (containing dirt and bacteria), or if the knife or utensil used to cut into the fruit is contaminated. Processing can also decrease risk, for example when raw milk is pasteurized it kills microbes during the heating step.

Cross-contamination can occur during food processing when microorganisms from one food item are transferred to another food item. This can happen when equipment, surfaces, or utensils are not properly cleaned and sanitized between uses. Insufficient cooking can increase the risk of contamination by allowing heat-resistant pathogens to survive. This can happen when food is not cooked to the appropriate temperature for the recommended length of time. Improper storage of processed foods can also increase contamination. Foods that are stored at the wrong temperature or for too long can become a breeding ground for microorganisms. Water is often used in food processing, and if it is contaminated with harmful microorganisms, it can lead to contamination of the final product. The use of recycled packaging materials can also increase contamination if they are not properly cleaned and sanitized before use. To minimize the risk of contamination during food processing, it is important to follow good manufacturing practices, implement appropriate food safety management systems, and regularly monitor and test products and equipment for potential contamination.

3.4 SUM UP

After studying this unit, you have understood about the growth of a microorganism and the phases involved. The unit has covered all the intrinsic factors like water activity, nutrient, pH, redox potential etc. and extrinsic factors such as temperature of storage, relative humidity and gas composition around the food that can affect the growth of a microorganism. Further, we have also discussed the effect of some implicit factors and processing factors also on the microbial growth.

3.5 CHECK YOUR PROGRESS

1. Describe the stages of bacterial growth.

.....

.....

.....

2. Define intrinsic factors affecting microbial growth.

.....

.....

.....

3. Explain the importance of water activity in growth of microbes.

.....

.....

.....

4. What are antimicrobial substances?

.....

.....

.....

5. Explain the different types of microbial interaction.

.....

.....

.....

6. What are processing factors?

.....

.....

.....

Unit-IV METHODS OF ISOLATION AND DETECTION OF MICROORGANISMS OR THEIR PRODUCTS IN FOOD

Structure

4.1 INTRODUCTION

This unit will give you an understanding about the method of isolation and detection of microorganisms in food. The unit will cover various conventional methods being used for detection of microorganisms. Different rapid techniques like ELISA, Radio immunoassay etc. based on immunological methods are also discussed. Further, we will also understand the principles of chemical methods used for rapid detection of microbes.

Objectives

After reading and learning this unit, you will be able to:

- discuss the methods of isolation and detection of microorganisms in food
- explain the various conventional methods in use
- discuss the immunological methods used
- describe the chemical methods for detection of microorganisms
- explain the principles of methods like ELISA, PCR etc.

4.2 NEED FOR DETECTION OF MICROORGANISMS

Microbiological examination of food is essential to assess its quality. This can be done to determine its shelf life, suitability for human consumption, or to confirm if it meets established microbiological standards. Another important reason for conducting a microbiological examination of food is to identify the cause of spoilage or detect the presence of pathogens that may have caused foodborne illness. Methods for estimating total microbial count differ significantly from those used for detecting pathogens or isolating them for further analysis.

Isolating specific pathogens, especially those present in low but significant numbers, can be challenging and often requires detailed procedures. This may involve enriching the culture in media that promote growth of the particular pathogen while inhibiting the growth of other microorganisms. Subsequently, selective diagnostic media may be used for isolation, followed by confirmatory tests to identify the specific pathogen.

4.3 HOMOGENIZATION OF FOOD SAMPLES

Microorganisms in food samples are commonly enumerated for plating using mechanical blenders. One such device is the Stomacher, which homogenizes specimens in a special plastic bag through vigorous pounding of two paddles. This pounding action shears food specimens, releasing microorganisms into the diluent. Several researchers have compared the Stomacher to a high-speed blender for food investigations, and plate counts from Stomacher-treated samples have been found to be comparable to those treated with a blender.

Food samples, especially solid or semi-solid samples, usually have non-uniform distribution of ingredients or components. Homogenization helps to ensure that the entire sample has consistent composition. Food samples taken for analysis or testing should be representative of the entire batch or lot. Homogenization helps to create a uniform mixture that accurately represents the overall composition of the food product. It is important to note that proper homogenization techniques should be selected based on the specific food sample and purpose of analysis or processing to ensure accurate and reliable results. Additionally, food safety and hygiene practices should always be followed during the homogenization process to prevent cross-contamination and maintain food safety standards.

4.4 CONVENTIONAL METHODS

4.4.1 Pour Plate Method

The pour plate method is a microbiological technique used for the quantitative analysis of microorganisms in a sample. This method is often used in environmental microbiology, food microbiology, and clinical microbiology for the enumeration of bacteria, yeasts, and molds in a sample.

The pour plate method involves a number of steps where a small volume of the sample to be analyzed is added to a sterile petri plate. Molten agar medium is added to the petri dish containing the sample. The agar medium is typically at a temperature of around 45-50°C to avoid damaging the microorganisms in the sample. The contents of the petri dish are mixed thoroughly to ensure even distribution of the sample throughout the agar medium (Fig 1). The agar medium is then allowed to solidify at room temperature. The petri dish is incubated at the appropriate temperature and for the appropriate time period for the specific

microorganisms to grow. After incubation, the petri dish is examined for the presence of colonies of microorganisms.

The pour plate method allows for the enumeration of microorganisms present in the sample by counting the number of colonies formed on the surface of the agar medium. The method is based on the principle that microorganisms will be evenly distributed throughout the agar medium as it solidifies, resulting in the growth of individual colonies that can be counted. One major advantage of the pour plate method is that it can be used to isolate individual colonies of microorganisms for further study or identification. Additionally, this method can be used for the detection and enumeration of microorganisms that may be present in low numbers in a sample. However, the method can be time-consuming and requires careful technique to ensure even distribution of the sample in the agar medium.

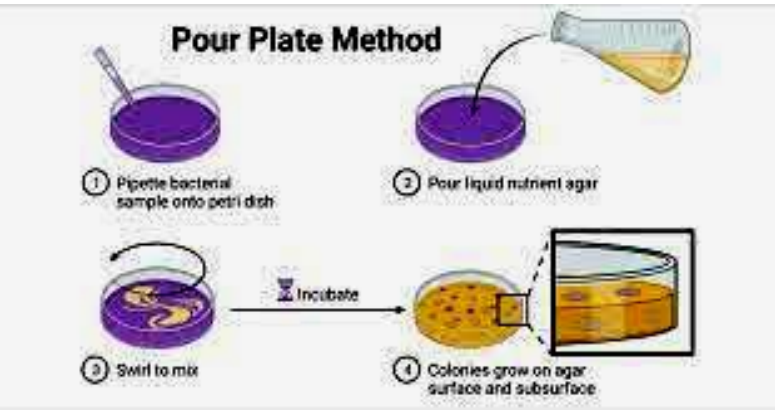

Fig 1: Illustration of Pour plate method

4.4.2 Spiral plating method

The spiral plating method is a microbiological technique used for the quantitative analysis of microorganisms in a sample. This method is also commonly used in food microbiology, clinical microbiology, and environmental microbiology for the enumeration of bacteria in a sample.

The spiral plating method involves the following steps i.e. a small volume of the sample to be analyzed is placed on a circular agar plate. A spiral plater, which is a specialized instrument with a rotating arm, is used to spread the sample in a spiral pattern across the surface of the agar plate (Fig 2). The agar plate is incubated at the appropriate temperature and for the appropriate time period in an incubator for the microorganisms to grow. After incubation, the plate is examined for the presence of colonies of microorganisms. The spiral

plating method allows for the enumeration of microorganisms present in the sample by counting the number of colonies formed on the surface of the agar plate. The method is based on the principle that as the sample is spread in a spiral pattern across the surface of the agar plate, the concentration of microorganisms decreases towards the outer edge of the spiral. This results in individual colonies that can be counted.

One advantage of the spiral plating method is that it is a fast and efficient way to analyze large numbers of samples. The method can also be used to analyze samples with a wide range of bacterial concentrations. Additionally, the method allows for the isolation of individual colonies of microorganisms for further study or identification. However, the spiral plating method requires specialized equipment, which may not be available in all laboratories. The method also requires careful technique to ensure even spreading of the sample in the spiral pattern.

Fig 2: Representation of Spiral plating method

4.4.3 Membrane filters

Membrane filter methods are commonly used to determine microbial numbers, where the membranes used have a pore size that can retain bacteria. After filtering a known volume of the sample, the membrane is placed on an agar plate or an absorbent pad saturated with the appropriate culture medium and incubated to allow for bacterial growth. The colonies that form on the membrane are then counted microscopically. This method is particularly useful for enumerating low numbers of bacteria.

The efficiency of membrane filter methods has been improved through the use of fluorescent dyes. Fluorescent dyes and epifluorescent microscopes have been widely employed since the early 1970s to specifically identify bacteria in water samples. While cellulose filters were initially used, polycarbonate Nucleopore filters are now recommended as they help retain all bacteria on top of the filter, enhancing the accuracy of the method.

4.4.4 Direct epifluorescence filter technique

The direct epifluorescent filter technique is commonly used for estimating bacterial counts in raw milk. This procedure was developed to provide a rapid method for assessing the hygienic quality of farm milks. It offers increased sensitivity (10^3-10^4 bacteria ml-^{1}) compared to conventional microscopy techniques, as it allows for concentration of bacteria from a larger volume of sample through filtration using a polycarbonate membrane filter. Bacteria are then counted directly under an epifluorescence microscope. In some cases, pretreatment of the sample may be necessary to enable filtration, such as treating milk with detergent and a protease enzyme. Polycarbonate membranes with standardized pores produced through neutron bombardment of a plastic film are preferred over cellulose acetate filters, which have irregular pores that can trap bacteria at various stages.

4.4.5 Agar Droplet method

The method proposed by Sharpe and Kilsby involves diluting the food sample in tubes of melted agar at 45°C. In the first tube, 1 mL of food homogenate is inoculated and mixed. Using a sterile capillary pipette, a line of 5 x 0.1-mL droplets is transferred to the bottom of an empty petri dish. Then, three drops (0.1 mL) from the first 9-mL tube are transferred to the second tube, mixed, and another line of 5 x 0.1-mL droplets is placed in the next petri dish. This process is repeated for the third tube of agar. The petri plates with agar droplets are then incubated for 24 hours, and colonies are enumerated using a 10x viewer. This method has been shown to yield comparable results to conventional plate counts when applied to pure cultures, meats, and vegetables. It is approximately three times faster, with 24-hour incubation giving counts similar to those obtained after 48 hours using conventional plate counts. Dilution blanks are not necessary, and only one petri dish per sample is required.

4.4.6 Most probable numbers technique

Most Probable Number (MPN) is used to estimate the concentration of viable microorganisms in a sample by means of replicating liquid broth growth in ten-fold dilutions. It is commonly used in estimating microbial populations in soils, waters, and agricultural products. MPN is most commonly applied for quality testing of water i.e to ensure whether the water is safe or not in terms of bacteria present in it. A group of bacteria commonly referred to as fecal coliforms act as an indicator of fecal contamination of water. The presence of very few fecal coliform bacteria would indicate that water probably contains no

disease-causing organisms, while the presence of large numbers of fecal coliform bacteria would indicate a very high probability that the water could contain disease-producing organisms making the water unsafe for consumption.

Water to be tested is diluted serially and inoculated in lactose broth, coliforms if present in water utilizes the lactose present in the medium to produce acid and gas. The presence of acid is indicated by the color change of the medium and the presence of gas is detected as gas bubbles collected in the inverted Durham tube present in the medium. The number of total coliforms is determined by counting the number of tubes giving positive reaction (i.e both color change and gas production) and comparing the pattern of positive results (the number of tubes showing growth at each dilution) with standard statistical tables.

4.4.7 Direct microscopic count (DMC)

The DMC (Direct Microscopic Count) process involves creating smears of food specimens or cultures on a microscope slide, staining them with a suitable dye, and then screening and counting cells using a microscope with an oil immersion objective. DMCs are commonly used in the dairy industry to assess the microbial quality of raw milk and other dairy products. The method involves adding 0.01 mL of a sample to a 1 cm2 area on a microscope slide, followed by fixing, defatting, and staining the organisms. A calibrated microscope is used to analyze the stained cells. DMC provides a rapid microbiological examination of food products such as dried and frozen foods, and offers advantages such as speed, simplicity, and the ability to assess cell morphology and use fluorescent probes for better efficiency. However, there are some limitations to DMC, including the inability to distinguish between viable and nonviable cells, difficulties in distinguishing food particles from microorganisms, non-uniform dispersion of microbial cells relative to single cells and clumps, variability in stain uptake by cells which may affect counting, and DMC counts are generally higher than counts by SPC (Standard Plate Count).

4.4.8 Contact Plate

The contact plate method is a microbiological technique used for the detection and enumeration of microorganisms on surfaces. This method is commonly used in industrial, pharmaceutical, and food processing environments to monitor for the presence of microorganisms that may cause contamination. The contact plate method involves the following steps:

- A sterile agar plate is pressed onto the surface to be tested for a predetermined amount of time and pressure, typically around 10-15 seconds and 2-3 pounds per square inch.
- The agar plate is then removed from the surface and incubated at the appropriate temperature and for the appropriate time period for the microorganisms of interest to grow.
- After incubation, the plate is examined for the presence of colonies of microorganisms.

The contact plate method allows for the enumeration of microorganisms present on the surface by counting the number of colonies formed on the surface of the agar plate. The method is based on the principle that microorganisms will be transferred from the surface to the agar medium as the plate is pressed onto the surface. Advantage of the contact plate method is that it is simple and easy to use, requiring minimal equipment and training. The method is also useful for monitoring the effectiveness of cleaning and disinfection procedures in industrial and food processing environments. However, the method has limitations, as it only detects microorganisms that can grow on the specific type of agar used in the plate, and may not detect all microorganisms present on the surface. Additionally, the method may not be suitable for surfaces that are difficult to press a plate onto, or surfaces that are uneven or porous.

4.5 IMMUNOLOGICAL METHODS

4.5.1 Fluorescent Antibody

This method has been widely used in both clinical and food microbiology since its industrialization in 1942. In the direct method, the antibody reacts with its antigen, forming an antigen-antibody complex that emits fluorescence and can be analyzed using a fluorescence microscope. Commonly used fluorescent markers include rhodamine B, fluorescein isocyanate, and fluorescein isothiocyanate. In the indirect method, the labeled compound is used to analyze the presence of the homologous antibody, while in the direct method, it detects the presence of the antigen.

The basic principle of fluorescent antibody staining involves using specific antibodies that are labeled with fluorescent dyes, which emit light when exposed to specific wavelengths of light. The fluorescently labeled antibodies can bind specifically to the target antigen or antibody in the sample, forming an immune complex. When the sample is then exposed to a

specific wavelength of light, the fluorescent dye emits light of a different wavelength, which can be detected and visualized using a fluorescence microscope or other fluorescence detection methods. Fluorescent antibody staining can offer high specificity and sensitivity, allowing for accurate detection and identification of target antigens or antibodies in complex biological samples. However, it requires careful selection and validation of antibodies, optimization of staining protocols, and proper handling and interpretation of results to ensure reliable and reproducible outcomes.

4.5.2 Enrichment serology

Enrichment serology typically involves using techniques or reagents that increase the sensitivity or specificity of antibody detection methods, such as enzyme-linked immunosorbent assays (ELISAs), chemiluminescent immunoassays (CLIAs), or other immunoassay techniques. The goal of enrichment serology is to improve the accuracy and reliability of antibody testing, particularly when the concentration of antibodies in a sample is low or when the antibodies of interest are difficult to detect due to interference from other substances in the sample. This can be achieved by various means, such as using specialized sample preparation techniques, optimizing assay conditions, or employing specific reagents or antibodies that enhance the binding or detection of target antibodies.

The use of enrichment serology (ES) is a faster method for recovering salmonellae from foods compared to the conventional culture method (CCM). The ES process involves enrichment in a nonselective medium for 18 hours, followed by selective enrichment in selenite-cystine and/or tetrathionate broth for 24 hours, selective enrichment in M broth for either 6-8 hours or 24 hours, and agglutination with polyvalent H antisera at 50°C for 1 hour. Results can be obtained in 50 hours. Overall, the ES method provides results in 32-50 hours, compared to 92-120 hours for CCM. The results obtained from ES are comparable to both CCM and FA methods, and no specialized equipment or training is needed.

4.5.3 Radioimmunoassay

Radioimmunoassay (RIA) is a laboratory technique used to measure the concentration of specific antigens or antibodies in a biological sample using radioactive isotopes as tracers. RIA is an immunoassay method that combines the principles of immunology and radioisotope detection to quantitatively determine the presence of a target molecule in a sample.

The basic principle of RIA involves the use of a radioactive antigen or antibody, known as a tracer, that competes with the non-radioactive antigen or antibody in the sample for binding to a limited amount of specific antibodies or antigens immobilized on a solid support, such as a microplate or a tube. After an incubation period, the solid support is washed to remove unbound components, and the radioactivity associated with the bound tracer is measured using a radiation detector, such as a gamma counter. The amount of tracer bound is inversely proportional to the concentration of the target antigen or antibody in the sample, allowing for quantification.

Solid-phase radioimmunoassay (RIA) refers to methods that utilize solid materials or surfaces where a monolayer of antibody molecules binds electrostatically. These solid materials can include polypropylene, polystyrene, and bromacetylcellulose. By iodination of enterotoxins, solid-phase RIA can be used to detect as little as 1 ng of toxin per gram. RIA methods can also be applied to assess foods for other biological hazards such as endotoxins and paralytic shellfish toxins. The use of 125I labeled homologous antibody filtered and washed on a Millipore membrane has allowed for the detection and recognition of bacterial cells within 8-10 minutes in some cases. RIA offers high sensitivity and specificity, making it a valuable tool for measuring low concentrations of antigens or antibodies in a wide range of biological samples, including serum, plasma, urine, and tissue extracts. It has been widely used in clinical diagnostics, research, and drug discovery for the measurement of hormones, enzymes, drugs, viral antigens, and other biomolecules.

4.5.4 ELISA

The enzyme-linked immunosorbent assay (ELISA) is a widely used method in immunology and molecular biology for detecting the presence of specific antigens or antibodies in a sample. The basic principle of ELISA involves the binding of an antigen or antibody to a solid surface, such as a microplate, followed by the addition of a labeled enzyme-conjugated antibody or antigen, and subsequent detection of the enzyme activity to determine the presence or quantity of the target molecule.

There are several different types of ELISA, including direct ELISA, indirect ELISA, sandwich ELISA, and competitive ELISA, each with its own variations and applications. In a direct ELISA, the antigen of interest is immobilized onto the solid surface, and a labeled antibody that specifically recognizes the antigen is directly added for detection. In an indirect ELISA, the antigen is immobilized, and an unlabeled primary antibody that specifically binds to the antigen is added, followed by a labeled secondary antibody that recognizes the primary

antibody for detection. Sandwich ELISA involves the capture of an antigen by immobilized antibodies, and subsequent detection using a labeled antibody that binds to a different epitope on the antigen. Competitive ELISA is used to measure the presence of an antibody in a sample by competing with a labeled antigen for binding to immobilized antibodies.

ELISA is a versatile and sensitive method that has numerous applications in research, clinical diagnostics, and food safety testing. It is commonly used for detecting pathogens, allergens, hormones, and other analytes in various sample types, such as serum, plasma, urine, and food samples. ELISA is known for its accuracy, reproducibility, and ability to analyze multiple samples in a high-throughput manner. However, careful validation and optimization are necessary to ensure reliable results, and proper controls and standards should be included in the assay for accurate interpretation of the data.

4.5.5 Gel Diffusion

Gel diffusion methods, also known as immunodiffusion methods, are laboratory techniques used to detect and quantify the presence of specific antigens or antibodies in a biological sample. These methods are based on the principle of antigen-antibody interactions leading to the formation of visible precipitate bands in a gel medium. There are two main types of gel diffusion methods:

- Single Radial Immunodiffusion (SRID): In SRID, a sample containing the antigen of interest is placed in a well made in a gel medium, typically agar, which contains a specific antibody against the antigen. Over time, the antigen and antibody diffuse from their respective wells and form a circular precipitin ring where they meet in the gel. The size of the ring is proportional to the concentration of the antigen in the sample. The ring can be visualized and measured to estimate the antigen concentration.
- Double Immunodiffusion (Ouchterlony Technique): In double immunodiffusion, both the antigen and antibody are incorporated into the gel medium. The antigen and antibody diffuse through the gel and form a visible precipitate where they encounter each other. The pattern of the precipitate, such as lines, arcs, or complexes, can provide information about the presence and relative concentrations of the antigen and antibody in the sample.

Gel diffusion methods are widely used in immunology and clinical diagnostics for detecting and measuring a variety of antigens and antibodies, including proteins, enzymes, hormones, and infectious agents. They are relatively simple and inexpensive techniques, and can be performed with basic laboratory equipment. However, they may have limitations in

terms of sensitivity, specificity, and precision compared to more advanced techniques like enzyme-linked immunosorbent assays (ELISAs) or molecular methods like polymerase chain reaction (PCR). Nevertheless, gel diffusion methods remain valuable tools in certain situations, particularly for qualitative or semi-quantitative analysis of antigen-antibody reactions, and they are often used in research, serological testing, and epidemiological studies.

4.5.6 Hemagglutination

Gel diffusion methods typically require at least 24 hours for obtaining results. However, there are two serologic methods that are faster, yielding results in 2-4 hours: hemagglutination-inhibition (HI) and reverse passive hemagglutination (RPH). In the HI test, specific antibody is kept stable while the enterotoxin (antigen) is diluted out. After incubation for approximately 20 minutes, treated sheep red blood cells (SRBCs) are added. Hemagglutination (HA) occurs only when the antibody is not bound by the antigen, in contrast to HI. In RPH, antitoxin globulin is directly attached to SRBCs and is used to detect the toxin. When diluted toxin preparations are added, the experiment is read for HA after incubation for 2 hours. HA occurs only where optimal levels of antigen-antibody complex are formed.

4.6 CHEMICAL METHODS

4.6.1 Thermostable nuclease

The thermostable nuclease test is a microbiological method used to detect the presence of thermostable nucleases, such as those produced by certain bacteria. The test is based on the ability of thermostable nucleases to degrade DNA at high temperatures, which can be detected using a gel electrophoresis assay. To perform the thermostable nuclease test, a bacterial culture is first grown in a suitable medium. The culture is then subjected to high temperature (usually 80-100°C) for a short period of time (usually 10-30 minutes) to denature any heat-labile nucleases that may be present. The heat-treated culture is then centrifuged to remove any bacterial cells or debris, and the supernatant is collected.

Next, a small amount of DNA is added to the supernatant, and the mixture is incubated at high temperature (usually 80-100°C) for a short period of time (usually 10-30 minutes) to allow any thermostable nucleases present in the supernatant to degrade the DNA. The degraded DNA fragments are then analyzed using gel electrophoresis, which separates

the fragments based on size. If thermostable nucleases are present in the supernatant, they will degrade the DNA, resulting in a pattern of DNA fragments on the gel that is different from that obtained from a control sample in which no thermostable nucleases are present. The presence of thermostable nucleases can be confirmed by comparing the pattern of DNA fragments obtained from the test sample to that obtained from the control sample.

The thermostable nuclease test is a simple and rapid method for detecting the presence of thermostable nucleases in bacterial cultures. It is useful for identifying bacteria that produce thermostable nucleases, which can be important for a variety of applications, such as in the production of heat-stable enzymes or in the diagnosis of infections caused by thermostable nuclease-producing bacteria. However, the test has some limitations, such as its inability to distinguish between different types of nucleases or to detect nucleases that are not thermostable.

The presence of *S. aureus* in food can be determined by testing for the presence of thermostable nuclease (DNase). This is possible because there is a strong correlation between the production of coagulase and thermostable nuclease by *S. aureus* strains, especially those that produce enterotoxins. The presence of *S. aureus* can be tested using thermostable nuclease because the enzyme is heat-stable, meaning it remains active even if the bacterial cells are damaged by temperature, chemicals, or bacteriophage, or if they are induced to l-forms. The heat-tolerant nuclease can be detected earlier than enterotoxins (around 3 hours versus several days). The nuclease is produced by enterotoxigenic cells before enterotoxins appear, making it a useful indicator for the presence of *S. aureus* in food.

4.6.2 Adenosine Triphosphate measurement

Adenosine Triphosphate (ATP) is a molecule that serves as the primary energy source for cells. ATP measurement is a widely used technique to determine the level of microbial contamination or cleanliness of a surface, liquid or air. The complete extraction and accurate quantity of cellular ATP can be considered equal to presence of specific groups of microorganisms similar to the method where endotoxins are considered for Gram-negative bacteria.

ATP measurement is based on the principle that living cells contain ATP, and the amount of ATP present is proportional to the number of living cells. ATP measurement uses a bioluminescence reaction, in which ATP reacts with luciferin and luciferase to produce light. The amount of light produced is measured by a luminometer and is proportional to the

amount of ATP present in the sample. To perform ATP measurement, a sample is collected from the surface, liquid or air and mixed with a reagent containing luciferin and luciferase. The mixture is then incubated for a short period of time to allow the ATP present in the sample to react with the reagent and produce light. The amount of light produced is measured using a luminometer, and the results are expressed as relative light units (RLUs).

ATP measurement can be used for a variety of applications, such as monitoring the effectiveness of cleaning and sanitization procedures, detecting microbial contamination in food, water, and other samples, and monitoring the quality of indoor air. It is a quick, simple, and sensitive method for detecting microbial contamination, and can provide results in a matter of seconds to minutes. However, it should be noted that ATP measurement does not differentiate between different types of microorganisms and cannot provide information on the specific type of microorganism present in the sample.

4.6.3 Radiometry

Radiometry can be used in the detection of microbes, particularly in the detection of radioactive isotopes that are produced by certain microbes. Some microorganisms are capable of producing radioactive isotopes as part of their metabolic processes. For example, certain strains of bacteria can produce radioactive carbon-14, which can be detected using radiometry.

One common method for detecting radioactive isotopes is through the use of radiometric assays, which involve labeling a molecule or substance with a radioactive isotope and then measuring the amount of radioactivity present. Radiometric assays can be used to detect the presence of specific microbes based on their ability to produce radioactive isotopes. Another application of radiometry in microbial detection is through the use of radiolabeling techniques. Radiolabeling involves attaching a radioactive isotope to a molecule or substance that is specifically recognized by the microbe of interest, such as a nutrient or antibody. The radiolabeled molecule or substance is then introduced to the sample, and the amount of radioactivity present is measured. The presence of the microbe can be inferred based on the amount of radioactivity detected.

Radiometry can also be used in combination with other detection methods, such as PCR and immunoassays, to improve the sensitivity and specificity of microbial detection. For example, radiolabeled probes can be used in PCR to detect specific sequences of DNA, or radiolabeled antibodies can be used in immunoassays to detect specific microbial antigens.

Radiometry can be a useful tool in the detection of microbes, particularly in the detection of radioactive isotopes produced by certain microorganisms. However, the use of radioactive materials requires strict safety precautions and regulatory compliance.

4.6.4 Polymerase Chain Reaction (PCR)

PCR stands for Polymerase Chain Reaction, which is a widely used laboratory technique to amplify a specific region of DNA in vitro. It was first developed in the 1980s by Kary Mullis and his colleagues, and has since revolutionized the field of molecular biology. The PCR process consists of a series of temperature cycles, during which the reaction mixture is subjected to different temperatures for specific periods of time. The PCR reaction mixture contains the template DNA, the primers, DNA polymerase enzyme, deoxynucleoside triphosphates (dNTPs), and buffer solution.

The first step of each cycle is a denaturation step, during which the reaction mixture is heated to a high temperature (usually 94-98°C) to separate the double-stranded DNA into two single strands (Fig. 3). The next step is the annealing step, during which the temperature is lowered to 50-65°C to allow the primers to anneal to their complementary sequences on the single-stranded DNA template. The third step is the extension step, during which the temperature is raised to 72°C to allow the DNA polymerase to synthesize new strands of DNA complementary to the single-stranded DNA template using the dNTPs as building blocks. After the first cycle, two copies of the target DNA region are present in the reaction mixture: one from each strand of the double-stranded DNA. In the subsequent cycles, the denaturation, annealing, and extension steps are repeated, resulting in an exponential increase in the number of copies of the target DNA region. This allows even a small amount of starting DNA to be amplified to detectable levels.

The amplified DNA products can be analyzed using a variety of techniques, such as gel electrophoresis, DNA sequencing, or hybridization-based assays. Gel electrophoresis separates the amplified DNA fragments by size, and the resulting band pattern can be visualized using stains or dyes. DNA sequencing can be used to determine the exact sequence of the amplified DNA products, which can be used for a variety of applications, such as identifying genetic mutations or characterizing genetic variation. Hybridization-based assays, such as TaqMan assays or SYBR Green assays, use fluorescent probes or dyes to detect the amplified DNA products in real time. The PCR process can be repeated multiple times, with each cycle producing a doubling of the amount of DNA. This exponential amplification

allows even small amounts of DNA to be amplified to detectable levels. The amplified DNA can be analyzed using a variety of methods, such as gel electrophoresis, DNA sequencing, or hybridization-based assays.

PCR is used in a wide range of applications, including genetic research, medical diagnosis, forensics, and biotechnology. It has enabled researchers to study the genetic material of organisms that were previously difficult to study, and has allowed for the development of new diagnostic tests for genetic disorders and infectious diseases.

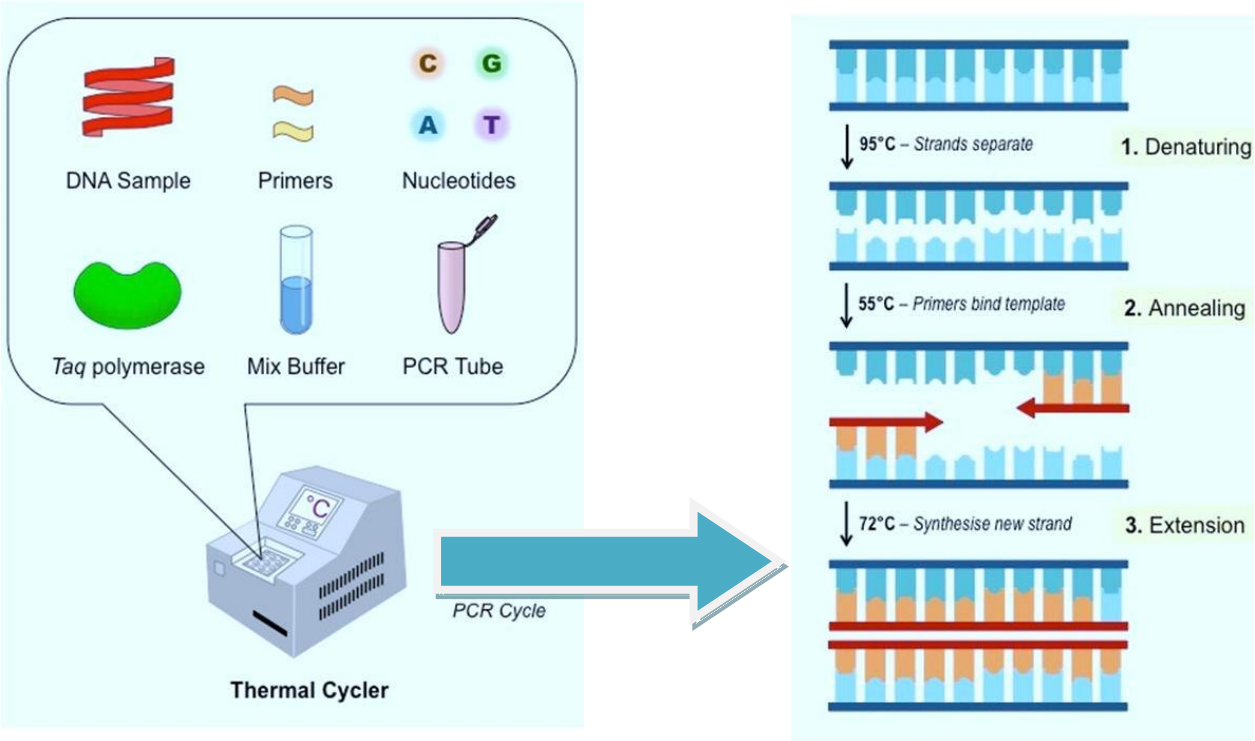

Fig 3: Pictorial representation of Polymerase Chain Reaction

4.7 SUM UP

In this unit we studied the need of detection of microorganisms in food. We looked at the concept and principles of method of isolation and detection of microorganisms in food. We covered various conventional methods being used for detection of microorganisms. We also discussed different rapid techniques like ELISA, Radio immunoassay etc. based on immunological methods. Further, we also studied the principles of chemical methods used for rapid detection of microbes.

4.8 CHECK YOUR PROGRESS

1. What is homogenization of food samples?

.....

.....

.....

2. Explain pour plate and spiral plating method.

.....

.....

.....

.....

3. Explain the ELISA method for detection of microorganisms.

.....

.....

.....

.....

4. Differentiate between conventional and rapid methods of microbe detection.

.....

.....

.....

.....

5. Explain the PCR method in detail.

.....

.....

.....

6. What is Adenosine Triphosphate measurement method for detection of microorganisms?

.....

.....

.....

.....

7. Write short notes on:

a) Radiometry

.....

.....

b) Gel Diffusion Method

.....

.....

Unit V SPOILAGE OF DIFFERENT GROUPS OF FOODS

Structure

5.1 INTRODUCTION

This unit will give you a detailed overview of microbial spoilage of different food groups. The unit gives you an understanding about concept of food spoilage and the factors affecting food spoilage. The unit will cover micro flora, contamination sources and spoilage occurring in plant foods category like cereals, fruits and vegetables. Microbial spoilage and contamination sources of animal foods and sea foods are also explained in detail.

Objectives

Learning this unit, will make you able to:

- define the concept of food spoilage
- explain the factors affecting the food spoilage
- discuss the composition, micro flora and contaminate of different food groups.
- describe the spoilage of cereals, cereal products, fruits and vegetables
- discuss the spoilage of meat, eggs, sea foods, milk and milk products

5.2 FOOD SPOILAGE

The term "spoil" refers to the process of food losing its good or effective qualities. When a food is spoiled, its characteristics are changed, making it no longer acceptable for consumption. These changes can be caused by various factors, including insect damage, drying out, discolouration, staling, or rancidity. However, microbial activity is often the main cause of food spoilage. Microbiological food spoilage can manifest in different ways, sometimes occurring in combination. This can include visible microbial growth such as

surface slime or colonies, degradation of structural components leading to loss of texture, and the production of chemical products through microbial metabolism, such as gas, pigments, polysaccharides, off odours, and flavours.

5.2 FACTORS AFFECTING SPOILAGE

The type of food spoilage caused by microorganisms and enzymes depends on the types and quantities of these agents present, as well as the environmental conditions. Raw foods typically contain various types of bacteria, yeasts, and molds, as well as plant and animal enzymes. The kind and number of microorganisms present in or on food are influenced by the type and extent of contamination. In some cases, due to environmental conditions, one type of organism may initiate spoilage, followed by one or more other types of organisms that produce secondary spoilage or a further succession of organisms and changes may be involved.

There are several factors that can contribute to microbial spoilage, including:

1. **Temperature:** Microorganisms thrive in warm and moist environments. Food that is stored at temperatures between 4°C to 60°C is particularly vulnerable to microbial growth and spoilage.
2. **pH:** The acidity of a food product can affect the growth and survival of microorganisms. Foods with a low pH, such as pickles and sauerkraut, are less susceptible to spoilage than those with a higher pH.
3. **Water activity:** The availability of water in a food product can affect the growth and survival of microorganisms. Foods with high water activity, such as fresh fruits and vegetables, are more susceptible to spoilage than those with lower water activity, such as dried fruits and jerky.
4. **Oxygen:** Some microorganisms require oxygen to grow, while others thrive in the absence of oxygen. Oxygen-sensitive microorganisms can cause spoilage in vacuum-packed and other oxygen-free food products.
5. **Nutrients:** Microorganisms require nutrients such as carbohydrates, proteins, and fats to grow and reproduce. Foods that are high in these nutrients, such as meat, dairy, and baked goods, are particularly susceptible to spoilage.
6. **Contamination:** Microorganisms can be introduced to food through contamination from equipment, surfaces, and other sources. Proper hygiene and sanitation practices can help reduce the risk of contamination and spoilage.

7. **Preservation methods:** Various preservation methods such as pasteurization, canning, freezing, drying, and the addition of preservatives can help prevent microbial spoilage by either reducing or eliminating the growth of microorganisms.

Classification of foods on the basis of shelf life:

1. Non perishable foods:

This category includes the foods which do not spoil unless handled and stored carelessly and that can be stored at least for several months. Examples of non-perishable foods include cereals, pulses, sugar etc.

2. Semi perishable foods:

Like the non-perishable foods, semi-perishable foods can also survive without any perceptible sign of spoilage for a couple of weeks or for a few months. If semi perishable foods are handled and stored properly, they shall remain unspoiled for a fairly long period. Examples in this category include cereal and pulse products like wheat flour, refined wheat flour, semolina, vermicelli, broken wheat, bengal gram flour (besan), potatoes, garlic, some fruits like apples, citrus fruits, fats and oils.

3. Perishable foods:

These are the foods which spoil easily within a day or two unless special methods are used to prevent such spoilage. All animal foods such as milk and milk products, meat and meat products, fish, poultry and eggs are included in this category. Most fruits and vegetables will also fall in this category.

5.4 CEREAL AND CEREAL PRODUCTS

Cereal grains, such as wheat, corn, rye, oat, rice, etc., are important nutrients and energy sources for humans. Cereal grains are the most commonly consumed food group worldwide and they are grown on about 60% of the cultivated land in the world. Cereals are consumed in various forms in the food industry. Cooked cereals are eaten directly after cooking (rice, maize). Flours are made by grinding cereals (such as wheat, maize, rice, and rye) and products processed from them, e.g. biscuits, cookies, etc. Bread is usually made from flours of wheat and rye by yeast fermentation. Manufactured dried cereal products are produced from wheat, maize, oats, and rice. Cereals are also used to produce dough, batter, pasta, noodles, pastries, cake, etc.

5.4.1 Composition and Microflora of Cereals

Cereals usually contain 70–75% carbohydrates, 8–15% protein, fat, fiber, vitamins, and minerals with almost neutral pH and hence are susceptible to microbial growth leading to spoilage. Microbial growth is normally prevented due to sufficiently low water activity (i.e. below 0.70). The most commonly associated bacterial families with cereals are *Bacillaceae, Micrococcaceae, Lactobacillaceae,* and *Pseudomonadaceae.* Yeast that is found in cereal includes *Candida, Cryptococcus, Pichia, Sporobolomyces, Rhodotorula, Trichosporon*. Mold spores in cereals and flour are chiefly *Aspergillus, Penicillium, Alternaria, Mucor, Cladosporium, Fusarium, Helminthosporium, Cladosporium* and *Rhizopus*. Mycotoxins are the toxic secondary metabolites produced by mold found in cereal crops under favorable growth conditions. The genera of molds producing mycotoxins are *Aspergillus, Penicillium,* and *Fusarium*. A high incidence of mycotoxin infections in cereals has been observed worldwide.

5.4.2 Spoilage of Cereals

The microbiology of cereals, from growth to harvest and storage, is dominated by molds. Two groups of fungi can be distinguished in this context. Field fungi are well adapted to the changing conditions on the surfaces of aging plant material in the field. Despite requiring relatively high water activities for optimal growth, genera such as Cladosporium, Alternaria, and Epicoccum are able to survive rapid changes that can occur from desiccation on a hot sunny day to cool and damp conditions at night. The genus Fusarium includes species that have both pathogenic and saprophytic activities.

The second category, storage fungi are well adapted to the storage conditions of cereals, and generally grow at lower water activities. The most important genera of storage fungi are Penicillium and Aspergillus, although species of Fusarium may also be involved in spoilage when grains are stored under moist conditions. Table 1 provides a list of microorganisms responsible for cereal spoilage. Water activity and temperature are considered the most important environmental factors influencing mold spoilage of cereals and the potential production of mycotoxins. Molds may grow very slowly at the lower limit of their water activity range, but once they start growing and metabolizing, they will produce water through respiration, causing the local water activity to rise and allowing for more rapid growth. This allows mesophilic mold spores to germinate and grow in an autocatalytic process. There is a sequence of observable consequences of mold growth on cereals, starting

with a decrease in grain germinability, followed by discoloration, production of mold metabolites including mycotoxins, demonstrable increase in temperature (self-heating), production of musty odors, caking, and rapid increase in water activity leading to complete decay with the growth of a wide range of microorganisms.

Table 1: List of microorganisms and the defect caused in cereals

Microorganisms	Defects caused in cereals
Alternaria, Fusarium, Cladosporium, and Botrytis	Blights and blemishes
Aspergillus fumigatus, A. penicillioides, A. ochraceus	Discolored germs
A. candidus	Powdery white patches
Claviceps purpurea	Ear rot (ergotism) in grain and corn
Eurotium	Discolored germs, green eye
Alternaria	Darkening of grains
Fusarium	Red streaking, particularly on maize
Penicillium	Blue coloration
A. flavus	Greenish discoloration
Fusarium or Alternaria	Pink or black tips in wheat

5.4.3 Spoilage of Cereal Products

Flour

The moisture content of the flour is less than 13% that prevents the growth of microorganisms. However, the addition of water to flour tends to make it susceptible to microbial growth in flour. The molds found in flours are mostly *Eurotium* species and *Aspergillus candidus*. The molds produce typical mycelium in flour. The spoilage flour contains many psychrotrophs, flat sour bacteria, and thermophilic spore-forming bacteria such as *Acetobacter* spp, *Bacillus* spp, Lactic acid bacteria. The presence of acid-forming bacteria in flour initiates acid fermentation followed by alcoholic fermentation of flour due to yeasts and then finally production of acetic acid will occur by *Acetobacte*r spp. *Bacillus* spp is known for producing lactic acid, gas, and acetoin in flour.

Bakery Product

Bakery products encompass a diverse range of items, such as leavened and unleavened bread, muffins, biscuits, cakes, cupcakes, doughnuts, pastries, rolls, buns, croissants, pancakes, waffles and sweet rolls. The nutrient content of bakery products includes carbohydrates, proteins, lipids, vitamins, and minerals. Therefore, bakery products are susceptible to microbial growth due to their high nutrient content and also because the most common factor of these products is water. The most famous bakery product that is consumed worldwide for a very long period is bread.

The presence of ambient storage temperatures, pH levels of the product about 5.4 - 7.5, as well as water activity within the range 0.75–0.98 provides favourable conditions for growth of spoilage mold, yeast, and rope bacteria in baked cereal foods. The most common source of microbial spoilage of bread is mold growth. The bacterial spoilage condition is known as 'rope' caused by the growth of the *Bacillus* species. The least common of all types of microbial spoilage in bread is that caused by certain types of yeast (Table 2). Yeasts that are involved in surface spoilage of bakery products belong to genus *Saccharomyces, Kluyveromyces, Pichia, Candida, Debaryomyces* and *Zygosaccharomyces.* Mold causing spoilage of cereal products includes *Penicillium, Aspergillus, Rhizopus, Fusarium, Monilia, Cladosporium, Endomyces* and *Mucor*.

Table 2: Defects observed on bread with their causative microorganism

Defect caused in bread	**Microorganisms**
White cottony mycelium and black dot	*Rhizopus stolonifer (bread mold)*
Green color	*Penicillium expansum, Penicillium stoloniferum*
greenish- or purplish-brown to black color	*Aspergillus niger*
Pink or reddish color on the bread	*Monilia sitophila*
Green and gray-green color	*Aspergillus glaucus*
Olive green color	*Aspergillus flavus*
Gray color	*Mucor*
Chalky defect	*Endomycopsis fibuligera, Trichosporon variable*
Ropiness	*Bacillus subtilis, Bacillus mesentericus, Bacillus licheniformis, Clostridium* spp, *Lactobacillus* spp, *Leuconostoc* spp

Defect caused in bread	Microorganisms
Red or bloody bread	*Serratia marcescens*
Alcoholic off-flavor	*Saccharomyces cerevisiae*

5.5 FRUITS AND VEGETABLES

Fruits and vegetables are rich source of energy, body-building nutrients, vitamins and minerals. The nutritional value of fruits and vegetables is highest when they are fresh, but it is not always possible to consume them instantly. During the harvest season, fresh produce is available in plenty amount, but at other times it is scarce. Moreover, most fruits and vegetables are only edible for a very short time, unless they are promptly and properly preserved.

5.5.1 Composition and Microflora

Fruits and vegetables have a high water activity as compared to meat, fish and cereals foods. Fruits and vegetables also have a much lower concentration of free amino acids and other nutrients than meat, fish, and milk. However, when fruits and berries ripen, endogeneous pectinases start to hydrolyse the pectin and this also makes the products more susceptible to microbial attacks. Spoilage in fruits and vegetable starts with the hydrolysis of the pectin. Once the pectinases have damaged the structure of the fruit/vegetable, other organisms start to contribute to the soft rot. Spoilage imparts the loss of original nutritive values, texture, flavour of fruits and vegetables and consumption such food stuffs may cause illness people.

There are many sources for spoilage; microbial spoilage, autolysis and other factors. Microbial spoilage contributed by yeast, moulds and bacteria. Majority of the microorganisms in vegetables are saprophytes, such as lactic acid bacteria, coryniforms, coliforms, micrococci, spore-formers, and pseudomonas, which may be come from the air, soil, and water. The microbes from enterobacteriaceae (Pseudomonas, Klebsiella, Enterobacter and Serratia) are the most frequent. The fungus namely Aureobasidium, Fusarium, and Alternaria, are also commonly present but lower in number as compare to bacteria. Yet, due to the lower pH of raw fruits, the major spoilage causing organisms are fungi, mostly moulds and yeasts, such as *Penicillum spp., Byssochlamys fulva, Sacharomyces cerevisiae, Aspergillus niger, B. nivea, Clostridium pasteurianum, Coletotrichum*

gloesporoides, Clostridium perfringes and *Lactobacillu*s spp. Psychrotrophic bacteria can grow in vegetable products; some of them are *Cytophaga jhonsonae*, *Xantomonas campestri, Erwina carotovora, Pseudomonas fluorescens, P. auriginosa, P. luteola, Bacillus* species, and *Vibrio fluvialis*. It is observed that around 20% of the fruits and vegetables are deteriorated by microorganisms.

5.5.2 Spoilage of Fruits and Vegetables

Saprophytic microorganisms

The microbial species that prevail on fuits and vegetables are commonly found on plants in the field or after harvest and probably originated from the epiphytic microflora of the raw materials. *P. fluorescens*, *E. agglomerans*, *E. herbicola* are major components of the epiphytic microflora of many vegetables.

Pectinolytic microorganisms

Pectinolytic isolates that are generally found on vegetables are *P. fluorescens* of different biovers, *P. paucimobilis, P. viridiflava, P. luteola, Xanthomonas maltophila, Flavobacterium spp., Cytophaga spp. or Vibriofluvialis*. Some pectinolytic fungi like Mucor spp., *Sclerotinia sclerotiorum* and yeasts like Trichosporon spp. have also been isolated from shredded carrots.

Yeast and molds

Low pH (<4.5) of most fruits means that spoilage is caused mainly by fungi. The pH range of most vegetables varies between 5.0 and 7.0 and thus spoilage may be caused by either fungi or bacteria. Downy mildew disease of plants, especially in cool, humid regions, is caused by several fungi, including species of Basidiophora, Peronospora, Phytophthora, Plasmopara, Bremia, Pseudoperonospora and *S. clerospora*. White, gray, bluish or violet downy patches of mildew form mostly on the undersides of leaves in damp weather. The black spots seen on tomatoes are usually caused by Alternaria, which appears when the weather is warm.

Table 1: General Types of Microbial Spoilage in Fruits and Vegetables

S.No.	Spoilage	Causative Microbe	Effect
1.	Bacterial soft rot	*Erwinia carotovora*	Watersoaked appearance with soft mushy consistency and a bad odor
2.	Gray mold rot	*Botrytis cinerea*	Favored by high humidity and a warm temperature occurs in oranges, grapes
3.	Rhizopus soft rot	*Rhizopus stolonifer*	Often soft and mushy cottony growth of the mold small black dots of sporangia often covers the food Sweet potatoes, tomatoes
4.	Anthracnose	*Collectotrichum musae*	Defect is spotting of leaves and fruits or seed pods Beans
5.	Alternaria Rot	*Alternaria tenuis*	Greenish brown early in the growth of the mold later turn to brown or black spots citrus fruits
6.	Blue mold rot	*Penicillium digitatum*	Bluish green color spores are produced on citrus fruits
7.	Downey mildew	*Phytophthora, Bremia*	Molds grow in white, woolly masses
8.	Watery soft rot	*Sclerotinia sclerotiorum*	Found mostly in vegetables like carrots
9.	Stem end rots	*Diplodia, Alternaria, Phomopsis, Fusarium*	Involve the stem end of citrus fruits
10.	Black mold rot	*Aspergillus niger*	Dark brown to black masses of spores of the mold termed "Smut", e.g., onions
11.	Black rot	*Alternaria, Ceratostomella, Physalospora*	Found in sweet potatoes
12.	Pink mold rot	*Trichothecium roseum*	Pink coloured spots or discolouration
13.	Fusarium rots	*Fusarium*	Potatoes
14.	Green mold rot	*Cladosporium*	Cherries, Peaches
15.	Brown rot	*Sclerolinia fructicola*	Peaches, Cherries

5.6 MEAT AND MEAT PRODUCTS

Meat is obtained from various species of birds like chicken, turkey, ducks, etc. and mammals i.e. pork, mutton, buffalo, sheep. After slaughtering, the carcasses and primary cuts obtained are processed to raw cuts or processed food products.

5.6.1 Composition and Microflora

Meat, being a nutrient-dense and protein-rich food, is highly perishable and has a limited shelf life without proper preservation. The quality and shelf life of meat are influenced by various factors, including temperature, which can impact its characteristics negatively. The biological and chemical composition of meat makes it susceptible to deterioration from the time of slaughter until consumption. Different meat products, such as ham, sausages, cooked meat, dry meats, smoked meats, vacuum-packed meat, minced meat, etc., are all prone to microbial spoilage. The lymph nodes of red-meat animals contain Staphylococci, streptococci, Clostridium, and Salmonella.

5.6.2 Contamination Sources

The healthy inner flesh of the meat contains few or no micro-organisms although they have been found in lymph node and bone marrow. Upon, death of animal, invasion of tissue by contaminating microorganisms takes place. In meat, microorganisms come from external source or from meat animal itself. Microbes can spread in the meat easily through blood, lymph vessels, spaces of connective tissue and further encourage spreading by grinding process of meat. However, the most significant contamination occurs during bleeding, handling, and processing due to external causes.

The animal's exterior (hides, hooves, and hair) and intestinal tract are the primary sources of bacteria during bleeding, skinning, and cutting. During the subsequent handling of the meat, contamination might originate from carts, crates, other containers, other infected meat, the air and employees. Particularly unwanted contamination is the introduction of psychrotrophic bacteria from any source, such as other meats that have been stored in the refrigerator. Specialized equipment, such as grinders, sausage stuffers, and casings, as well as additives in specialised products, such as fillers and spices, may introduce significant quantities of pathogens.

5.6.3 Spoilage of Meat under aerobic conditions

(i) Bacterial spoilage of meat:

- **Surface spoilage:**

It is caused by *Pseudomonas, Acenatobacter, Streptococcus, Leuconostoc, Bacillus* and *Micrococcus.* Temperature and available moisture influence type of microorganisms causing slime.

- **Change in color of meat:**

Red color of meat may be changed into green brown or grey due to production of oxidising agent, H_2S, etc. by microorganisms. For example, *Lactobacillus* and *Leuconostoc* cause greening of sausage.

- **Change in fat:**

Fat of meat may become rancid due to lipase producing microorganisms such as *Pseudomonas* and *Achromobacter.*

- **Surface color due to pigmented bacteria:**

Serratia marcescens give red spots. *Pseudomonas syncyanea* give blue color, *Chromobacterium lividum* gives greenish blue to brownish black color, *Flavobacterium* gives yellow color.

- **Phosphorescence:**

Phosphorescence in meat is caused by growth of luminous bacteria e.g. proliferation of *Photobacterium* sp. on surface of meat.

- **Off odors and off taste:**

Undesirable odor and taste called taint are caused by many bacteria due to production of volatile acids such as formic acid, acetic acid, butyric acid etc. Actinomycetes give musty or earthy flavor.

ii. Fungal spoilage of meat:

- **Stickness:**

Many molds grow on surface of meat and make it sticky to touch. Whiskers, a defect occurs when meat is kept at temperature near freezing, mold grow slowly

without sporulation on surface producing while cottony growth. It may be caused by *Thamnidium, Mucor mucedo, Mucor racemosus* etc.

- **Black spot**: Black coloured discolouration is caused on surface of meat due to growth of *Cladosporium herbarum.*
- **White spot**: This defect is caused by growth of *Sporotrichum carnis.*
- **Green spot:** It is caused by growth of *Penicillium* species.
- **Change in fat**: Many molds produce lipase and cause hydrolytic rancidity of fat.
- **Off odor and off taste:** Some mold growth contributes to musty flavor in meat.
- **By yeast**: Under aerobic condition, yeast grow on surface of meat causing sliminess, rancidity of fat, off odor and taste and discolorations like white, pink, brown spots.

5.6.4 Spoilage of meat under anaerobic conditions:

In anaerobic condition, anaerobic or facultative anaerobic bacteria spoil the meat.

- **Souring:**

It is caused by formic acid, acetic acid, butyric acid, propionic acid, higher fatty acids and other organic acids. e.g. lactic acid produced by bacteria. Souring may also be caused by foods own enzyme.

- **Putrefaction:**

It means decomposition of meat protein under anaerobic conditions alongwith production of foul smelling compounds like hydrogen sulphide, mercapatans, skatole, indole, etc. It is usually caused by *Clostridium* species but species of *Pseudomonas proteus* and Alkaligens may cause putrefaction.

- **Taint:**

It refers to any undesirable odor or taste.

5.7 EGGS

The eggs most commonly consumed by humans are the eggs are of hens, ducks, and quails. Eggs are a highly nutritious food that contains proteins, minerals, fats, iron, phosphorus vitamins (A, B, D, E, and K).

5.7.1 Composition and microflora

The chicken's egg, in particular, is very popular consists of the yolk (30-33%), albumen (60%), and the shell (9-12%). Egg yolk is about 50% of proteins of which the main ones are lipoproteins, lipovitellins, and lipovitellinin. Egg albumen is about 12% protein of which are ovalbumin (54%), Conalbumin (13%), Avidin (0.05%), ovotransferrin (10%), ovomucoid (11%), ovomucin (1.5-3%), and lysozyme (3.5%). The shell of the egg is a rigid structure made largely of calcium carbonate on an organic matrix. Internally, there are two shell membranes and the inner one that acts as a barrier when an organism penetrates the shell. Eggs and other egg products such as poached egg, scrambled egg, fried egg or omelets, liquid, dried, or frozen egg products can undergo microbial spoilages.

The microflora of the eggshell is dominated by Gram-positive bacteria such as *Staphylococcus, Streptococcus, Aerococcus, Bacillus,* and *Micrococcus* and Gram-negative bacteria, such as *Salmonella, Escherichia,* and *Alcaligenes* spp. The bacteria such as *Pseudomonas, Proteus, Alcaligenes, Enterobacter, Serratia, Stenotrophomonas, Cloaca, Acinetobacter, Moraxella,* and *Citrobacter* spp. are responsible for making egg rotten with off-odors and color change. Yellow pigmentation of the shell membrane occurs in eggs due to the action of *Flavobacterium or Cytophaga* species. The mold that is responsible for causing egg spoilages is the species such as *Penicillium, Cladosporium, Alternaria.* The early mold growth in egg surface is termed pin-spot molding and the final stage of spoilages is termed fungal rotting.

5.7.2 Contamination Sources

Freshly laid egg is sterile but the egg shell soon becomes contaminated by fecal matter of hen by nest, by washing water, by handling and by other material in which it is stored. Some of the reasons are listed below:

- by the fecal matters of the chicken
- by specks of dirt or materials used for the cage or nest
- water used during washing of eggs
- equipment used during processing and handling
- materials used to pack the eggs
- the temperature used during storage

- presence of eggshell cracks or micro-cracks

The eggshell serves as a physical barrier, protecting the egg white from invading bacteria. It creates an unfavorable environment for microbial growth, acting as a defense mechanism against bacterial contamination. The egg consists of antimicrobial substances, such as lysozyme, ovotransferrin, proteinase inhibitors (cystatin, ovomucoid, and ovoinhibitor), and vitamin-binding proteins (riboflavin binding protein, avidin- and thiamin-binding proteins). The organism must contaminate the shell, penetrate the pores of the shell, grow through shell membrane to reach albumen and finally to yolk to cause of spoilage of egg.

5.7.3 Spoilage of eggs:

(i) *Bacterial spoilage of egg:*

Bacteria are more common spoilage organism than mold. When bacteria grow within the egg, they decompose the content and form byproduct. These result in characteristic odor, appearance or color from which various microorganisms acquire their name.

- **Green rot:**

It is caused by *Pseudomonas fluorescence.* Green egg white shows fluorescence when exposed to UV light. In later stage of spoilage, egg yolk disintegrates and masks green color of egg white. Odor is lacking or appears fruity or sweetish.

- **Colorless rot:**

It may be caused by *Pseudomonas, Acetobacter, Acinatobacter* and *coliform.* In later stage of spoilage, egg yolk disintegrates or at least has incrustations.

- **Black rot:**

It is caused by *Proteus* and sometimes *Pseudomonas* and *aeromonas.* Egg yolk blackens and then breakdown to give whole egg content muddy brown color. Odor is putrified due to H_2S.

- **Pink rot:**

It is caused by *Pseudomonas* usually at the later stage of green rot. They are similar to colorless rot except that pink coloration occurs in yolk and white.

- **Red rot:**

It is caused by *Serrotia marcesceus.* These eggs are distinguished by a rod dissociation of egg white and the surface of the yolk in ammonical i.e. putrified odor.

- **Custard rot:**

  In this rot, yolk is incrusted with custard like material and occasionally have green to olive pigment. The albumin becomes thin with orange coloration. This type of spoilage is caused by Citrobacter and *Proteus vulgaris.*

(ii) *Fungal spoilage of egg*

- **Pin spot molding:**

In this case, compact colonies of small size appear on the shell as well as on the inner surface of the shell. The color of pin spots varies with the type of mold. For example: *Cladosporium* give black spot and *Sporotrichum* give pink spot.

- **Superficial fungal spoilage:**

This occurs if eggs are stored in atmosphere of high humidity. In this case, molds grow on shell in the form of whiskers.

- **Fungal rotting:**

The mold mycelium grows through cracks and pores in the egg shell. Jellying of egg white may occur and colored spots may be produced. Hypha of mold grows through the yolk membrane and ruptures it, so that yolk mixes with the white. Molds causing spoilage of egg include *Penicillium, Sporotrichum, Mucor, Botrytis, Alternaria, Thamnidium* etc.

5.8 SEAFOOD

Fish is one of the most consumed seafood and it is a highly perishable food product. Fish and fish products are widely consumed as it is a good nutrition source due to their high protein content, unsaturated fatty acids, especially omega-3 fatty acids. The biological and chemical nature of fish leads to its deterioration after it is caught. The spoilage process (Rigor mortis) will start within 12 h. The deterioration occurs very quickly due to the metabolic activity of microorganisms, endogenous enzymatic activity (autolysis), and the chemical oxidation of lipids.

5.8.1 Composition and Microflora

Fish contain important nutritional and digestive proteins, essential amino acids, lipid-soluble vitamins, micronutrients, and highly unsaturated fatty acids. It contains water (75–85%) and has a high water activity (0.98–0.99) which makes it prone to microbial growth. There are three modes of fish spoilage: Oxidation, Enzymatic and Microbial spoilage. Fish flesh is composed of protein, fats, carbohydrates, water, and amino acid compounds such as trimethylamine oxide (TMAO), urea, taurine, creatine, free amino acids, and trace glucose, etc. The internal tissue of fish is generally considered sterile. Bacteria are present on the slime layer of the skin, gill surfaces, and the intestine.

The flora of living fish is contingent on the microbial composition of the waterways in which they reside. Bacteria from genus Acinetobacter, Pseudomonas, Alcaligenes, Moraxella, Flavobacterium, Corynebacterium, Micrococcus, Sarcina, Vibrio, Serratia and Bacillus have been found in fish slime. The majority of the bacteria on fish from northern waters are psychrophiles, whereas tropical fish carry more mesophiles. Freshwater fish carry freshwater bacteria, which include Aeromonas, Lactobacillus, Brevibacterium, Alcaligenes, and Streptococcus in addition to members of most genera found in salt water.

5.8.2 Contamination Sources

Contamination of fish with bacteria from various sources (mud, water, handlers, contact surfaces, slime etc.) increase bacterial load. Bacteria from slime, gill and intestine invade the flesh and cause spoilage. There may be as few as 100 or as many as several million microbes per square centimetre in the slime and on the skin of newly captured saltwater fish, while the digestive contents may contain between 1,000 and 100 million bacteria per millilitre. In general, greater the load of bacteria of fish the more rapid the spoilage. In ungutted fish (whole fish) decay of food in the gut may release odorous substances enabling diffusion of decomposition products into the flesh. Gutting the fish on boat spreads intestinal and surface slime bacteria to flesh. But, thorough cleaning will remove most bacteria, and adequate chilling will inhibit bacterial growth. Any damage to fish skin or mucous membrane will reduce the keeping quality of the product.

5.8.3 Spoilage of Fish

The microbial growth in fish is the main cause of fish spoilage and produces amines, biogenic amines, organic acids, alcohols, aldehydes, and ketones with unpleasant and off-flavors (Table 3). The high water activity, low acidity (pH > 6) of fish result in the fast

growth of microorganisms that leads to undesirable changes in appearance, texture, flavor, and odor, reducing its quality.

At room temperature, *Bacillus, Clostridium, Escherichia, Micrococcus, Proteus, Sarcina*, and *Serratia* may predominate. For unpreserved fish, spoilage is caused by Gram-negative, fermentative bacteria (such as *Vibrionaceae*), whereas psychrotolerant Gram-negative bacteria (such as *Pseudomonas* spp. and *Shewanella* spp.) tend to spoil chilled fish. The fish spoilage is also caused by psychrotrophic, aerobic, or facultative anaerobic Gram-negative bacteria such as *Pseudomonas, Moraxella, Acinetobacter, Shewanella putrifaciens, Vibrio, Flavobacterium, Photobacterium*, and *Aeromonas* Gram-positive bacteria such as *Staphylococcus spp., Micrococcus, Bacillus, Clostridium, Corynebacterium, Brochothric thermosphacta*, and *Streptococcus* are found in fish. Lactic acid bacteria (LAB) can predominate in fish storage under vacuum or CO2 storage. Some parasites can also be transmitted by fish, including tapeworm (*Diphyllobothrium latum*), nematodes (*Anisakis simplex* and *Capillaria philippinensis*), and trematodes (*Opisthorchis* and *Paragonimus*).

Table 3: Spoilage compounds produced in fish by different spoilage bacteria

Spoilage bacteria	**Spoilage compounds produced**
Shewanella putrefaciens	TMA, H_2S, CH_3SH, $(CH_3)2S$, Hypoxanthine, and acids
Vibrionaceae	TMA and H_2S
Pseudomonas spp., *Enterobacteriaceae*	CH_3SH, $(CH_3)2S$, ketones, esters, aldehydes, NH_3, and hypoxanthine
Photobacterium phosphoreum	TMA and hypoxanthine
Aerobic spoilers	NH_3, acetic, butyric, and propionic acids
Yeast, Anaerobic rods	Ketones, esters, aldehydes, NH_3, and acids
Lactic acid bacteria	H_2S, ketones, esters, aldehydes, NH_3, and acids

5.9 MILK AND MILK PRODUCTS

Microbial spoilage of milk is a significant problem in the dairy industry, as it can lead to significant economic losses and pose a risk to public health. The microbial spoilage of milk can be caused by various microorganisms, including bacteria, yeasts, and molds, which can thrive in the nutrient-rich environment provided by milk.

5.9.1 Composition and Microflora of Raw Milk

Milk is a complex mixture of various components, including water, fats, proteins, carbohydrates, vitamins, and minerals. The composition of milk can vary depending on various factors, including the breed of the animal, its diet, and stage of lactation. For example, milk from cows that are fed a diet rich in beta-carotene may contain higher levels of vitamin A, while milk from cows that are fed a diet low in iodine may contain lower levels of iodine. Similarly, milk produced during the early stages of lactation may have higher protein and fat content than milk produced later in lactation.

Milk is an ideal medium for microbial growth due to its high water activity, moderate pH (6.4-6.6), and abundant supply of nutrients. As a result, strict hygiene standards are required in milk production and processing, as recognized by food hygiene legislation in many countries where milk was the first food to be regulated. Although milk possesses some antimicrobial properties, they are typically present in low concentrations in cow's milk and do not significantly impact its shelf life or safety. Common psychrotrophic species found in raw milk include Gram-negative rods such as Acinetobacter, Alcaligenes, Pseudomonas, Flavobacterium and Gram-positive such as Bacillus spp.as well as psychrotrophic coliforms like Aerobacter spp.

5.9.2 Contamination Sources

The main sources that contribute to the presence of microbes in milk are the udder interior, the teat exterior and teat surroundings alongwith the milking process and milk handling equipment. Milk that is obtained aseptically from a healthy cow typically contains a low number of microorganisms, usually less than 10^2-10^3 cfu ml^{-1}, and milk from some quarters of the udder may even be sterile. The most commonly isolated organisms are micrococci, streptococci and *Corynebacterium bovis*. The exterior of the udder and its immediate surrounding areas can be contaminated with organisms from the cow's general environment, with contamination being less of an issue during summer months when cows graze in open pasture and more severe when cows are housed indoors in wet and humid conditions. Heavily contaminated teats have been reported to contribute up to 10^5 cfu ml^{-1} to the milk. Bedding and manure can be a source of human pathogens such as E. *coli*, Campylobacter and Salmonella while Bacillus species may be introduced from soil. The primary source of microorganisms in raw milk is milk-handling equipment such as teat cups, pipework, milk holders, and storage tanks. As the quality of the milk deteriorates, the

proportion of microflora derived from this source increases. To prevent microbial spoilage of milk, various measures can be taken, including proper hygiene and sanitation practices, adequate cooling and storage, and the use of preservatives such as antibiotics, bacteriocins, and organic acids. Pasteurization is also a critical step in ensuring the safety and quality of milk, as it can eliminate most pathogenic microorganisms and reduce the number of spoilage bacteria.

5.9.3 Spoilage of Milk

Psychrotrophic bacteria are responsible for the spoilage of milk as they can grow and multiply at low temperatures, including in refrigerated milk, and can produce enzymes that break down milk proteins and fats, leading to the production of specific off-flavors and odors. The enzymes produced by these bacteria can result in the degradation of casein, which is the primary protein in milk, and the release of free fatty acids, leading to rancidity and off-flavors in the milk.

In addition to psychrotrophic bacteria, other bacteria such as lactic acid bacteria, coliforms, and staphylococci can also cause spoilage of milk. Lactic acid bacteria are responsible for the fermentation of lactose, producing lactic acid, which can lead to the souring of milk. Coliform bacteria can produce gas and cause the milk to become slimy or stringy, while staphylococci can produce a variety of enzymes that can cause discoloration and off-flavors. Yeasts and molds can also cause spoilage of milk, especially in unpasteurized milk or milk that has been improperly stored. Yeasts can produce alcohol and gas, resulting in the souring of milk and the formation of a foamy layer on the surface. Molds can cause the milk to become slimy or develop a musty odor.

- **Souring:**

The souring of milk is evident through the sour flavor and the coagulation of milk, forming solid curds. Various bacteria, such as lactic acid bacteria, coliforms, and others, ferment the sugars present in milk and produce acid. At temperatures ranging from 10-37°C, Streptococcus lactis is most likely to cause souring, with possible growth of *Coliforms, Enterococci, Lactobacillus,* and *Micrococcus*. At higher temperatures, between 37-50°C, *Streptococcus thermophilus* and *Streptococcus faecalis* may produce around 1% acid, followed by Lactobacillus, which produces more acid. Minimal souring occurs in milk held at refrigeration temperatures.

Pasteurization of milk eliminates most of the active acid-forming bacteria, but allows for the survival of thermoduric lactic acid bacteria, such as *Enterococcus, Streptococcus thermophilus, Lactobacillus*, and others. Bacteria other than lactic acid bacteria can also produce acid, especially when conditions are unfavorable for lactic acid bacteria. For example, *coliforms* can produce acetic acid, formic acid, ethanol, CO_2, H_2, etc. Similarly, *Clostridium* can produce butyric acid.

- **Gas production (Strong fermentation of milk):**

Organisms that ferment sugar in milk produce both gas and acid. The main gas-producing bacteria include *Coliforms, Clostridium*, Heterofermentative Lactic acid bacteria, Propionic bacteria, and others. *Coliforms, Clostridium*, and *Bacillus* produce both hydrogen (H_2) and carbon dioxide (CO_2), while others produce only CO_2. The presence of gas in milk can be observed by foam at the top of liquid milk, gas bubbles trapped in curd, and the formation of curd. Excessive gas production can cause cracking or breakdown of curd, resulting in a phenomenon known as "stormy fermentation" of milk. *Clostridium perfringens* is primarily associated with stormy fermentation in milk.

- **Proteolysis:**

Proteolysis is promoted largely during storage at lower temperatures, which can result in the destruction of lactic acid bacteria or the distribution of already produced acid by molds and yeasts. Proteolytic organisms can cause acid proteolysis, where acid production and proteolysis occur simultaneously. Examples of bacteria that cause acid proteolysis include *Micrococcus, Streptococcus faecalis* var *liquefaciens*, and some lactose-fermenting proteolytic *Bacillus* species. Another defect in milk is known as sweet curdling, which is caused by microorganisms that produce a renin-like enzyme without producing acidity. Bacillus cereus is known to cause sweet curdling in milk.

- **Ropiness/ sliminess:**

Ropiness in milk can occur due to both bacterial and non-bacterial causes. Non-bacterial ropiness can be caused by the thickness of cream or by the presence of a film of casein or lactalbumin during cooling. Bacterial ropiness, on the other hand, is caused by the slimy capsular material produced by certain bacteria, which usually develops at low storage temperatures. Examples of bacteria known to produce ropiness in milk include *Alcaligenes*

viscolactis, Micrococcus freudenreichii, Enterobacter aerogenes, Klebsiella oxytoca and *Escherichia coli (E. coli).*

- **Change in milk fat:**

Different types of bacteria, yeast, and mold have the ability to break down fat in milk, leading to rancidity. Examples of lipolytic species include *Proteus, Pseudomonas fragi, Staphylococcus, Bacillus, Micrococcus, Clostridium,* and others. *Pseudomonas fragi* and *Staphylococcus aureus* are known to produce lipase enzymes that are resistant to heat.

- **Alkali production:**

Production of alkali in milk occurs due to growth of *Pseudomonas fluorescence* and *Alcaligens viscolactis*. Alkali is produced due to development of ammonia from urea and carbonate from organic acids.

- **Flavor defects:**
  - **Acid flavor:** Production of acidic flavor may be classified as aromatic or sharp. Sharp flavor is developed due to undesirable production of acetic acid, formic acid, butyric acid etc. by growth of *Coliform* and *Clostridium.* Aromatic flavor in milk is desirable, and is caused by *Streptococcus lactis* and *Leuconostoc* growing together.
  - **Caramel or burnt flavor:** It is produced by growth of *Streptococcus lactic var. maltigens.*
  - **Bitter flavor**: Bitterness is caused in milk due to growth of proteolytic organism.
  - **Other flavor**: A particular earthy flavor is caused by *Actinomycetes,* fruity flavor growth of *Pseudomonas fragi,* soapiness caused by *Pseudomonas sapolactic* etc.
- **Color defects:**

  Pigmented bacteria growth produces undesirable color in milk, for example:
  - **Blue milk**: Blue coloured discoloyration appears in milk due to growth of *Pseudomonas syncyaneum*
  - **Red milk**: Two important strains responsible for this defect are *Serratia marcescencs* and *Micrococcus roseus.*
  - **Yellow milk**: Growth of *Pseudomonas synxantha* and flavobacterium causes this defect.
  - **Brown milk**: It is caused bygrowth of *Pseudomonas putrefaciens* and also by enzymatic oxidation of tyrosin by *Pseudomonas fluorescence.*

5.9.4 Spoilage of Milk Products:

(i) Spoilage of Butter

- **Color defect:**

There are various non-microbial causes of color defects in butter. These include pink coloration, which can be caused by sulphur dioxide refrigerant, and surface darkening, which can occur due to water evaporation from the surface. On the other hand, microbial discoloration of butter depends on the type of microorganism involved. For instance, *Stemphylium* can cause black spots, *Penicillium* can cause green spots, *Alternaria* or *Phoma* can cause brown spots, and *Pseudomonas nigrificans* can cause reddish-brown spots.

- **Flavor defect:**

Cream and butter have a tendency to absorb moisture from surrounding environment. Some flavors may be developed in butter during microbial growth also, as given below:

- Fishiness – fish like flavour caused by growth of *Aeromonas hydrophila.*
- Ester – fruity flavour is caused by *Pseudomonas fragi.*
- Rancid odor – may be caused by lipase producing microorganism.
- Yeasty flavor - caused by growth of yeast.

(ii) Spoilage of Cheese

- **Spoilage during manufacturing:**

In the manufacturing process of most cheeses, a lactic starter culture is typically added to facilitate lactic acid fermentation. However, if these starter cultures are not effective or if there is heavy contamination, it can result in the growth of undesirable organisms that can bring about negative changes in the cheese. For example, when the starter culture is not effective, *Clostridium* and *Bacillus* may grow and cause the formation of holes and other changes in the cheese. Acid proteolytic bacteria can also produce a bitter flavor, while *Leuconostoc* may create holes in the cheese. Additionally, various organisms can cause proteolysis, gas production, sliminess, and off-flavors, all of which can damage the quality of the cheese. If the cheese has a low acidity due to failure of the starter culture or addition of cream, it can become slimy due to the presence of *Alcaligenes*, *Melalcaligenes*, and *Pseudomonas fragi*.

- **Spoilage during ripening:**

Through the ripening process of cheese, spoilage can occur due to enzymes released from autolyzed bacteria or the growth of microorganisms. For instance, gas production by *Clostridium*, heterofermentative lactis, *Propionibacterium*, yeast, and others can cause the formation of eyes or cracking in cheese. *Clostridium* can also produce undesirable flavors, such as butyric acid. Certain lactic streptococci may contribute to a bitter flavor, while other bacteria and yeast can produce sweet, fruity, or yeasty flavors. In cheeses with insufficient acidity, putrefaction can be caused by anaerobic *Clostridium*. Microorganisms can also cause discoloration on the surface of cheese, such as blue-green or black discoloration resulting from the reaction of H_2S produced by microorganisms with metal or metallic salts. Oxidation of tyrosine by bacteria can give rise to reddish-brown or greyish-brown coloration. *Propionibacterium* can grow as yellow, pink, or brown-colored complexes.

Molds can also contribute to spoilage in cheese. For example, *Cladosporium* can grow on the surface and cause black discoloration. Oospora (*Geotrichum*), also known as dairy mold, can grow on the surface of soft cheese, leading to gradual liquefaction of the curd underneath. *Oospora crustacea* can cause red spots. *Penicillium puberulum* and other green-spored species can grow on the surface or into holes in the cheese, resulting in green coloration. *Monilia nigra* can grow on the surface of hard cheese, producing black discs.

5.10 SUM UP

The unit helped you to understand the overview of microbial spoilage of different food groups. In this unit we gave an understanding about concept of food spoilage and discussed the factors affecting food spoilage. The unit covered the composition, microflora, contamination sources and spoilage occurring in plant foods category like cereals, cereal products, fruits and vegetables. Microbial spoilage and contamination sources of animal foods and seafoods were also discussed.

5.11 CHECK YOUR PROGRESS

1. Define the term food spoilage.

.....

.....

2. Explain the factors affecting food spoilage.

.....

.....

.....

3. Give the classification of foods on basis of their shelf life.

.....

.....

.....

4. Write a short note on spoilage of cereals and their causative micro organism.

.....

.....

.....

5. Why are fruits, vegetables and milk highly perishable?

.....

.....

.....

6. Explain spoilage of meat under aerobic conditions.

.....

.....

.....

7. What are the contamination sources of eggs?

.....

.....

.....

8. What are the compounds produced in fish during spoilage?

.....

.....

.....

9. Explain spoilage of milk in detail.

.....

.....

.....

.....

.....

10. Write short notes on:

a) Spoilage of butter

.....

.....

.....

b) Spoilage of cheese

.....

.....

.....

UNIT-VI FOOD PRESERVATION

Structure

6.1 INTRODUCTION

This unit will introduce you to food preservation, its importance and methods employed. The unit gives you an understanding about different physical methods used for food preservation like drying, freeze drying, irradiation, heat treatment etc. The unit will also cover chemical and natural preservatives used in food products. Further, biological preservation methods will also be explained in this unit.

Objectives

After studying this unit, you will be able to:

- define food preservation and its principles
- understand the mechanism and application of food preservation methods
- comprehend the comparative advantages and efficiency of these techniques
- explain the physical food preservation methods
- discuss the chemical and natural food preservatives used in food industry.
- describe biological preservation methods
- discuss the emerging trends in food processing and preservation

6.2 FOOD PRESERVATION

Food spoilage can occur due to various factors, such as contamination by microorganisms, infestation by insects, degradation by endogenous enzymes present naturally in the food, and physical or chemical changes like tearing of plant or animal tissues or oxidation of food constituents. These factors can render food unsuitable for human consumption. Therefore, food preservation methods are required to prevent or slow down food spoilage and extend food storage. Food preservation involves inhibiting the growth of bacteria, fungi (such as yeasts), and other microorganisms, as well as slowing down the oxidation of fats that can cause rancidity. It may also include processes that prevent visual deterioration, such as enzymatic browning in apples after cutting during food preparation. Many food preservation methods involve a combination of different techniques.

It is well known that chemicals, microbes from the suuroundings and enzymes present in the food itself may cause spoilage to food products. Besides, food and food products have to be transported from one place to another place for distribution and marketing. During transit, there are chances to deteriorate the food, loss or decrease in morphological attraction

and reduction in nutritional value of the food. Therefore, it is important to do efforts for food preservation for longer shelf life, stability in quality, maintaining the morphological attraction and no change in taste. The preservation and processing of food is not as simple or straightforward as it was in the past. A number of new preservation techniques are being developed to satisfy current demands of economic preservation and consumer satisfaction in nutritional and sensory aspects, convenience, safety, absence of chemical preservatives, price, and environmental safety. Understanding the effects of each preservation method on food has therefore become critical in all aspects.

Principle of Food Preservation

1. To reduce microbial contamination.
2. To eliminate contaminating pathogens.
3. To prevent microbial growth.
4. To diminish food spoilage and food poisoning.

6.3 PHYSICAL METHODS

6.3.1 Drying

Drying is the process of preserving food by removing water from it. Drying of foods is done by various methods like sun drying, air drying, heat drying, wind drying, or drying near an open fire. Removing water prevents decay and the growth of microorganisms. This method of food preservation has been known since ancient times. Principle of drying is based on water activity, lesser the water activity more will be the life of the food product. Dehydration reduces the water content of the food products leading to reduction in water activity, hence more food product shelf life. Molds, yeast and bacteria need water to grow. When foods are sufficiently dehydrated (dried) microorganism cannot grow and foods will not spoil. Dried fruits and fruit leathers may be used as snack foods; dried vegetables may be added to soups, stews or casseroles.

Drying or dehydration is one of the oldest methods of preserving food. Primitive societies practised the drying of meat and fish in the sun long before recorded history. Today the drying of foods is still important as a method of preservation. Dried foods can be stored for long periods without deterioration occurring. The principal reasons for this are that the

microorganisms which cause food spoilage and decay are unable to grow and multiply in the absence of sufficient water and many of the enzymes which promote undesired changes in the chemical composition of the food cannot function without water. The low water content attained by drying extends the shelf life of dried foods without the need for refrigerated storage or transportation. As well, available surplus can be converted to stable forms. For example, liquid milk is highly perishable, whereas milk powder is more stable and easier to preserve and handle. Other examples of dehydrated products in this category include egg and juice powders. Usually, a significant reduction in weight and bulk volume occurs during drying, which can lead to savings in the cost of transportation and storage. The rapid reconstitution characteristics and relatively good organoleptic qualities of many modern dehydrated products make them acceptable as convenience foods.

Examples of such foods include instant coffee, tea, milk, chocolate, instant drinks, soup mixes and instant meals containing dried vegetables, breakfast cereals, and cereal products such as rice, baby foods containing dried cereals, pasta, dried vegetables (such as potato flakes or granules), peas, beans, carrots, dried meat and fish ingredients, dried fruits for use as snacks or in desserts or baked products, and many more for use in home cooking. To provide such a comprehensive range of products, it is obvious that food dehydration constitutes a large and very significant part of manufacturing or food processing activities worldwide. Dehydration accomplishes preservation in two major ways. First, it removes the water necessary for the growth of microorganisms and for the enzymatic activity. Second, by removing the water, it increases the osmotic pressure by concentrating salts, sugars, and acids, creating a chemical environment unfavorable for the growth of many microorganisms. The microbial stability of dehydrated foods results from the interruption of vital processes essential to microbial growth or spore germination. A dehydrated product remains stable only when it is protected from the subsequent exposure to the surrounding environment (e.g. water, air, sunlight and contaminants). Hence, appropriate packaging of a dried product is an important consideration.

6.3.2 Freeze drying

Freeze drying is a food preservation process that removes moisture from food by sublimation, which is the direct conversion of ice into water vapor without going through the liquid phase. Freeze drying is also known as lyophilization and is commonly used to preserve foods such as fruits, vegetables, meats, and seafood, as well as pharmaceuticals and other biological materials. During the freeze-drying process, food is first frozen and then placed in

a vacuum chamber, where the pressure is reduced to a level that allows ice to sublimate. Heat is then applied to the food, which causes the ice to evaporate and exit the food as water vapor. The water vapor is then captured by a condenser, which converts it back into ice, and the process is repeated until most of the moisture is removed from the food.

One of the benefits of freeze drying is that it preserves the nutritional and sensory properties of the food. Because freeze drying involves low temperatures and minimal processing, it can help to maintain the color, texture, and flavor of the food. Freeze-dried foods can also have a longer shelf life than fresh or canned foods, as the removal of moisture helps to prevent spoilage and bacterial growth. Freeze-dried foods can be rehydrated quickly by adding water, which makes them a convenient and portable option for camping, hiking, or other outdoor activities. Freeze-dried foods are also used by astronauts and military personnel, as they are lightweight, easy to transport, and have a long shelf life. One of the drawbacks of freeze drying is that it can be an expensive process, as it requires specialized equipment and can take a long time to complete. Additionally, freeze drying may not be suitable for all types of food, as some foods may not rehydrate well or may lose some of their nutritional value during the process.

Thus, we can say that freeze drying is a food preservation technique that uses sublimation to remove moisture from food. It is a useful technique for preserving the nutritional and sensory properties of food, and can be a convenient and portable option for outdoor activities. However, it can be an expensive process and may not be suitable for all types of food.

6.3.3 Low temperature preservation

Temperature management is a critical tool for controlling physical and biological deteriorations in food. The application of low-temperature treatment during handling, transportation, and storage is the most straightforward and efficient method of extending shelf life without compromising quality. Low-temperature treatment involves the removal of heat energy from food, leading to a decrease in temperature or a change in the state of water to ice. To achieve effective preservation, low temperature can be combined with other preservation techniques, also known as hurdles.

Since most of the biological, biochemical, physiological, and microbial activities increase or decrease with temperature, thus control of temperature (refrigeration) remains the most widely used method today to keep food fresh. Refrigeration and freezing are low-

temperature methods utilized for food preservation. As the spoilage activities are not completely stopped, refrigeration only provides temporary shelf-life extension. On the other hand, freezing terminates most of these microbiological and physiological activities (except chemical and some enzymatic changes). The freezing process can provide a long storage life, especially when the product is frozen and stored at temperatures below-18^{o}C.

Refrigeration

Refrigeration involves storing food at temperatures above the freezing point but below 16°C down to -2°C. Commercial and household refrigerators typically operate at temperatures ranging from 0 to 7.2°C, depending on the type of food being stored. Refrigeration is commonly used to preserve perishable foods for days or weeks. While refrigeration does not necessarily kill microorganisms, it helps to inactivate enzymes that contribute to rapid food deterioration. This technique helps to maintain the sensory and quality attributes of many foods in a natural state. Lower temperatures in refrigeration inhibit microbial growth, and slow down enzymatic and chemical processes, thereby extending the shelf life of food.

Freezing

Freezing involves storing food at temperatures below the freezing point, resulting in the food being in a frozen condition. Generally, a temperature of -18°C or below is required for proper freezing. While microbiologically -18°C storage is not strictly necessary, as most pathogens do not grow below 3.3°C and food spoilage organisms do not grow below -9.5°C, the application of -18°C is sufficient to retard enzymatic deterioration and slow nonenzymatic reactions in most cases. Freezing can preserve foods for months to years, depending on the proper packaging method used. Food spoilage microorganisms can grow rapidly at temperatures above 10°C, but psychrotrophs can grow below 0°C as long as there is unfrozen water. Therefore, making water unavailable by converting it into crystal form through freezing can prevent microbial growth or destroy microbial cells to some extent.

Crystallization of water also increases the concentration of solutes, which in turn increases osmotic pressure or reduces water activity, preventing microbial growth. Additionally, low temperatures lower enzyme activity and minimize chemical reactions and microbial growth. Freezing process is of two types, viz. Slow freezing process and quick freezing process. Slow freezing is also known as sharp freezing where the food is frozen under temperatures ranging from −4^{o}C to −29^{o}C. Freezing may require three to seventy-two

hours under such conditions. The temperatures used in the quick freezing process range from −32°C to −40°C. It freezes food rapidly so that fine crystals are formed. The time taken for quick freezing is significantly lower than that of slow freezing. In quick freezing, large quantities of food can be frozen in a short period of time. The use of very low temperature for both freezing and holding frozen products adds to the cost but of desirable for many products in terms of retention of palatability and nutritive value.

Although most foods contain water that turns into ice during freezing, the initial freezing point of most foods ranges from −0.5°C to −2.2°C. Foods freeze at temperatures lower than the freezing point of pure water because the water in the foods is not pure water and, when removing heat energy from the food, the freezing point is depressed (lowered) due to the increase in solute concentration in the ice-water sections of the material. Therefore, the food will begin to freeze at temperatures lower than 0 to 0.01°C (Fig.1). This is called the freezing point depression.

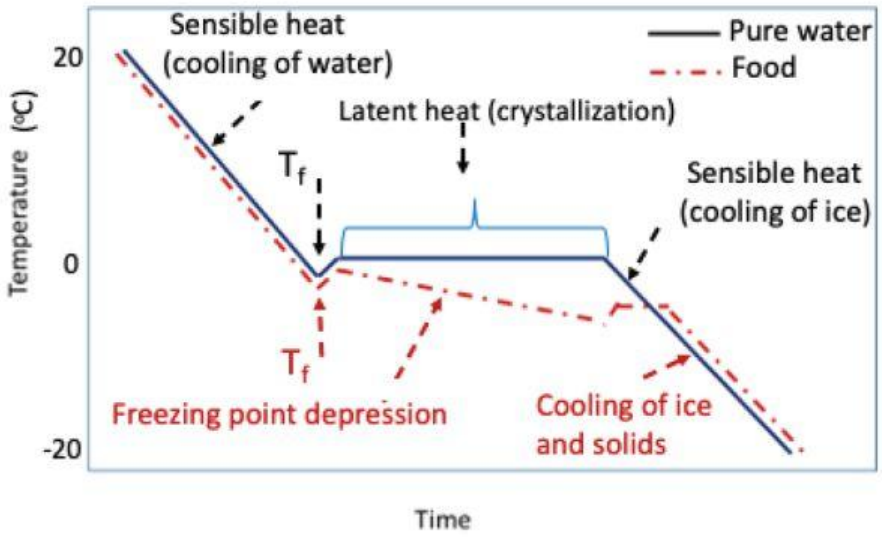

Fig 1: Freezing curve depicting freezing point depression

6.3.4 Heat Treatment

Thermal processes are primarily designed to eliminate or reduce the number of microorganisms of public health significance to an acceptable level (commercial sterility) and provide conditions that limit the growth of pathogenic and spoilage microorganisms. Pasteurization treatments rely on storage of processed foods under refrigerated conditions for a specified maximum period whereas; sterilization processes are intended to produce shelf-stable products having a long storage life. Destruction of *C. botulinum* is the main criterion,

from a public health point of view, in the sterilization of low acid foods (pH>4.5), whereas other spoilage type microorganisms are employed for acid foods. For thermal processing of acid foods and pasteurization of dairy products, inactivation of heat-resistant enzymes (pectinesterase, phosphatase, and peroxidase) is often used as basis. In conventional thermal processes, most enzymes are inactivated either because the processes are so designed using them as indicators, or their heat resistance is lower than other indicator microorganisms.

Blanching perhaps represents the least severe heat of the above processes; however, nutrient loss during blanching can occur due to reasons other than heat, such as leaching. Steam and hot water blanching are the two most commonly used blanching techniques. These conventional processes are simple and inexpensive but are also energy intensive, resulting in considerable leaching of soluble components (which occur both during heating and cooling), and produce large quantities of effluent. Whereas texture degradation is characteristic of most heat treatments, low-temperature blanching has been shown to improve the texture of some products (carrots, beans, potatoes, tomatoes, cauliflower) due to activation of the pectin methyl esterase enzyme.

Pasteurization

Pasteurization is a heat treatment applied to foods, which is less drastic than sterilization, but which is sufficient to inactivate particularly pathogenic organisms of importance in a specific foodstuff. Pasteurization inactivates most viable vegetative forms of microorganisms but not heat-resistant spores. We have learnt that the nutritional and sensory characteristics of most foods are only slightly affected by the pasteurization process because of its mild heat treatment. However, because it is only a temporary method of shelf-life extension, the product quality continues to change (deteriorate) during storage. Pasteurization is a thermal process that involves heating food or beverages to a specific temperature and holding it at that temperature for a set amount of time. The exact temperature and time depend on the type of food or beverage being pasteurized and the specific microorganisms that need to be eliminated.

The most common method of pasteurization is called "high-temperature short-time" (HTST) pasteurization, which involves heating the food or beverage to a temperature of 72°C (161°F) for 15 seconds, followed by rapid cooling. This process can eliminate most harmful bacteria while preserving the nutritional and sensory qualities of the food or beverage. Another method of pasteurization is called "ultra-high-temperature" (UHT) pasteurization,

which involves heating the food or beverage to a temperature of 135°C (275°F) for a few seconds, followed by aseptic packaging. This process can extend the shelf life of the food or beverage to several months without refrigeration. Both HTST and UHT pasteurization can eliminate pathogenic microorganisms such as Salmonella, Listeria, and *E. coli*, as well as spoilage organisms such as yeasts and molds. However, some heat-resistant bacteria such as *Bacillus cereus* and *Clostridium sporogenes* may survive pasteurization.

Sterilization

Sterilization processes are more severe with respect to the heat treatment given generally to achieve commercial sterility. Sterilization of food is a process of completely eliminating all viable microorganisms, including bacteria, viruses, and spores, from food. This process is typically accomplished through the application of heat, radiation, or chemicals. Obviously, these products will be subjected to a nutrient loss. The nutrients that are more sensitive to destruction by heat include vitamin A, B1, B6, B12, C, D, E, folic acid, inositol, and pantothenic acid, and amino acids such as lysine and threonine. Because of the possibility of using numerous time temperature combinations for achieving thermal sterilization, the influence of the process cannot be easily quantified. The severity of the heat treatment is determined by the pH of the food (low-acid foods require more severe heat treatment to ensure the destruction of *C. botulinum*); the composition of the food (protein, fats, and high concentrations of sucrose increase the heat resistance of microorganisms); the heating behavior of the food (conduction, convection); the nature, size, and shape of the container; as well as the nature and mode of application of the heating medium.

One of the most common methods of sterilizing food is through the use of heat, specifically through the use of a retort. A retort is a sealed container that is heated to a high temperature for a set period of time. The heat and pressure created within the retort can eliminate all microorganisms present in the food, including spores that are resistant to other forms of sterilization. The time and temperature required for sterilization depend on the type of food being sterilized and the specific microorganisms present. In general, a temperature of 121°C (250°F) is required to achieve sterilization, and the food must be held at this temperature for a minimum of 15 minutes. However, some foods may require higher temperatures or longer times to achieve complete sterilization. Another method of sterilizing food is through the use of radiation, specifically ionizing radiation such as gamma rays. This process can destroy all microorganisms present in the food, but it can also affect the nutritional quality and sensory characteristics of the food. Chemical sterilization involves the

use of chemical agents, such as hydrogen peroxide or chlorine dioxide, to eliminate microorganisms. However, these methods are generally not used in food production as they can leave chemical residues that may be harmful to consumers.

Microwave Sterilization

Microwave sterilization is a thermal process. A microwave oven works by passing non ionizing microwave radiation, usually at a frequency of 2.125 GHz (a wavelength of 12.212 cm), through the food. Microwave radiation falls between radio and infrared frequencies. Microwave heating takes place due to the polarization effect of electromagnetic radiation at frequencies between 300 MHz and 300 GHz. It delivers energy to the food package under pressure and controlled temperature to achieve inactivation of bacteria harmful for humans. Most processed foods today are heat treated to kill bacteria. Prolong exposure to high heat often diminishes product quality. Microwaves interact with polar water molecules and charged ions. The friction resulting from molecules aligning in rapidly alternating electromagnetic field generates the heat within food. Since the heat is produced directly in the food, the thermal processing time is sharply reduced. The colour, texture and other sensory attributes of foods processed by microwave sterilization are often better compared with those of conventionally retorted foods while meeting microbial safety requirements. The microwave sterilization technology uses the combination of 915 MHz microwave and conventional heating to improve heating uniformity. Microwave ovens use electromagnetic radiation to excite water molecules in food. The actual waves penetrate only about 10 inches from the source of the radiation. Within the food, the waves only penetrate 3/12 to 1 inch on all sides. As a result, the actual ovens must be limited in size. Heat is produced within the food by the friction of water molecules, which spreads to the centre of the food by conduction. Small portions are cooked rapidly in microwave ovens. As the quantity of food increases, however, the efficiency is lost.

Microwave heating has also found applications in the food industry, including tempering of frozen foods for further processing, pre-cooking of foods for institutional use and final drying of products like pasta. In those applications, microwave heating demonstrates significant advantages over conventional methods in reducing process time and improving food quality. The shelf life of a product is determined by its microbiological safety and sensory attributes. In general, microwave sterilization can achieve the same reduction of bacterial population as conventional retorting. Products intended for microwave sterilization are usually packaged in plastic trays or pouches. The ability of plastics to withstand oxygen

permeation will affect the organoleptic or sensory acceptance of the product during storage. Normal shelf life expectancy of microwave-sterilized products pre-packaged in plastic containers or pouches is 2-3 years or longer. With innovative plastic technologies coming to the market, the new generations of plastics may increase the expected shelf life even longer.

6.3.5 Irradiation

Food irradiation involves exposing food to ionizing radiation, such as gamma rays, x-rays, or electron beams. The ionizing radiation disrupts the DNA or cellular structure of microorganisms, such as bacteria, viruses, and parasites, and kills them or prevents them from reproducing. This helps to reduce the risk of foodborne illness and extends the shelf life of the food. The process of food irradiation does not make the food radioactive or significantly change its nutritional value, flavor, or texture. The ionizing radiation passes through the food quickly and does not leave any residual radiation. However, it is important to note that the effects of food irradiation on the quality and sensory attributes of food may vary depending on the type of food, the dose of radiation, and the conditions of storage and handling.

Food irradiation is used to treat a wide variety of foods, including fruits, vegetables, meats, poultry, seafood, spices, and herbs. In the United States, the use of irradiation is approved by the U.S. Food and Drug Administration (FDA) for several purposes, including reducing the levels of harmful bacteria such as *E. coli* and Salmonella in meat and poultry, and controlling insects and parasites in fruits and vegetables. While food irradiation has been shown to be safe and effective, some concerns have been raised about its use. One concern is that food irradiation may create harmful byproducts, such as free radicals, that could cause cellular damage or cancer. However, studies have shown that the levels of free radicals produced by food irradiation are lower than those produced by many common cooking methods, such as grilling or frying.

Another concern is that the use of food irradiation may lead to the use of lower quality or contaminated foods, since the radiation may be used to mask poor sanitation or processing practices. However, food irradiation is not a substitute for good food handling and sanitation practices, and irradiated food must still meet all applicable food safety standards and regulations. Irradiation process includes radappertization, radicidation and raurization. Radappertization is equivalent to radiation sterilization or "commercial sterility," as it is understood in the canning industry. Typical levels of irradiation are 3(MK) kGy. Radicidation

refers to the reduction of the number of viable specific nonspore forming pathogens, other than viruses, so that none is detectable by any standard method. Typical levels to achieve this process are 2.5-10 kGy. Radurization may be considered equivalent to pasteurization. It refers to the enhancement of the keeping quality of a food by causing substantial reduction in the numbers of viable specific spoilage microbes by radiation. Common dose levels are 0.75-2.5 kGy for fresh meats, poultry, seafood, fruits, vegetables, and cereal grains. Food irradiation is a safe and effective technology that can help to reduce the risk of foodborne illness and extend the shelf life of food. However, it is important to understand its potential benefits and limitations, and to use it in conjunction with good food handling and sanitation practices.

6.3.6 High Pressure Processing

High pressure processing (HPP) is a food processing technology that uses high pressure to inactivate or kill bacteria, viruses, and other microorganisms in food. HPP is a non-thermal pasteurization technique, meaning that it does not use heat to kill microorganisms, which can sometimes damage the texture, flavor, and nutritional quality of food. During HPP, food is placed in a chamber and subjected to pressures ranging from 100 to 800 megapascals (MPa), which is equivalent to 10,000 to 80,000 times atmospheric pressure. The high pressure can effectively destroy or inactivate microorganisms that cause foodborne illness, such as *Listeria*, *Salmonella*, and *E. coli*, while preserving the nutritional and sensory properties of the food.

HPP is used to process a variety of foods, including meats, poultry, seafood, fruits, vegetables, and juices. The process can extend the shelf life of foods by reducing the number of spoilage microorganisms, which can help to reduce food waste and improve food safety. HPP can also help to maintain the fresh flavor, color, and texture of foods, which can be beneficial for foods that are sensitive to heat or other processing methods. One of the advantages of HPP is that it can be used to process foods without the use of preservatives, chemicals, or heat, which can be important for consumers who are looking for minimally processed or natural foods. However, HPP does have some limitations, including its high cost, the need for specialized equipment, and the fact that it may not be effective against all types of microorganisms or pathogens. HPP is a safe and effective food processing technology that can help to improve the safety and quality of food products. Its use is regulated by government agencies, such as the U.S. Food and Drug Administration, to ensure that it is used in a safe and effective manner.

6.3.7 Infra red radiation processing

Infrared food processing involves using infrared radiation to cook or heat food. Infrared radiation is a form of electromagnetic radiation that has a longer wavelength than visible light but shorter than radio waves. This type of radiation can be absorbed by the surface of food, and the energy is converted into heat, which cooks or heats the food. Infrared food processing can be used for a variety of applications, such as cooking, baking, roasting, drying, and thawing. It is a fast and efficient method of food processing, as the heat is generated directly in the food, rather than being transferred through a medium such as air or water. One of the advantages of infrared food processing is that it can cook or heat food quickly, which can save time and energy. It can also provide more precise control over the cooking process, as the heat can be directed to specific areas of the food.

However, there are also some limitations to infrared food processing. For example, it may not be suitable for cooking certain types of food, such as those that are very thick or have a high moisture content. It can also lead to uneven cooking if the food is not positioned correctly in the infrared radiation field.

6.3.8 Ultrasonics

Ultrasonics is the use of high-frequency sound waves to process materials. Ultrasonics in food processing can be used for a variety of applications, such as cleaning, homogenization, emulsification, and preservation. One of the primary applications of ultrasonics in food processing is the use of high-frequency sound waves to break down cell walls and disrupt microorganisms, which can help to extend the shelf life of food. This is known as ultrasonic preservation or sonication, and it can be used to preserve a variety of food products, including fruits, vegetables, meats, and dairy products.

Ultrasonics can also be used to emulsify liquids, such as oils and water, which can be useful in the production of sauces, dressings, and other food products. In addition, ultrasonics can be used to mix and homogenize food products, which can help to ensure consistency in texture and flavor. Another application of ultrasonics in food processing is the cleaning of food processing equipment. Ultrasonic cleaning can be an effective method of removing stubborn deposits and contaminants from equipment, such as molds, utensils, and surfaces.

Overall, ultrasonics is a versatile technology that can be used in a variety of food processing applications. While there are some limitations to its use, such as the potential for damage to

sensitive food products, it can provide an efficient and effective method of processing and preserving food.

6.3.9 Pulsed electric field

PEF (pulsed electric field) is a food processing technology that uses short bursts of high-voltage electric fields to inactivate microorganisms, enzymes, and other unwanted substances in food products. PEF has a wide range of applications in the food industry, including pasteurization, sterilization, and extraction. During PEF processing, food products are placed between two electrodes and subjected to a series of high-voltage electrical pulses. These pulses create tiny holes in the cell membranes of microorganisms, causing them to rupture and die. The electrical pulses can also break down enzymes and other unwanted substances in the food product.

One of the primary advantages of PEF processing is that it can be used to preserve the nutritional quality, flavor, and texture of food products, while still achieving a high level of microbial inactivation. This is because PEF processing uses a relatively low temperature and a short processing time, which minimizes damage to the food product. PEF processing can be used for a variety of food products, including juices, dairy products, sauces, and soups. It can also be used for the extraction of valuable compounds from food products, such as pigments, flavors, and antioxidants. Overall, PEF is a promising food processing technology that offers a range of benefits, including improved food safety, extended shelf life, and enhanced nutritional quality. While there are still some challenges to its widespread adoption, such as high capital costs and limited knowledge of its effects on certain food products, PEF has the potential to revolutionize the food industry in the years to come.

6.3.10 Ohmic heating

It is a food processing technology that uses electrical current to heat food products directly. Unlike traditional heating methods, such as convection or conduction, ohmic heating heats the entire volume of the food product at once, resulting in faster and more uniform heating. Ohmic heating works by passing an electrical current through a food product, which heats the product through resistance. The electrical current is applied using two electrodes, which are placed in contact with the food product. The resistance to the electrical current generates heat, which heats the food product.

Primary advantage of ohmic heating is that it can heat food products quickly and evenly, which can help to preserve the nutritional quality, flavor, and texture of the product. Ohmic heating can also be used for a wide range of food products, including liquids, semi-

solids, and particulate foods. Ohmic heating can be used for a variety of food processing applications, such as pasteurization, sterilization, and cooking. It can also be used for other purposes, such as thawing frozen foods and reducing the viscosity of food products.

Ohmic heating is a promising food processing technology that offers a range of benefits, including improved food quality, increased efficiency, and reduced energy consumption. While there are still some challenges to its widespread adoption, such as high capital costs and limited knowledge of its effects on certain food products, ohmic heating has the potential to revolutionize the food industry in the years to come.

6.4 CHEMICAL METHODS

6.4.1 Chemical Preservatives

Preservatives are substances that have the capability to inhibit, retard, or arrest the growth of microorganisms or prevent deterioration caused by their presence, or mask the evidence of such deterioration. They do not include substances that act by inhibiting chemical reactions that can limit shelf-life, such as antioxidants used to control rancidity or oxidative discoloration. Additionally, certain food additives, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and phosphates used as acidity regulators and emulsifiers, may contribute to antimicrobial activity but are not considered primary preservatives. Preservatives can be microbicidal, killing the target organisms, or microbistatic, preventing their growth. The effectiveness of preservatives is often dose-dependent, with higher concentrations having lethal effects on microorganisms, while the lower concentrations permitted in foods tend to be microbistatic. As a result, chemical preservatives are useful for controlling low levels of contamination and should not be considered a substitute for good hygiene practices.

Common preservatives are benzoic acid and benzoates which are used in acidic foods such as jams, salad dressing, juices, pickles, carbonated drinks, soy sauce among others. Sorbic acid and sorbates are used as preservatives in cheese, wine and baked foods among others. Sulphur dioxide and sulphites are used in fruits and wine. Similarly, propionic acid and propionates are used in baked foods. Nitrides and nitrates are used in many foods as preservatives and functional ingredients. These are critical components used to cure meat, and they are known to be multifunctional food additives and potent antioxidants

6.4.2 Natural Preservatives

Since long time, chemical preservatives such as sorbate and benzoate have been used as reliable preservative factors to control a number of microbial hazards. However, such compounds do not satisfy the concept of “natural” and “healthy” food that consumers prefer and that the food industry, consequently, needs to manufacture. The negative reaction to chemical preservatives in our society is strongly increasing, despite the fact that such compounds are as yet indispensable in food processing. As a result, replacement of chemicals by more natural alternatives can only be relevant.

Many plants contain compounds that have some antimicrobial activity, collectively referred to as “green chemicals” or “biopreservatives”. Interest in naturally occurring antimicrobial systems has expanded in recent years in response to consumers requirements for fresher, more natural additive free foods. A range of herbs and spices are known to possess antibacterial activity as a consequence of their chemical composition. Antimicrobial agents can occur in foods of both animal and vegetable origin. Herbs and spices have been used for centuries by many cultures to improve the flavor and aroma of foods. Essential oils show antimicrobial properties, and are defined as a group of odorous compounds, soluble in alcohol and to a limited extent in water, consisting of a mixture of esters, aldehydes, ketones, and terpenes. They not only provide flavor to the product, but also preservation activity. Scientific studies have identified the active antimicrobial agents of many herbs and spices. These include eugenol, allicin, cinnamic aldehyde, allyl isothiocyanate, isothymol and thymol etc. Citric, succinic, malic, and tartaric acids are commonly found in fruits (e.g., citrus, rhubarb, grapes, and pineapples) and vegetables (e.g., broccoli and carrots). Through their use as acidulants or antioxidants in foods, their antimicrobial properties provide additional benefit. The majority of antimicrobial plant compounds are identified as secondary metabolites, mainly being of terpenoid or phenolic biosynthetic origin. In general, herbs and spices and several of their antimicrobial constituents are GRAS, either because of their traditional use without any documented detrimental impact or because of dedicated toxicological studies.

6.5 BIOLOGICALLY BASED PRESERVATION METHODS

With respect to the natural antimicrobial activity derived from microorganisms (referred to as biopreservatives), the most promising ongoing development in food

preservation is the use of Lactic acid bacteria (LAB). LAB's are GRAS organisms, and have a long and safe tradition in food fermentation practices. The use of these organisms or the antimicrobial compounds they produce has been successfully achieved in many different types of foods. Most prominently, bacteriocins produced by LAB have been under investigation worldwide for food preservation purposes. Bacteriocins are proteins with a rather narrow antimicrobial spectrum, as compared to traditional preservatives. The many different antimicrobials they produce are able to counteract a wide range of competitors that would cause problems in the fermentation process. In recent years, some research has been developed to use LAB in food processing applications where the outgrowth of specific problem microorganisms is to be controlled. In this case, the selected LAB are referred to as protective cultures and should affect pathogens or spoilage microorganisms without any negative impact on the sensory or organoleptic characteristics of the food product.

Nisin is a protein consisting of amino acids, which is stable on autoclaving and effectively inhibits growth of important Gram-positive foodborne pathogens like *L. monocytogenes* and *S. aureus*, and prevents outgrowth of spores of many species of Clostridium and Bacillus. It is especially active in acidic food matrixes. The bacteriocin is produced by some strains of *Lactococcus lactis* subsp. lactis. Consumers are taking a greater interest in the quality of foods and are creating a demand for chemical free, "natural health" foods. This has stimulated extensive research into the applications of LAB for both the control of pathogenic and spoilage microorganisms and also for health promotion. A range of potential health benefits has been associated with the consumption of LAB. Some benefits are as a consequence of their growth and activity during food fermentations, and some from the resultant colonization of the gastrointestinal tract.

Fermented foods can be a dietary source of the live organisms known as probiotics. As per the definition provided by the World Health Organization, probiotics are live microorganisms that, on ingestion in sufficient amounts, provide a health benefit to the host. Probiotics can benefit the health of human hosts as well as the health of animals. Probiotic cultured dairy products include yogurts with live *Lactobacillus* and *Bifidobacterium* bacteria. These bacteria are considered exclusively beneficial, with no harmful effects. Microbes that inhabit our bodies are active partners in gut health, neurological health, and also play roles in bowel disease, obesity, and asthma. Prebiotics are the nutrients necessary for probiotic microbial growth, such as carbohydrates, vitamins, and proteins (the substrates). In this view of probiotics and health, it is not only what you eat that promotes health, but what your gut

bacteria eat, which keeps your immune system and gut healthy. Prebiotics are often non-digestible to the human or animal host, but are good sources of food (substrates) to the microorganism. Fermented foods that contain prebiotics include vegetables, cheese and dairy products, soy and miso fermented products, and fermented cereals that contain glucans, oligosaccharides and polyphenolic compounds.

6.6 SUM UP

This unit explained the concept of food preservation, its principles and methods employed. The unit gave you a detailed understanding about different physical methods used for food preservation like drying, freeze drying, irradiation, heat treatment etc. The unit also covered chemical and natural preservatives used in food products. Further, biological preservation methods have also been explained in this unit.

6.7 CHECK YOUR PROGRESS

1. Explain the term 'food preservation'.

.....

.....

.....

2. Define freeze drying.

.....

.....

3. How does low temperature cause preservation of foods?

.....

.....

.....

4. Differentiate between pasteurization and sterilization.

.....

.....

.....

5. Explain food irradiation.

.....

.....

.....

6. Write a short note on use of chemical preservatives in food.

.....

.....

.....

7. Why natural preservatives are preferred over chemical preservatives?

.....

.....

.....

8. Explain Pulsed electric field processing.

.....

.....

.....

9. Write a short note on High pressure processing.

.....

.....

.....

Unit-VII FOOD BORNE DISEASES

Structure

7.1 INTRODUCTION

This unit will introduce you to various food borne diseases. It will give you a detailed overview of different bacteria, viruses, parasites and fungi responsible for food borne diseases. The unit gives you an understanding about concept of food poisoning and helps you to distinguish between food infection and food intoxication. The unit will also explain the about the organism involved, symptoms, contamination sources and foods related to various food illness. The unit will also cover the different types of mycotoxins produced by fungi growth in foods. The methods of prevention of food illness are also explained in detail.

Objectives

Learning this unit will make you:

- understand the concept of food poisoning
- differentiate between food infection and food intoxication
- discuss the different bacteria, viruses and parasites-based food borne illness.
- explain the symptoms and contamination sources of various food illness
- describe the different types of mycotoxins produced by fungi in foods
- discuss the methods of prevention of food illness

7.2 FOOD BORNE DISEASES

Foodborne diseases are illnesses caused by consuming contaminated food or beverages. These diseases can be caused by a variety of microorganisms, including bacteria, viruses, parasites, and toxins produced by microorganisms (Table 1). These pathogens cause illness by infecting the body of a host. Infectious pathogens damage tissues and cells during growth in the host and sometimes release toxins that cause illness. Contaminated food can cause a range of symptoms, from mild to severe, and can affect individuals of all ages. Symptoms can include nausea, vomiting, diarrhea, abdominal pain, fever, and dehydration. In some cases, foodborne illnesses can lead to more serious health problems, such as kidney failure, meningitis, and even death.

Contamination of food can occur at various stages of the food chain, including during production, processing, transportation, storage, and preparation. Certain types of food are more prone to contamination than others, including raw or undercooked meat, poultry, fish,

and shellfish, as well as raw fruits and vegetables that have not been properly washed. Preventing foodborne illnesses involves a combination of proper food handling and preparation, including washing hands and surfaces, cooking food to the proper temperature, and storing food at the appropriate temperature. Proper food safety practices can help to reduce the risk of contamination and ensure that food is safe to eat.

In addition to individual efforts, regulatory agencies play an important role in ensuring the safety of the food supply. Governments around the world have established food safety standards and regulations, and food producers and handlers are required to adhere to these standards to minimize the risk of foodborne illness. Regular monitoring and inspection of food production facilities and food products can help to identify potential sources of contamination and prevent outbreaks of foodborne illness. Food borne illness can be of two types:

- **Foodborne infection** is caused by eating food contaminated with pathogens, including bacteria, parasites, and viruses. Once ingested, these pathogens infect the body, often in the intestinal tract causing infection and disease.
- **Foodborne intoxication** is caused by ingesting food that contains pre-formed toxins produced by pathogens. Toxins may be produced by bacteria, mushrooms, moulds, or marine organisms. During intoxication, the pathogen may no longer be present in the food.

Table 1: List of Various organisms causing food borne illness

Bacteria	Parasite	Virus
E.coli O157:H7	*Cryptosporidium*	Hepatitis A virus (HAV)
Bacillus cereus	*Cyclospora*	Norovirus
Campylobacter jejuni	*Giardia*	Rotavirus
Clostridium botulinum	*Toxoplasma gondii*	
Clostridium perfringens	*Trichinella*	
Listeria monocytogenes		
Salmonella spp.		
Shigella spp.		
Vibrio spp.		
Yersinia enterocolytica		

7.3 BACTERIAL DISEASES

7.3.1 Salmonellosis

Salmonellosis is a form of food infection that may result when foods containing Salmonella bacteria are consumed. The Salmonella family includes more than 2300 serotypes, but two types, *Salmonella enteritidis* and *Salmonella typhimurium* are the most common and account for half of the infections. Once eaten, the bacteria may continue to live and grow in the intestine, set up an infection and cause illness. The possibility and severity of the illness depends largely on the size of the dose, the resistance of the host and the specific strain of Salmonella causing the illness. Salmonella bacteria are spread through indirect or direct contact with the intestinal contents or excrement of animals, including humans. For example, they may be spread to food by hands that are not washed after using the toilet. They also may be spread to raw meat during processing so that it is contaminated when brought into the kitchen. Because of this, it is important to make sure hands and working surfaces are thoroughly washed after contact with raw meat, fish and poultry and before working with foods that require no further cooking. Salmonella bacteria grow at temperatures between 41 and 113 degrees F. They are readily destroyed by cooking to 160 F and do not grow at refrigerator or freezer temperatures. They do survive refrigeration and freezing, however, and will begin to grow again once warmed to room temperature.

Symptoms of salmonellosis include headache, diarrhea, abdominal pain, nausea, chills, fever and vomiting. These occur within 8 to 72 hours after eating contaminated food and may last four to seven days. Infants, young children, pregnant women, the elderly or people already ill have the least resistance to disease effects. Foods commonly involved include eggs or any egg-based food, salads (such as tuna, chicken, or potato), poultry, beef, pork, processed meats, meat pies, fish, cream desserts and fillings, sandwich fillings, raw sprouts, and milk products. These foods may be contaminated at any point where the food is handled or processed from the time of slaughter or harvest until it is eaten.

7.3.2 Campylobacteriosis

Campylobacteriosis or Campylobacter enteritis is caused by consuming food or water contaminated with the bacteria *Campylobacter jejuni. C. jejuni* commonly is found in the intestinal tracts of healthy animals (especially chickens) and in untreated surface water. Raw and inadequately cooked foods of animal origin and non-chlorinated water are the most

common sources of human infection (e.g., raw milk, undercooked chicken, raw hamburger, raw shellfish). The organism grows best in a reduced oxygen environment, is easily killed by heat (120 F), is inhibited by acid, salt and drying, and will not multiply at temperatures below 85 F. Diarrhea, nausea, abdominal cramps, muscle pain, headache and fever are common symptoms. Onset usually occurs two to ten days after eating contaminated food. Duration is two to seven days, but can be weeks with such complications as urinary tract infections and reactive arthritis. Meningitis, recurrent colitis, acute cholecystitis, and Guillain-Barre syndrome are rare complications. Deaths, also rare, have been reported. Preventive measures for Campylobacter infections include pasteurizing milk; avoiding postpasteurization contamination; cooking raw meat, poultry and fish; and preventing cross-contamination between raw and cooked or ready-toeat foods.

7.3.3 Listeriosis

Prior to the 1980s, listeriosis, the disease caused by *Listeria monocytogenes*, was primarily of veterinary concern, where it was associated with abortions and encephalitis in sheep and cattle. As a result of its wide distribution in the environment, its ability to survive for long periods under adverse conditions, and its ability to grow at refrigeration temperatures, Listeria has since become recognized as an important foodborne pathogen. *L. monocytogenes* is frequently carried by humans and animals. The organism can grow in the pH range of 4.4 to 9.6. It is salt tolerant and relatively resistant to drying, but easily destroyed by heat. (It grows between 32 F and 113 F). Listeriosis primarily affects newborn infants, pregnant women, the elderly and those with compromised immune systems. In a healthy non-pregnant person, listeriosis may occur as a mild illness with fever, headaches, nausea and vomiting. Among pregnant women, intrauterine or cervical infections may result in spontaneous abortion or still birth. Infants born alive may develop meningitis. The mortality rate in diagnosed cases is 20 to 25 percent. The incubation period is a few days to several weeks. Recent cases have involved raw milk, soft cheeses made with raw milk, and raw or refrigerated ready-to-eat meat, poultry or fish products. Preventive measures for listeriosis include maintaining good sanitation, turning over refrigerated readyto-eat foods quickly, pasteurizing milk, avoiding post-pasteurization contamination, and cooking foods thoroughly.

7.3.4 Staphylococcal Intoxication

Staphylococcus bacteria are found on the skin and in the nose and throat of most people; people with colds and sinus infections are often carriers. Infected wounds, pimples,

boils and acne are generally rich sources. Staphylococcus is also widespread in untreated water, raw milk and sewage. When Staphylococcus bacteria get into warm food and multiply, they produce a toxin or poison that causes illness. The toxin is not detectable by taste or smell. While the bacteria itself can be killed by temperatures of 120 F, its toxin is heat resistant; therefore, it is important to stop the staphylococcus organism from growing. Foods commonly involved in staphylococcal intoxication include protein foods such as ham, processed meats, tuna, chicken, sandwich fillings, cream fillings, potato and meat salads, custards, milk products and creamed potatoes. Foods that are handled frequently during preparation are prime targets for staphylococci contamination. Symptoms include abdominal cramps, vomiting, severe diarrhea and exhaustion. These usually appear within one to eight hours after eating staphylococcus infected food and last one or two days. The illness seldom is fatal. Keep food clean to prevent its contamination, keep it either hot (above 140 F) or cold (below 40 F) during serving time, and as quickly as possible refrigerate or freeze leftovers and foods to be served later.

7.3.5 *Clostridium perfringens* Foodborne Illness

Clostridium perfringens belong to the same genus as the botulinum organism. However, the disease produced by *C. perfringens* is not as severe as botulism and few deaths have occurred. Spores are found in soil, nonpotable water, unprocessed foods and the intestinal tract of animals and humans. Meat and poultry are frequently contaminated with these spores from one or more sources during processing. Spores of some strains are so heat resistant that they survive boiling for four or more hours. Furthermore, cooking drives off oxygen, kills competitive organisms and heatshocks the spores, all of which promote germination. Once the spores have germinated, a warm, moist, protein-rich environment with little or no oxygen is necessary for growth. If such conditions exist (i.e., holding meats at warm room temperature for several hours or cooling large pots of gravy or meat too slowly in the refrigerator), sufficient numbers of vegetative cells may be produced to cause illness. Foods commonly involved in *C. perfringens* illness include cooked, cooled, or reheated meats, poultry, stews, meat pies, casseroles, and gravies. Symptoms occur within eight to 24 hours after contaminated food is eaten. They include acute abdominal pain and diarrhea, nausea, vomiting and fever.

7.3.6 *E. coli* Hemorrhagic Colitis

Escherichia coli belongs to a family of microorganisms called coliforms. Many strains of *E. coli* live peacefully in the gut, helping keep the growth of more harmful microorganisms in check. However, one strain, *E. coli* O157:H7, causes a distinctive and sometimes deadly disease. Symptoms begin with nonbloody diarrhea one to five days after eating contaminated food, and progress to bloody diarrhea, severe abdominal pain and moderate dehydration. In young children, hemolytic uremic syndrome (HUS) is a serious complication that can lead to renal failure and death. In adults, the complications sometimes lead to thrombocytopenic purpura (TPP), characterized by cerebral nervous system deterioration, seizures and strokes. Ground beef is the food most associated with E. *coli* O157:H7 outbreaks, but other foods also have been implicated. These include raw milk, unpasteurized apple juice and cider, dry-cured salami, sprouts, lettuce, spinach, and untreated water. Infected food handlers with the disease likely help spread the bacteria. Preventive strategies for *E. coli* infections include thorough washing and other measures to reduce the presence of the microorganism on raw food, thorough cooking of raw animal products, and avoiding recontamination of cooked meat with raw meat.

7.4 VIRAL DISEASES

Foodborne infections caused by viruses are transmitted through the ingestion of food and water. They show high resistance to environmental factors, such as low pH (acidity) and heat. This makes them highly persistent so that they can remain infective for over a month in food and water. As they originate within the intestines of humans and animals, these viruses are predominantly spread through feces and other body fluids. The contamination of food stuff with pathogenic viruses is often caused by poor hygienic practices in the production line or contact of the food with animal waste or sewage.

Foods most commonly associated with foodborne viruses include shellfish, which are harvested near human sewage outlets, undercooked meats as well as fruit and vegetables which are grown on animal waste fertilized grounds. Gastroenteritis and hepatitis are the most commonly reported syndromes of foodborne viruses. Although many different types of gastrointestinal viruses can be found in humans, gastroenteritis caused by the human norovirus and hepatitis A virus (HAV) are predominantly reported with foodborne viruses. Other viruses including enterovirus, sapovirus, rotavirus, astrovirus, adenovirus and Hepatitis E virus have also been associated with the transmissions through food and water.

Norovirus causes gastroenteritis leading to diarrhoea, vomiting, fever, headaches and abdominal pain. The disease is self-limiting, typically 12−48 hours up to 3 days for the majority of people, with a low infection dose of 10−100 virus particles. Prolonged virus shedding of up to 8 weeks may occur in asymptomatic people and immunosuppressed individuals. Headache and low-grade fever may occur and there is anecdotal evidence that there may be other diseases caused by Norovirus including infant necrotising enterocolitis. The primary route of transmission is person-to-person transmission through the faecal−oral and vomit-oral routes and indirectly through food (ready to eat including leafy vegetables and herbs, berries and foods handled after cooking), water and environment.

Rotaviruses are a group of non-enveloped viruses in the Reoviridae family, consisting of 8 species (named A−H). Rotavirus A is endemic worldwide, causing approximately 80% of rotavirus gastroenteritis in humans, particularly through waterborne infection, with rotavirus B and C also being human pathogens. Rotavirus gastroenteritis is a self-limiting, mild to severe disease characterised by vomiting, watery diarrhoea and low-grade fever. Symptoms usually start 1−2 days after infection with vomiting followed by 3−7 days of diarrhoea. Outbreaks caused by Group B rotavirus have been reported in the elderly and adults with Group C rotavirus being associated with sporadic cases of diarrhoea in children in many countries. Rotavirus gastroenteritis is a common and highly contagious form of viral gastroenteritis that affects primarily infants and young children. The virus is transmitted through contact with contaminated surfaces, objects, or food, as well as through person-to-person contact. Symptoms of rotavirus gastroenteritis typically include fever, vomiting, and diarrhea, which can be severe and can last for several days. Infants and young children may also experience dehydration, which can be serious and even life-threatening if not promptly treated. Rotavirus is the leading cause of severe diarrhea in children worldwide and is responsible for an estimated 200,000 deaths each year, mostly in developing countries. Treatment of rotavirus gastroenteritis typically involves managing symptoms and preventing dehydration. This may include giving oral rehydration solutions, which contain a balance of salts and sugars to replace fluids lost through diarrhea and vomiting. In severe cases, hospitalization may be necessary to provide intravenous fluids and electrolytes.

Another common foodborne virus is Hepatitis A, which causes liver inflammation. Symptoms include fever, headache, nausea and vomiting, diarrhea, abdominal pain and jaundice. The virus is responsible for 50% of hepatitis cases and is often self-limiting which means the pathogen clears up or its host dies. However, Hepatitis A virus can rarely cause

liver failure (requiring a liver transplant) or death. The potential of these viruses to spread across borders is immense due to the unrestricted travel conditions in many parts of the world. Due to the lack of appropriate detection methods to quantify the viral load present and the low levels of virus present in food, the risk of spreading remains.

7.5 FOOD BORNE PARASITES

Parasites are tiny organisms that live inside another organism. Some of the important food borne parasites are discussed below:

7.5.1 *Cryptosporidium parvum*

Cryptosporidium parvum is a single-celled parasite that can infect the small intestine of humans and animals. It is spread through the consumption of contaminated food or water, or may be transmitted through contact with infected individuals or animals. Symptoms of cryptosporidiosis, the illness caused by Cryptosporidium infection, can include diarrhea, abdominal cramping, nausea, and vomiting. In healthy individuals, the illness usually resolves within a few days to a few weeks, but it can be more severe and long-lasting in people with weakened immune systems, such as those with HIV/AIDS.

Cryptosporidiosis can be diagnosed through laboratory tests that detect the parasite in stool samples. Treatment typically involves managing symptoms, such as dehydration, and allowing the body's immune system to fight off the infection. In severe cases, antiparasitic medications may be used. Preventing Cryptosporidium infection involves practicing good hygiene and avoiding contact with contaminated water and food sources. This can include washing hands thoroughly with soap and water, especially after using the restroom or changing diapers, avoiding drinking untreated water from rivers, lakes, or wells, and properly washing and cooking food. In areas where cryptosporidiosis is common, water should be boiled or treated with chlorine before consumption.

7.5.2 *Giardia intestinalis*

It is also known as *Giardia lamblia*, and is a single-celled parasite that can infect the small intestine of humans and animals. It is one of the most common parasites found in humans, and is transmitted through the intake of contaminated water or food, or through contact with infected individuals or animals. Symptoms of giardiasis, the illness caused by Giardia infection, can include diarrhea, abdominal pain, bloating, gas, and nausea. In some cases, individuals may also experience weight loss, fatigue, and dehydration.

Giardiasis can be diagnosed through laboratory tests that detect the parasite in stool samples. Treatment typically involves a course of antibiotics, which can help to alleviate symptoms and clear the infection. Preventing Giardia infection involves practicing good hygiene and avoiding contact with contaminated water and food sources. This can include washing hands thoroughly with soap and water, especially after using the restroom or changing diapers, avoiding drinking untreated water from rivers, lakes, or wells, and properly washing and cooking food. In areas where giardiasis is common, water should be boiled or treated with chlorine before consumption.

7.5.3 *Trichinella spiralis*

Trichinella spiralis is a parasitic roundworm that can infect humans and animals, such as pigs and wild game, that are commonly consumed by humans. The parasite is transmitted through the ingestion of undercooked or raw meat containing Trichinella larvae. Symptoms of trichinellosis, the illness caused by Trichinella infection, can include abdominal pain, diarrhea, fever, muscle pain, and swelling around the eyes. In severe cases, trichinellosis can lead to more serious complications, such as heart and lung problems.

Trichinellosis can be diagnosed through laboratory tests that detect the presence of antibodies to the parasite in blood samples. Treatment typically involves managing symptoms and using antiparasitic medications to eliminate the infection. Preventing Trichinella infection involves properly cooking meat to a safe temperature, which can kill Trichinella larvae. For pork, this means cooking it to an internal temperature of 145°F (63°C) and allowing it to rest for at least 3 minutes before carving or consuming. Foods that come into contact with contaminated water during growth or preparation can become contaminated with these parasites. Food handlers who are infected with these parasites can also contaminate foods if they do not thoroughly wash their hands after using the bathroom and before handling food. *Trichinella spiralis* is a type of roundworm parasite. People may be infected with this parasite by consuming raw or undercooked pork or wild game.

7.6 MYCOTOXINS

Mycotoxins have been defined as “fungal metabolites which when ingested, inhaled or absorbed through the skin, can cause disease or death in humans and domestic animals, including birds”. Only a small number of such compounds are classified as mycotoxins; i.e.,

they have been demonstrated to cause illness in humans or domestic animals. Mycotoxin production occurs only as a result of fungal growth. However, if environmental conditions, particularly of temperature and water activity (aw), are conducive to fungal growth, toxin production may occur at any period during growing, harvesting, drying, or storage of food commodities. Mycotoxins may occur in processed foods, but are much less important than when they occur in commodities such as grains or nuts. Mycotoxins are typically chemically stable once formed, and persist in food even after the destruction of the fungi that produced them. Molecular structures of mycotoxins vary widely, so their effects on human and animal health also vary widely. They may be neurotoxins, teratogens, nephrotoxins, hepatotoxins, immunosuppressive agents or carcinogens. The most important mycotoxins are aflatoxins, ochratoxin A, fumonisins, deoxynivalenol and zearalenone.

7.6.1 Aflatoxins

Aflatoxins are a group of toxic and carcinogenic compounds produced by certain species of fungi, particularly *Aspergillus flavus* and *Aspergillus parasiticus*. These fungi can grow on a variety of crops, including peanuts, corn, cottonseed, and tree nuts, particularly in warm and humid conditions. Aflatoxins are classified into several different types, with aflatoxin B1 being the most toxic and carcinogenic. Aflatoxin B1 is also the most common type of aflatoxin found in contaminated food. Consumption of foods contaminated with aflatoxins can have serious health consequences, including liver damage, immune suppression, and an increased risk of liver cancer. Aflatoxins are particularly dangerous for young children and individuals with weakened immune systems.

Aflatoxins can be controlled through a variety of methods, including good agricultural practices, proper storage conditions, and food processing techniques such as sorting and dehulling. The use of fungicides can also help to prevent fungal growth and reduce the risk of aflatoxin contamination. Food manufacturers and regulatory agencies around the world have established strict limits on the allowable levels of aflatoxins in food products. These limits vary depending on the type of food and the country or region where it is produced and consumed.

7.6.2 Ochratoxin

Ochratoxin is a toxic and carcinogenic compound produced by several species of fungi, particularly *Aspergillus ochraceus* and *Penicillium verrucosum*. These fungi can grow on a variety of crops, including cereal grains, coffee, and grapes. Like aflatoxin, ochratoxin

can be harmful to human health. Chronic exposure to ochratoxin has been linked to kidney damage and an increased risk of kidney cancer in humans. Ochratoxin has also been shown to be immunosuppressive and genotoxic, meaning that it can damage DNA and increase the risk of cancer. Ochratoxin contamination is a particular concern in certain food products, such as wine and coffee, where fungal growth is common. It can also occur in stored grains, particularly in warm and humid conditions.

Control of ochratoxin contamination is achieved through a combination of good agricultural practices, proper storage conditions, and food processing techniques. For example, reducing moisture levels during storage can help to prevent fungal growth and reduce the risk of ochratoxin contamination. In the wine industry, careful monitoring of grape quality and storage conditions can help to prevent fungal growth and reduce the risk of ochratoxin contamination in finished products. Regulatory agencies around the world have established limits on the allowable levels of ochratoxin in food products, with specific limits varying depending on the type of food and the country or region where it is produced and consumed.

7.6.3 Control of Mycotoxins

Control of mycotoxins involves a range of preventive measures aimed at reducing the growth of fungi that produce mycotoxins and minimizing the exposure of humans and animals to these toxins. Some of the key strategies for controlling mycotoxins include:

- Good agricultural practices like proper cultivation, harvesting, and storage of crops can reduce the risk of fungal growth and mycotoxin contamination. This includes techniques such as crop rotation, pest management, and the use of fungicides.
- Proper storage conditions: Storage conditions can significantly affect the growth of fungi and the production of mycotoxins. Proper storage conditions include maintaining low moisture levels, controlling temperature and humidity, and preventing insect infestations.
- Food processing techniques: Processing techniques such as sorting, cleaning, and dehulling can reduce the levels of mycotoxins in food products. For example, sorting can remove visibly contaminated grains, while dehulling can remove mycotoxin-contaminated outer layers of grains.
- Regulations and monitoring: Regulatory agencies around the world have established limits on the allowable levels of mycotoxins in food products. Regular monitoring of

crops and food products can help to ensure compliance with these regulations and identify potential sources of mycotoxin contamination.

- Education and awareness: Educating farmers, food handlers, and consumers about the risks of mycotoxin contamination and the strategies for prevention can help to minimize exposure to these toxins.

Overall, the control of mycotoxins requires a comprehensive approach that involves prevention, monitoring, and regulatory oversight. By implementing these strategies, it is possible to reduce the risk of mycotoxin contamination and protect human and animal health.

7.7 PREVENTING FOODBORNE ILLNESS

The following food handling practices have been identified as essential in preventing bacterial foodborne illness:

- Keep packages of raw meat and poultry separate from other foods, particularly foods to be eaten without further cooking. Use plastic bags or other packaging to prevent raw juices from dripping on other foods or refrigerator surfaces.
- Buy products labeled “keep refrigerated” only if they are stored in a refrigerated case. Refrigerate promptly.
- Buy dated products before the label sell-by, use-by or pull-by date has expired.
- Wash hands (gloved or not) with soap and water for 20 seconds before preparing foods and after handling raw meat or poultry, touching animals etc.
- Rinse raw produce thoroughly under running tap water before eating.
- Scrub containers and utensils used in handling uncooked foods with hot, soapy water before using with ready-to-serve foods.
- Use separate cutting boards to help prevent contamination between raw and cooked foods.
- Serve cooked products on clean plates with clean utensils and clean hands.
- Keep hot foods hot (above 140 F) and cold foods cold (below 40 F).
- Refrigerate or freeze cooked leftovers in small, covered shallow containers (2 inches deep or less) within two hours after cooking. Leave airspace around containers to help ensure rapid, even cooling.
- Use cooked leftovers within 4 days. Don’t taste leftovers to determine safety.

- If reheating leftovers, cover and reheat to appropriate temperature before serving.
- Discard outdated, unsafe or possibly unsafe leftovers in the garbage disposal or in tightlywrapped packages.

7.8 SUM UP

In this unit we studied in detail about various food borne diseases. It gave you a detailed overview of different bacteria, viruses, parasites and fungi responsible for food borne diseases. The unit made you understanding about concept of food poisoning and helped you in differentiating between food infection and food intoxication. The unit explained about the organism involved, their symptoms, contamination sources and common foods related to various food illness. The unit also covered the different types of mycotoxins produced by fungi during its growth in foods. The various methods of prevention of food illness are also explained in detail.

7.9 CHECK YOUR PROGRESS

1. Differentiate between food infection and food intoxication.

   .....

   .....

   .....

2. List some bacteria that cause food borne illness.

   .....

   .....

   .....

3. Write a short note on salmonellosis.

   .....

   .....

   .....

4. Explain food borne illness caused by virus.

.....

.....

.....

5. How can we prevent parasite infection in food?

.....

.....

.....

6. Define mycotoxins.

.....

.....

.....

7. Explain the measures involved in control of mycotoxins.

.....

.....

.....

Unit-VIII: INDICATORS OF FOOD SAFETY AND QUALITY: MICROBIOLOGICAL CRITERIA OF FOODS AND THEIR SIGNIFICANCE

Structure

8.1 INTRODUCTION

Unit 8 of this course will introduce you concept of food safety and food quality specifically in microbial terms. The unit will explain the relation of food quality with microbiological criteria. You will also understand the terms microbiological standard, specification, guidelines etc. The unit will also help you understand the difference between index and indicator microorganisms alongwith discussion of some indicator microorganisms

in food. Further, methods for control of microbiological qualityof foods will also be explained in this unit.

Objectives

After studying this unit, you will be able to:

- define food safety and food quality
- understand the relation of food quality with microbiological criteria
- comprehend the terms microbiological standard, specification, guidelines
- explain the difference between index and indicator microorganisms
- discuss some indicator microorganisms in food related to product quality
- describe control of microbiological quality of foods
- discuss the do's and dont's in a food plant to ensure the product quality

8.2 FOOD SAFETY AND FOOD QUALITY

Food plays a critical role in determining our health, nutritional status, and productivity. Therefore, it is imperative that the food we consume is free from plagiarism and safe for consumption. Unsafe food can lead to a large number of foodborne diseases. You may have seen reports in the newspapers about health problems caused by contaminated or adulterated foods. Food-borne illness is a huge world wide problem related to public health concern. Food-borne illness can not only result in mortality but can damage trade and tourism, lead to loss of earnings, unemployment and litigation and thus can impede economic growth, and therefore food safety and quality have gained worldwide significance. Ensuring the safety and quality of food is essential both at the household level and in large-scale food production, processing, and fresh food preparation and service, to prevent plagiarism and protect public health. Advancement in technology and processing, higher per capita incomes and better purchasing power as well as increased consumer demand have led to a variety of processed foods, food for health / functional foods being manufactured. Safety of such foods needs to be assessed. The quality of food stuff, raw as well as processed is of public health concern and must be addressed. In the past decade, safety challenges faced globally as well as in India have changed significantly and issues related to food quality and food safety have gained tremendous importance.

Food Safety

Food safety is an essential scientific discipline that involves proper handling, preparation, and storage of food to prevent foodborne illnesses. This requires following specific routines to avoid severe health hazards, and food safety often overlaps with food defense to protect consumers from harm. In the food industry, food safety considerations include the origins of food, such as food labeling, food hygiene, food additives, and pesticide residues, as well as policies on biotechnology, guidelines for governmental import and export inspection, and certification systems for foods. On the other hand, market-to-consumer practices typically involve ensuring that food deemed safe in the market is delivered and prepared safely for the consumer. Food can transmit diseases from person to person, and it can also serve as a breeding ground for bacteria that cause food poisoning. Developed countries usually have complex standards for food preparation, while less developed countries often struggle with the availability of safe water, which is a critical issue for food safety.

Food Quality

Food quality refers to a set of characteristics that impact a product's desirability and value to consumers. This encompasses negative factors, such as spoilage, contamination, adulteration, and food safety hazards, as well as positive factors, such as color, flavor, and texture. It is therefore a holistic concept integrating factors such as nutritional traits, sensorial properties (colour, texture, shape, appearance, taste, flavour, odour), social considerations, safety. Safety is a preliminary attribute and precursor of quality. In order to ensure that foods are safe and of good quality, across the world various governments and international bodies have laid down food standards that manufacturers/suppliers are expected to adhere to.

8.3 QUALITY AND CRITERIA

The concept of quality in food can be defined as the "degree of excellence" of a product, which refers to how well it serves its intended purpose. In the context of food microbiology, quality encompasses three major characteristics:

- Safety: A food shall not contain levels of pathogens or toxins that can cause illness on its consumption.

- Shelf-life: A food should not contain microorganisms that would cause it to spoil or become unacceptable in oraganoleptic features within an unacceptable short period of time.
- Consistency: A food should maintain consistent quality in terms of safety and shelf-life over time.

These aspects of quality in food are essential to ensure that food products are safe for consumption, have an acceptable shelf-life, and are consistent in their quality characteristics. Consumers have a low tolerance for food products that exhibit significant variations in shelf-life and safety between batches. The principles and concepts for establishing microbiological criteria were developed by the International Commission on Microbiological Specifications for Foods (ICMSF) in the mid-1980s. These concepts have been used to develop recommendations for criteria for foods in international trade or for specific criteria for pathogens such as *Listeria monocytogenes*. They have also been the basis of the Codex Alimentarius Commission (CAC) document "*Principles for the Establishment and Application of Microbiological Criteria for Foods*". While CAC recognizes only the general category "microbiological criterion," other national, transnational, and international organizations, trade associations, and other stakeholders in the food chain often describe microbiological standards, guidelines, or specifications that can be differentiated as follows:

- *Microbiological standard* is a mandatory criterion included into a law or a regulatory ordinance.
- *Microbiological guideline* is an advisory criterion issued by either authorities, industry associations, or food manufacturers. Such guidelines are indicative of what can be expected for certain microbiological parameters when a food is manufactured according to best hygienic or manufacturing practices.
- *Microbiological specification* is an element of purchasing agreements between a buyer and a supplier of a raw material or a food product. Their use may be mandatory or advisory depending on the agreements between the two parties. Microbiological criteria have traditionally been developed around significant pathogens, relevant commensals and hygiene indicators as reflected in the ICMSF cases. They are widely used today to discriminate between acceptable and unacceptable lots of food products.

8.4 SAMPLING PLANS

Traditionally and historically, microbiological criteria have been established to determine whether a lot of product was suitable for commercial distribution and consumption. Acceptability of such a product was defined as the compliance to requirements for certain microorganisms (including parasites) and/or their toxins or metabolites. Requirements for microbiological criteria in food products are typically expressed as the absence or maximum allowable numbers or concentrations of specific parameters per unit(s) of mass, volume, area, or lot. These criteria are established to ensure that food products meet certain safety and quality standards and to prevent potential health hazards associated with microbial contamination. Microbiological criteria have been and are still widely used to make a decision upon analysis of a food for defined parameters. The analytical results obtained are compared to the established requirements and serve as a basis to decide whether a lot is acceptable or needs to be rejected.

The design of meaningful microbiological criteria used for lot acceptance is therefore a key step in the decision process. It must be considered that the development of such criteria is a complex process requiring the appropriate knowledge and information, resources, and efforts. They should therefore be developed only when there are clear benefits and justified need and when they are effective and practical in serving their purpose. Microbiological criteria, if deemed necessary and justified, are established to allow assessment of one or more of the objectives listed below:

- the safety of a specific food
- the hygienic quality of a food, in particular the adherence to good hygiene and manufacturing practices
- the suitability of a food or raw material for a particular usage such as its consumption or further processing
- the acceptability of a food or ingredient manufactured under unknown conditions

The foundation for the establishment of microbiological criteria was initially laid during joint consultations between the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). These organizations worked together to develop guidelines and principles for microbiological criteria in food safety to ensure the protection of public health and the maintenance of food quality standards. Since then, the outcome of these consultations has been continuously evolving with the input of the ICMSF, national

governments, and academic researchers. These efforts resulted in the preparation of a document on the principles for the establishment of microbiological criteria issued by CAC.

The revision of this document was initiated in 2010 to take into account new developments in food safety management systems. To fulfill the purpose of the objectives, a microbiological criterion needs to be established for a specific food or a defined category of food showing similar characteristics, taking into account a number of elements including the following: the identification of recognized or potential health hazards associated with the food product, the microbiological quality of raw materials used in the food manufacturing process, the impact of processing on the microbiological status of the food, the possibility and potential consequences of microbial contamination during processing, subsequent handling, storage and use of the food product. These factors are taken into account to establish microbiological criteria that ensure the safety and quality of the food product.

A microbiological criterion is then established by translating the general principles given above into specific elements that should consist of the following:

- a description of the specific microorganism of concern and/or toxins/metabolites produced by it, where applicable, as well as the reason for the concern microbiological limits considered as appropriate to fulfill the objective the specific point(s) of the food chain at which the criterion would apply
- the number of analytical units that need to comply with the established limits
- a sampling plan defining the number of samples to be drawn for analysis
- the procedures to sample and handle the the size of the analytical unit as well as the appropriate analytical methods (qualitative or quantitative) capable of providing the adequate response with respect to the limits
- actions to be taken when the criterion or individual elements thereof are not met

8.5 INDEX AND INDICATOR MICROORGANISMS

Microorganisms need to be relevant for the specific food and its process and may encompass bacteria, viruses, yeasts, molds, algae, parasitic protozoa, and helminths as well as their toxins or metabolites. Microbiological criteria include normally relevant pathogens, hygiene indicators, and/or spoilage organisms. The current approach in the formatting of recently issued microbiological criteria is to make a clear distinction between food safety-related parameters and process hygiene indicators. The different elements constituting a

microbiological criterion define the requirements and their stringency. Over time, two different terms have been used to describe and define such microorganisms, namely, index organisms and indicator organisms, and they are well established today.

Index microorganisms have been defined as microorganisms, groups of microorganisms, or microbial metabolites whose presence in numbers exceeding a specified limit would indicate the possible presence of pathogens showing a similar behavior and ecology. The intended role and purpose of index organisms are therefore to be used as a direct predictor of the presence of a specific pathogen. Such a prediction, however, is possible only if a statistically valid correlation has been established between the organism used as index and the "associated" specific pathogen.

Indicator microorganisms, on the other hand, have been defined as microorganisms, groups of microorganisms, or microbial metabolites whose presence in numbers exceeding specific limits would indicate a failure in the adherence to GHPs. The intended role and purpose of indicator organisms are thus to serve as an indirect predictor of the presence of a pathogen. It is therefore an indication of an increased risk related to a deviation of the implemented hygiene control measures. The direct testing for the specific pathogen cannot, however, be replaced by the sole testing for indicators.

Along with hygiene indicators, microorganisms, groups of microorganisms, or metabolites are also qualified as quality indicators. These quality indicators are usually related to physicochemical or organoleptical parameters and hence quality attributes of a food product. Their presence and growth can be associated with defective control measures leading to spoilage of food products. The use of indicators has been discussed by many authors, in particular in trying to define appropriate or ideal properties to fulfill their role both in raw materials, processing environment, and processing lines and in the end product. Methods for indicator organisms need to fulfill the same requirements as the one for pathogens, i.e., they need to be reliable and validated; in addition, they should be more rapid and less expensive. Quantitative results should show a correlation between indicator concentration and level of the pathogen.

8.6 INDICATORS OF PRODUCT QUALITY

Microbial product quality or shelf-life indicators are organisms and/or their metabolic products whose presence in given foods at certain levels may be used to assess existing

quality or, better, to predict product shelf life. The indicator organisms should meet the following criteria:

1. They should be present and detectable in all foods whose quality (or lack thereof) is to be assessed.
2. Their growth and numbers should have a direct negative correlation with product quality. 3. They should be easily detected and enumerated and be clearly distinguishable from other organisms.
4. They should be enumerable in a short period of time, ideally within a working day.
5. Their growth should not be affected adversely by other components of the food microbiota.

In general, the most reliable indicators of product quality tend to be product specific. When a single organism is the cause of spoilage, its numbers can be monitored by selective culturing or by a method such as impedance with the use of an appropriate selective medium. The overall microbial quality of the products is a function of the number of organisms present, and shelf life can be increased by their control. In effect, microbial quality indicators are spoilage organisms whose increasing numbers result in loss of product quality. The products have confined biota, and decay is commonly the consequence of the development of a solitary organism. Microbial quality indicators are deterioration creatures whose expanding numbers bring about loss of product quality. Metabolic products might be utilized to evaluate and foresee microbial quality in certain products. Microbial indicators are mostly utilized to focus on examination of food safety and sanitation rather than quality. A food safety indicator should meet certain significant measures:

- Effectively and quickly perceivable.
- Effectively recognizable from other members of the food biota have a background marked by consistent relationship with the pathogen whose presence it is to indicate.
- Consistently be available when the pathogen of concern is available.
- An organism whose numbers ideally should connect with those of the pathogen of concern.
- Have development prerequisites and a development rate rising to those of the microorganism.
- Absent from food sources that are liberated from the pathogen with the exception of maybe at certain base numbers.

8.7 CONTROL OF MICROBIOLOGICAL QUALITY OF FOODS

The conventional method for managing microbiological quality at its source has typically based on a combination of a skilled workforce, thorough inspection of facilities, and supervision of operations, along with microbiological testing of not only finished products, but also ingredients, products in progress, equipment, environment, and personnel. This comprehensive approach aims to ensure that all aspects of the production process meet the required microbiological standards to maintain food safety and quality.

8.7.1 Training of Food Handlers

Food handlers should receive training on the fundamental concepts and requirements of food and personal hygiene, including specific aspects relevant to their particular food-processing operation. The level of training may vary depending on the type of operation and the job description of the employee, but at a minimum, induction training with regular updates or refresher courses should be provided. The training should aim to provide food handlers with an understanding of the basic principles of hygiene, the reasons for its importance, and practical ways to achieve it. Some key concepts that should be emphasized in such training include:

(1) Microbes are considered as the main reason of food spoilage and foodborne illness. They should have knowledge of the characteristics of the common food poisoning.

(2) Methods to prevent food poisoning by controlling microbial growth, their survival and cross contamination.

(3) Standard requirements of personal hygiene of food handlers. These are mainly to prevent food contamination through the food handler. Microbes like *S. aureus, Salmonella* can be a part of skin microflora or they may carry these microbes from the outside premises to the plant, e.g. *Listeria, B. cereus*.

(4) Basic principles of food handling and food storage like importance and correct use of refrigerators and freezers, temperature monitoring, stock rotation etc.

(5) Proper cleaning and sanitation process and the need of the rigrous cleaning.

(6) Information about the common pests found in food plant premises and methods to be adopted for their elimination and control.

(7) Brief knowledge of the requirements of current food legislation in the country.

These aspects should be exaplained and informed with supplementary material related to the specific food being handled. Some important instructions of personal hygiene for food handlers are given below:

- Wash hands regularly, especially after using the washroom, before handling food or equipment, after handling raw foods, after touching hair, after eating, smoking, coughing or blowing the nose, and after handling waste food, garbage, or chemicals.
- Keep fingernails short and clean.
- Cover any cuts, spots, or boils with a waterproof dressing.
- Keep hair clean and covered to prevent hair/dandruff from entering food.
- Always wear clean protective clothing, including footwear, in food processing areas.
- Do not smoke, chew gum, tobacco, betel nut, fingernails, or anything else.
- Do not taste food.
- Do not spit, sneeze, or cough over food.
- Do not pick nose, ears, or any other body site.
- Do not wear jewelry when handling food.
- Do not wear protective clothing outside the production areas.

8.7.2 Facilities and Process

The environment where processing of food is done, plays a crucial role in determining product quality. The premises should be adequately sized for the intended scale of operation and located in areas that are free from issues such as pest infestations, objectionable odors, smoke, or dust. The site should be easily accessible by metalled roads and have reliable supplies of power and potable water suitable for the intended purpose. Proper attention should also be given to the provision of facilities for efficient disposal of processing wastes.

Buildings must be constructed soundly and maintained in good repair to protect raw materials, equipment, personnel, and products from contamination and prevent the entry of pests. The areas surrounding the plant should be well-maintained, with regular lawn cutting and a grass-free strip of gravel or tarmac around the buildings. Well-kept grounds not only enhance aesthetics but also aid in pest control, as features such as ponds may attract birds and insects and are therefore not advisable.

It is essential that the buildings provide a comfortable and pleasant working environment that promotes good hygienic practices. They should have adequate lighting, ventilation, and size to maintain appropriate separation between processes to prevent cross-contamination. Some process areas may require features such as temperature and relative humidity control, as well as positive pressure with filtered air, for the well-being of both personnel and products. In processing areas, floors should be made of durable, impervious, non-slip, washable materials, and free from cracks or crevices that could harbor contamination. Where applicable, floors should be gently sloped towards floor drains with trapped outlets. Internal walls should be smooth, impervious, easy to clean and disinfect, and light in color. The junction between floors and walls should be covered to facilitate cleaning.

Food processing facilities should have light-colored ceilings that are easy to clean and constructed to minimize condensation, mould growth, and flaking. Any pipework, light fittings, or other services should avoid creating difficult-to-clean recesses or overhead condensation. A false ceiling may be used to separate processing areas from overhead services in particularly sensitive areas. Light fittings should be covered to prevent food contamination in the event of a bulb or fluorescent tube shattering. Windows should have sloped sills and may be covered with well-maintained fly screens in some climates. All entrances to the plant should be protected by close-fitting, self-closing doors to prevent the ingress of birds and other pests. Air curtains may also be used to protect some work areas.

Toilets and changing facilities should be clean, comfortable, well-lit, and provide secure storage for employees' belongings. Toilets should not open directly onto food-processing areas and must be provided with hand-washing facilities supplied with hot water, soap, and hand-drying facilities. Ideally, taps and soap dispensers should be of the non-hand-operated type, and single-use disposable towels or an air blower should be provided for hand-drying. Hand washing facilities should also be available elsewhere in the plant wherever the process demands. The overall layout of the plant should ensure a smooth flow-through from raw materials reception and storage to product storage and dispatch. Areas may be designated as 'high risk' or 'low risk' depending on the sensitivity of the materials being handled and the processes used. High and low-risk areas of a production process should be physically separated, should use different sets of equipment and utensils, and workers should be prevented from passing from one area to the other without changing their protective clothing and washing their hands. The same rules governing access, behavior, and the wearing of

protective clothing apply to management, visitors, and anyone requiring to visit the processing area.

8.7.3 Equipment

Equipment and its failures can be a potent source of product contamination. The design of hygienic food-processing equipment should aim to efficiently and economically perform the prescribed task while protecting the food from contamination. The basic principles of hygienic design, adapted from the Institute of Food Science and Technology (UK) publication 'Good Manufacturing Practice: A Guide to its Responsible Management', with slight modification, are as follows:

- All surfaces that come in contact with food should be inert to the food under conditions of use and must not release any substance that could migrate to or be absorbed by the food.
- All working surfaces in contact with food should be microbiologically cleanable, smooth, and non-porous to prevent particles from getting caught in small surface crevices, which could be difficult to dislodge and act as a potential source of contamination.
- All surfaces must be visible for inspection, or the equipment must be easily dismantled for inspection, or it must be demonstrated that routine cleaning procedures eliminate the possibility of contamination.
- Equipment surfaces in contact with food must be easily avialable for manual cleaning, or if clean-in-place (CIP) techniques, it should be demonstrated that the results achieved with CIP are equivalent to those obtained with manual cleaning.
- All internal surfaces should be designed in a way that the equipment is or self-draining without any dead spaces that can trap food and allow microbial growth.
- Equipment must be designed to protect the food from external contamination and should not self contaminate through leaking glands, lubricant drips or inappropriate modifications or adaptations.
- External surfaces of equipment should be designed to avoid the harboring of soils, microorganisms, or pests on equipment, floors, walls, and supports.
- Whereever appropriate, equipment should be fitted with devices that can monitor and record its performance, such as temperature/time, flow, pH, or weight.

8.7.4 Cleaning and Disinfection

In the course of its use, food processing equipment will inevitably become soiled with food residues, which can negatively impact its performance and potentially serve as a source of microbiological contamination. Therefore, proper and frequent cleaning of both premises and equipment is crucial to maintain hygiene in food processing operations. Cleaning should be considered an integral and essential part of the production process, rather than a rushed or superficial end-of-shift task.

It's important to note that visual cleanliness alone may not be sufficient, as surfaces that appear clean may still harbor viable microorganisms that can contaminate the food product. Therefore, cleaning operations in food processing serve two purposes: physical cleaning to remove visible soil from surfaces, which can protect microorganisms and serve as a source of nutrients, and microbiological cleaning, also known as sanitizing or disinfection, to reduce the numbers of adhering microorganisms to acceptable levels after physical cleaning.

A two-stage cleaning process is generally recommended for best results, although combined detergent/sanitizers may be used in cases of light soiling for simplicity. In a general cleaning/disinfecting procedure, gross debris should be removed first by brushing or scraping, possibly followed by a pre-rinse with clean, potable (drinking quality) water. This should be followed by a more thorough cleaning step that involves the application of a detergent solution. The composition of the detergent will depend on the nature of the soil to be removed, but it typically contains surfactants, which are compounds that have both polar (hydrophilic) and nonpolar (hydrophobic) portions. Surfactants help reduce the surface tension of the aqueous phase in the detergent, improving its penetrating and wetting ability, and contributing to other useful properties such as emulsification, dispersion, and suspension.

8.8 SUM UP

This unit explained the concept of food safety and food quality. The unit also explained the relation of food quality with microbiological criteria. The terms microbiological standard, specification, guidelines etc were discussed in relation to food quality. The unit also helped you to understand the difference between index and indicator microorganisms as well asdiscussed, some important indicator microorganisms in food.

Lastly, methods for control of microbiological quality of foods like training, equipment, facilities, cleaning etc. were also described.

8.9 CHECK YOUR PROGRESS

1. Define:

   (a) Food Safety

   .....

   .....

   (b) Food Quality

   .....

   .....

   (c) Microbiological guidelines

   .....

   .....

   (d) Index microorganisms

   .....

   .....

2. Write the full form of:

   (a) ICMSF

   .....

   (b) CAC

   .....

   (c) FAO

   .....

(d) WHO

.....

3. What are indicator microorganisms?

.....

.....

.....

4. Write a short note on training of food handlers to maintain microbial quality of foods.

.....

.....

.....

5. How can equipments serve as the source of food contamination?

.....

.....

.....

Unit-IX THE HACCP SYSTEM AND FOOD SAFETY USED IN CONTROLLING MICROBIOLOGICAL HAZARDS

Structure

9.1 INTRODUCTION

This unit will acquaint you with concept of food safety and its concern for public health. It will introduce you to the principles of Food Safety Management System. The unit gives you an understanding about basics of GMP, GHP, HACCP and Codex Alimentarius. The unit will also cover the need of HACCP and terminology associated with HACCP. The seven principles of HACCP are explained in detail. The implementation of HACCP, benefits and Applications of HACCP are also discussed.

Objectives

After learning this unit, you will be able to:

- explain the concept of food safety
- define food hazards
- understand the importance of food safety management systems
- explain the principles of GMP and GHP
- understand the terminology of HACCP
- describe the principles of HACCP and its implementation
- explain the benefits and applications of HACCP

9.2 CONCEPT OF FOOD SAFETY

Food safety is a broad discipline that encompasses the handling, preparation, and storage of food in a manner that prevents foodborne illnesses. It involves a set of routines and practices that must be followed to avoid potential health hazards. Food safety often intersects with food defense, which aims to prevent harm to consumers. When considering practices from industry to market, food safety considerations include the origin of food, food labeling, food hygiene, food additives, pesticide residues, biotechnology policies, and governmental import/export inspection and certification systems for foods. When considering practices from market to consumer, the primary concern is that food should be safe in the market, and there is a focus on safe delivery and preparation of food for consumers.

The quality of food stuff, raw as well as processed is of public health concern and must be addressed. In the past decade, safety challenges faced globally as well as in India

have changed significantly and issues related to food quality and food safety have gained tremendous importance. A number of factors are responsible for this:

- With swift changes in lifestyle patterns and eating habits, large numbers of people are not eating home made food. In commercial settings, foods are prepared in bulk handled by many persons, thus there are more chances of food getting contaminated. Further, food items are prepared many hours in advance, and may spoil if not stored appropriately.
- There are many processed and packaged foods in the market and safety of these foods is very important.
- Spices and condiments, oilseeds were processed at home in former times and purity of these were not a concern. In today's world, pre- packaged individual spices, condiments, spice powders and mixes are in demand, especially in cities and metros. Quality of even raw food stuff besides processed foods is of public health concern and must be addressed.
- Logistics governing transport of bulk food is complex and there is a long gap between processing and consumption. Thus, risk assessment and safety management during mass production and mass distribution is critical.
- Microbial adaptations, antibiotic resistance, altered human susceptibility and international traveling have all contributed to increasing incidence of food-borne microbial diseases. Nearly half of all known food-borne pathogens have been discovered during the past 25-30 years. There are still many food borne illnesses of unknown etiology. This is an issue of global public health concern and there is a need to detect, identify and recognise emerging pathogens and establish active surveillance networks, nationally and internationally.
- India being a signatory of non-tariff agreement of World Trade Organisation (WTO), has provided huge opportunities to world markets and access to all countries to participate in international trade. In this scenario, it has become essential for every country to protect the safety and quality of foods and also ensure that imported foods are of good quality and safe to eat. Efficient food standards and control systems are necessary to safeguard domestic food production and facilitate international trade with other countries. All food manufacturers are required to meet the given standards of quality and safety, and need to have their products regularly tested.

Owing to the above factors, there is a growing concern for safe, wholesome and nutritious foods in a highly dynamic food business environment, which in turn has greatly expanded the scope and has increased career opportunities in this sector.

9.3 FOOD HAZARDS

Food safety means assurance that food is acceptable for human consumption according to its intended use. An understanding of food safety is improved by defining two other concepts i.e. toxicity and hazard. Toxicity refers to the inherent ability of a substance to cause harm or injury under any circumstances. Hazard, on the other hand, is the relative likelihood that harm or injury will occur when a substance is not used as directed, in the prescribed amount or manner. Hazards can be physical, chemical and biological causing harmful / adverse effects on the health of consumers.

Physical hazard is any physical material not normally found in food, which causes illness or injury and includes wood, stones, parts of pests, hair etc.

Chemical hazards are substances that can be added to food, either intentionally or unintentionally, and pose a risk to health and safety. This category of hazards includes pesticides, chemical residues, toxic metals, polychlorinated biphenyls, preservatives, food colours and other additives.

Biological hazards are living organisms and include microbiological organisms. Those micro-organisms which are associated with food and cause diseases are termed food-borne pathogens. There are two types of food-borne diseases from microbial pathogens; infections and poisoning.

Among the various hazards, biological hazards pose a significant threat of food-borne illnesses. Despite all the efforts in the field of food safety, food-borne pathogens are still a critical concern with new pathogens that continue to emerge.

9.3.1 SOURCES OF HAZARDS

The emergence of pathogens is influenced by various factors, including the human host, animal hosts and their interactions with humans, the characteristics of the pathogen itself, as well as environmental factors such as food production, processing, handling, and storage practices (Fig 1).

Raw Materials

Raw materials are the primary source of contamination. Failure to follow basic quality assurance procedures (identification and labelling, Storage conditions, Handling requirements, Preparation and processing and Isolation of unsuitable raw materials) on raw materials may lead to food products that are unsafe for consumption.

Processing Steps

Uncontrolled processing operations can lead to hazardous conditions. Failure to maintain processing conditions such as temperature or time delay in processing, incorrect formulations and procedures and following unauthorized processing techniques may all result in contamination or microbial growth. Poor cleaning practices may leave excess cleaning chemical residues on plant and equipments.

Machinery

Unclean and unhygienic equipment can easily promote the growth of microorganisms. Preventive maintenance of machinery is an important aspect in a safety management program. If safety requirements are ignored, the layout of the machinery and equipment can be a potential hazard. The machinery should be examined at intervals to ensure a safe operation.

Handling of Food

Personal hygiene is extremely important in any food serving establishment. If adequate precautions are not taken, food handlers can transmit pathogenic bacteria. Personal articles such as jewellery can get mixed with foods during preparation.

Environmental Conditions

Hazards due to environmental conditions may affect raw materials, processing and machinery. Pollution of water and soil can have alarming results through the food chain. Environmental contamination may also be due to foreign matter, chemicals such as sprays and contaminants in water.

Fig. 1: Sources of Food Hazards

9.4 FOOD SAFETY MANAGEMENT SYSTEMS

Over the years, issues related to food safety and quality have gone beyond just the avoidance of food-borne pathogens, chemical toxicants and other hazards. A food hazard can can be caused in food at any stage of the food chain, thus, proper monitoring and control is required through out the food chain. Food safety and quality can be ensured through Good Manufacturing Practices (GMP), Good Handling Practices (GHP) and Hazard Analysis Critical Control Points (HACCP).

9.4.1 Good Manufacturing Practices (GMP)

Good Manufacturing Practices (GMPs) lie at the Heart of Quality. GMPs are also known as current Good Manufacturing Practices (cGMPs), are a series of manufacturing and administrative procedures aimed at ensuring that products are consistently made to meet specifications and customer expectations. In relation to food, GMP results in safe and quality food. The three elements of GMP are Food Safety, Good Practice and Quality. A preventive approach to the safety of foods is more effective than testing or inspection of processed foods at the final stage. GMP assures food safety through vigilant measures at the source product design and process control. It enables to minimise or eliminate contamination and false labelling, thereby protecting the consumer from being misled and helping in purchasing products that are not harmful. GMP is a good business tool that helps to refine compliance and performance by the manufacturers/producers.

9.4.2 Good Handling Practices (GHP)

These are a set of guidelines and practices that are designed to ensure that food is produced, processed, handled, and stored in a safe and hygienic manner to prevent contamination and maintain food safety. GHP includes practices such as personal hygiene of food handlers, proper cleaning and sanitization of food contact surfaces, safe storage and handling of raw and cooked foods, and proper temperature control during food production and storage. GHP is an important aspect of food safety management systems and is often implemented in food production facilities, restaurants, and other food handling establishments to minimize the risk of foodborne illnesses and ensure the safety of consumers.

9.4.3 Hazard Analysis Critical Control Point (HACCP)

HACCP is a means of providing assurance about safety of food. HACCP is an approach to food manufacture and storage in which raw materials and each individual step in a specific process are considered in detail and evaluated for its potential to contribute to the development of pathogenic micro organisms or other food hazards. It involves identifying potential hazards, assessing their likelihood of occurrence at various stages in the food chain, including raw material procurement, manufacturing, distribution, and product usage, and defining effective measures to control and mitigate these hazards.

9.5 CODEX ALIMENTARIUS

The Codex Alimentarius is a collection of internationally adopted food standards, codes of practice, guidelines and recommendations. They have been created for the purpose of protecting the health of consumers and ensuring fair practices in the food trade. Recognizing the importance of HACCP to food control, the twentieth session of the Codex Alimentarius Commission, held in Geneva, Switzerland from 28 June to 7 July 1993, where *Guidelines for the implementation of the Hazard Analysis Critical Control Point* (*HACCP*) *system* were adopted. The Codex General Principles of Food Hygiene lay a firm foundation for ensuring food hygiene. They follow the food chain from primary production through the consumer, highlighting the key hygiene controls at each stage and recommending a HACCP approach wherever possible to enhance food safety. These controls are internationally recognized as essential to ensure the safety and suitability of food for human consumption and international trade.

Codex Alimentarius is literally translated from Latin, a **"food code"**. It comprises a series of general and specific food safety standards that have been formulated with the objective of protecting consumer health and ensuring fair practices in the food trade. Food put on the market for local consumption or export must be safe to eat and of good quality. In addition, food should not carry disease-causing organisms that could harm animals or plants in importing countries. Codex Alimentarius is run by the Codex Alimentarius Commission, which is an intergovernmental body where all member countries have a vote. Various specialist committees are responsible for drafting standards, which are then adopted by the Codex Commission.

9.6 WHY IMPLEMENT HACCP?

It is a proactive perspective to ensure food safety. End product inspection and testing, although important, is time consuming, expensive and detects the problems only after they occur. In contrast, HACCP (Hazard Analysis and Critical Control Points) empowers the detection of hazards at any stage of processing or manufacturing, allowing for timely corrective action to ensure the production of safe and high-quality end products. It enables producers, processors, distributors, and exporters to optimize resource utilization in a cost-effective manner to ensure food safety. FSSA, 2006 places primary responsibility for safe food with producers and suppliers through HACCP, GMP, GHP. These guidelines are important for consumer protection, international food trade as well as to attain good quality products, consistently.

9.7 TERMINOLOGY

The following terms are used in discussion of HACCP that must be clearly understood to effectively develop and implement a plan.

1. ***Acceptable Level*** means that the presence of hazard which does not pose the likelihood of causing an unacceptable health risk.
2. ***Control point*** refers to a point within a food system where a loss of control would not result in an unacceptable health risk.
3. ***Critical control*** point is defined in the food Code, as a point at which loss of control may result in an unacceptable health risk.
4. ***Critical Limit*** is the highest or lowest value that a physical, biological, or chemical parameter must be regulated to at a critical control point in order to reduce the possibility of the identified food safety hazard from occurring.
5. ***Deviation*** refers to the inability to meet the essential critical limit at a critical control point.
6. ***HACCP plan*** as defined in the Food Code, means a written document that delineates the formal procedures for following the HACCP principles developed by The National Advisory Committee on Microbiological Criteria in Foods.
7. ***Hazard,*** as defined in the Food Code, means a biological, chemical, or physical property that may cause an unacceptable consumer health risk.

8. ***Monitoring*** refers to a planned series of observations or measurements of critical limits that are aimed at generating a precise record and verifying that the critical limit maintains product safety. "Continuous monitoring" refers to an unbroken record of data.
9. ***Preventive*** measure means an action to exclude, destroy, eliminate, or reduce a hazard and prevent recontamination through effective means.
10. ***Risk*** means an estimate of the likely occurrence of a hazard.
11. ***Sensitive ingredient*** means any ingredient historically associated with a known microbiological hazard that causes of contributes to production of a potentially hazardous food as defined in the Food Code.
12. ***Verification*** means methods, procedures, and tests used to determine if the HACCP system in use in compliance with the HACCP plan.

9.8 THE HAZARD ANALYSIS AND CRITICAL CONTROL POINT (HACCP) CONCEPT

In the modern food industry, the Hazard Analysis Critical Control Point (HACCP) concept has largely replaced the traditional approach based on Good Manufacturing Practice. HACCP is a rule-based approach that leverages our knowledge of food microbiology to control microbiological quality more efficiently. While it can also be applied to physical and chemical factors affecting food safety or acceptability, this text will focus on microbiological hazards. It is important to note that HACCP is primarily a preventative approach to quality assurance, and it can be used to design quality and develop novel products during their development. The concept was initially developed as part of the United States space program by the Pillsbury Company, the National Aeronautics and Space Administration (NASA), and the US Army Natick Laboratories. They used it to apply the same zero defects philosophy to food for astronauts as to other items of their equipment. HACCP is based on an engineering system called the Failure Modes Analysis Scheme, which examines a result and all its components with questioning at each step "What can go wrong?"

In 1973, the US Food and Drug Administration approved HACCP for the examination of low-acid canned food. Since then, it has been increasingly applied to all aspects of food manufacture, food processing, and food service, at all scales of operation from large industrial concerns to cottage industries and even domestic food preparation. The meaning of the terms "hazard" and "threat" in the HACCP system differs from their general everyday usage as synonyms. In HACCP, a hazard is a source of risk defined as the unacceptable

contamination, development, or survival of a microorganism that can compromise safety or shelf-life, and/or the unacceptable creation or persistence in food of microbial metabolites affecting safety or shelf-life. Individual hazards can be evaluated in terms of their severity and threat, with a hazard to food safety being more severe than one to shelf-life. For instance, botulism is a more severe hazard than Staphylococcus aureus food poisoning. Risk is an estimate of the likely incidence of a hazard, and although C. botulinum is a more severe hazard, epidemiological evidence indicates that the risk it poses is typically very low.

Best HACCP can be planned and conducted only by involvement of a multidisciplinary team including microbiologist, process supervisor, engineer, and quality assurance manager, where all can contribute their expertise and knowledge to solve the problem. Input from production personnel will also ensure compliance with the plan by those who will have to execute it. Specific microbial hazards should be identified in the study's terms of reference to allow the team to define specific controls. The selection of hazards to be considered will entirely depend on whether there is epidemiological evidence linking a particular microorganism with the food being processed. In the absence of any such confirmation, product’s physical and chemical characteristics and its end use by the customer must offer the basis for selection.

Scope of HACCP

- Aggressive competition required organization to reduce costs while maintaining quality.
- Increasing consumer awareness and legal liability to produce safe food
- Changes in processed technology, increased automation, complex packaging solutions, new ingredients and improved formulations.
- Greater emphasis on sensory evaluations and complex distribution networks leading to reduce delivery times.

9.9 PRINCIPLES OF HACCP

In past few years national and international bodies have agreed on a definition based on seven important principles of a HACCP system:

(1) Conduct a hazard analysis.

(2) Determine the Critical Control Points (CCPs).

(3) Establish critical limits.

(4) Establish a system to monitor control of the CCP.

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

(6) Establish procedures to verify that the HACCP system is working effectively.

(7) Establish documentation concerning all procedures and records

To apply these principles in practice it is necessary to go through a series of steps outlined in Table 1.

Table 1: Steps in the application of HACCP

Sl. No.	Steps Required	HACCP Principle Involved
1	Assemble the HACCP team	
2	Describe the product	
3	Identify intended use	
4	Construct flow diagram	
5	On-site confirmation of flow diagram	
6	List all potential hazards	Principle 1
7	Conduct a hazard analysis	
8	Determine control measures	
9	Determine CCPs	Principle 2
10	Establish critical limit for each CCP	Principle 3
11	Establish a monitoring system for each CCP	Principle 4
12	Establish corrective action for deviations that may occur	Principle 5
13	Estabish verification procedures	Principle 6
14	Estabish documentation of procedures and records	Principle 7

9.9.1 Hazard Analysis

Hazard Analysis is a crucial step in the HACCP system and involves identifying potential hazards in the food production process that could pose a threat to consumer safety. It is important to approach this step systematically by examining all raw materials,

ingredients, and processing steps, as well as potential sources of contamination throughout packaging, distribution, and storage.

The hazard analysis must identify raw materials and ingredients that may contain harmful microorganisms or toxic metabolites, as well as the likelihood of these hazards occurring and the severity of their adverse health effects. The potential for contamination at different stages in processing must also be evaluated, as well as the physical and chemical characteristics of intermediates and products that could promote microbial growth or the production of toxic metabolites.

To effectively control these hazards, measures such as lethal or bacteriostatic process steps must be identified. The expertise of a food microbiologist is crucial in this step to distinguish between microbiologically sensitive raw materials and ingredients, and to provide quantitative tools such as predictive models to estimate potential microbial growth or survival at each step.

9.9.2 Identification of Critical Control Points (CCPs)

Identification of Critical Control Points (CCPs) is a crucial step in HACCP, which involves identifying specific points in the production process where potential hazards can be controlled or eliminated to ensure the safety of the final product. CCPs are the points in the production process where a failure to control a hazard would result in an unacceptable risk to the consumer or product.

To identify CCPs, a hazard analysis is conducted to identify potential hazards and the steps in the production process where they are likely to occur. Then, the decision tree is used to determine which of these steps should be designated as CCPs. Decision trees are designed to help identify CCPs by asking a series of questions, such as whether a control measure exists at a particular step or if a hazard can be eliminated or reduced to an acceptable level. Once the CCPs have been identified, specific control measures are implemented to ensure that the hazards are effectively controlled or eliminated. The control measures may include process modifications, such as temperature or pH adjustments, the use of microbial inhibitors, or changes to equipment design, employee hygiene, or sanitation practices.

The effective identification of CCPs requires a thorough understanding of the production process, the hazards that may be present, and the control measures that can be implemented to ensure the safety of the final product. The process should be reviewed

regularly to ensure that CCPs remain effective and that new hazards are identified and controlled as necessary.

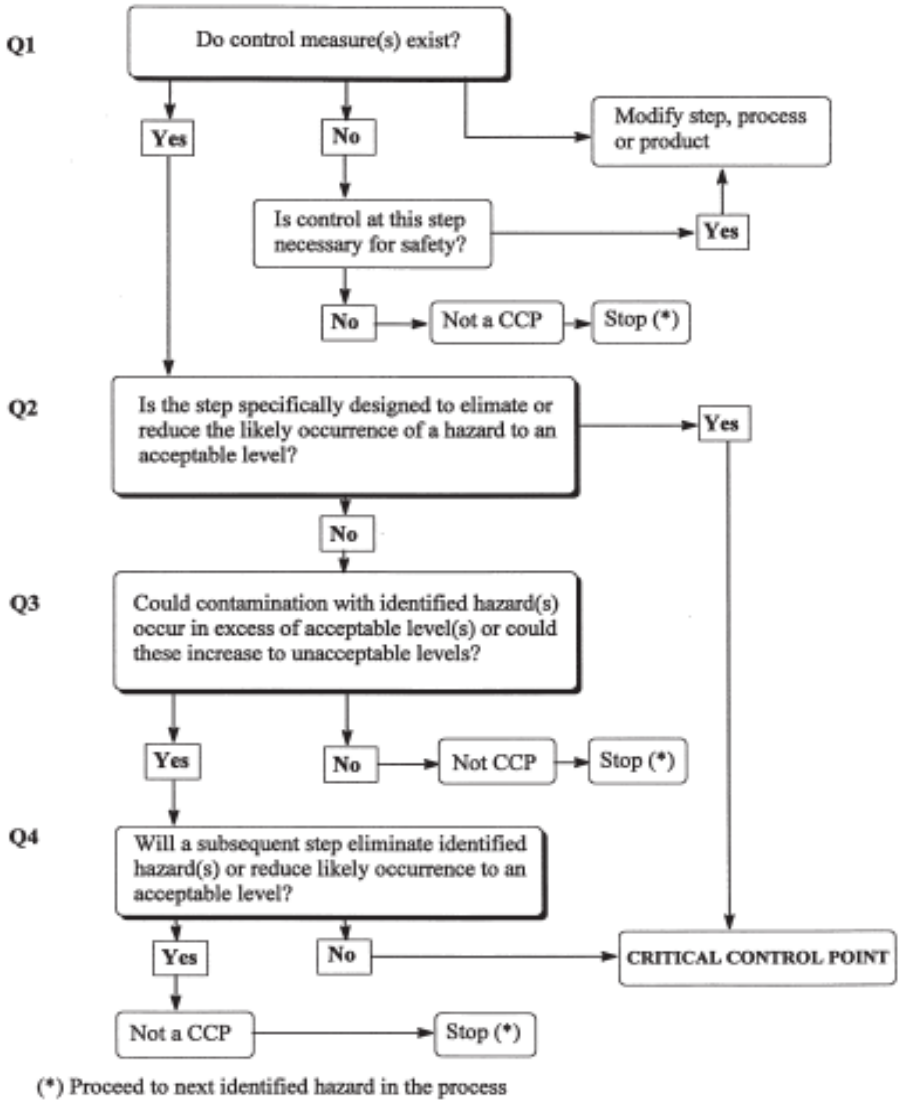

Fig 2: Decision tree

9.9.3 Establishment of CCP Critical Limits

After identifying the CCPs, the next step is to establish critical limits for each CCP. Critical limits are specific parameters or values that must be met to ensure that the hazard is controlled at the CCP. These limits can be based on regulatory requirements, scientific studies, industry standards, or other sources of information. Critical limits can be physical, chemical, or biological parameters, such as temperature, pH, water activity, microbial count, and so on. For example, the critical limit for a cooking CCP may be a minimum temperature

of 165°F for a specific time period, while the critical limit for a storage CCP may be a maximum temperature of 40°F.

It is important to establish critical limits that are measurable and can be monitored in real-time or near-real-time. This enables the identification of deviations from the critical limits and the implementation of corrective actions to prevent the production of unsafe food products. The establishment of critical limits must be based on scientific and technical information, and involve the collaboration of food safety experts, microbiologists, process engineers, and other relevant personnel. The process of establishing critical limits should also take into account the characteristics of the product, the production process, and the intended use of the product.

Once the critical limits have been established, they must be documented and communicated to all relevant personnel involved in the production process. They should also be regularly reviewed and updated to ensure that they are still appropriate and effective in controlling the identified hazards.

9.9.4 Monitoring Procedures for CCPs

After establishing the criteria for critical limits for each CCP, it is important to implement monitoring procedures to ensure that these limits are consistently met. Monitoring involves the measurement or observation of a parameter or CCP at a specific point in the process, which enables the identification of any deviations from the critical limits.

Monitoring procedures should be designed to provide a timely indication of process control and the ability to detect any loss of control before a hazard occurs. These procedures should include the use of appropriate equipment, sampling plans, and analytical methods. For example, a monitoring procedure for a CCP that involves cooking temperatures may include the use of a temperature probe to measure the internal temperature of the food product during the cooking process.

The frequency of monitoring should be established based on the level of risk associated with the CCP and the likelihood of deviations occurring. Monitoring should be frequent enough to detect any loss of control before a hazard occurs. However, overly frequent monitoring can be resource-intensive and may not add significant value. Monitoring records should be kept to document the results of monitoring activities, including the date and time of the measurement or observation, the individual responsible for the monitoring, and the results obtained. These records are important for verifying that the process is under

control and for identifying any trends or patterns that may indicate a need for corrective action.

Monitoring procedures for CCPs are critical to ensure that critical limits are consistently met and that deviations from these limits are detected in a timely manner. The frequency of monitoring should be established based on the level of risk associated with the CCP, and monitoring records should be kept to document the results of monitoring activities.

9.9.5 Protocols for CCP Deviations

These are the procedures that should be followed when a deviation from the established critical limit occurs at a CCP. Deviations can occur due to equipment failure, human error, or any other unforeseen circumstances. The protocols should be designed to ensure that the deviation is identified, documented, and resolved in a timely and appropriate manner to prevent any potential harm to the consumer or product. It is important to have protocols in place for CCP deviations to ensure that any potential hazards are identified and addressed promptly.

9.9.6 Verification

Verification is the process of evaluating the effectiveness of the HACCP system in controlling hazards and ensuring the safety of the food product. This involves a series of activities to ensure that the system is working as intended and that the identified hazards are being effectively controlled.

It includes activities such as reviewing and updating the HACCP plan, reviewing and evaluating monitoring data and records, conducting internal audits, and verifying that corrective actions have been taken when necessary. The goal of verification is to ensure that the HACCP system is operating effectively and that any deficiencies or weaknesses are identified and corrected. The verification process should be conducted by trained individuals who are independent of the HACCP team and who have the necessary expertise to evaluate the effectiveness of the system. There are several different methods that can be used to verify the HACCP system, including: Internal Audits, Product Testing, equipment Calibration, Process Verification, Record Review and Challenge Testing.

Verification should be conducted on an ongoing basis to ensure that the HACCP system remains effective over time. Any issues or problems that are identified during the verification process should be addressed immediately to prevent the production of unsafe

food. The results of verification should be documented, and any necessary corrective actions should be taken to ensure that the HACCP system remains effective.

9.9.7 Record Keeping

Record keeping is an essential part of a HACCP plan. The purpose of record keeping is to provide a written history of the HACCP system, including all activities and procedures that have been followed, and any deviations that have occurred. This information is used to demonstrate that the system is being properly implemented and that the products are safe for consumption. Records should be kept for all CCPs, and should include information such as monitoring results, corrective actions taken, and verification activities. Records should also include information about product specifications, supplier information, and any other relevant information that pertains to the safety of the product.

The type of records that should be kept will vary depending on the nature of the product and the specific hazards that are being controlled. For example, records might include temperature logs for refrigeration units, pH readings for acidified products, or microbial test results for ready-to-eat products. The HACCP team should establish procedures for maintaining and reviewing records, and for ensuring that all necessary information is recorded accurately and completely. Records should be kept in a secure location and should be easily accessible for review by regulatory agencies or auditors.

Effective record keeping is critical for the success of a HACCP plan. It provides a means of tracking the performance of the system, identifying areas for improvement, and demonstrating compliance with regulatory requirements.

9.10 BENEFITS OF IMPLEMENTING THE HACCP SYSTEM

Application of an effective HACCP system has clear benefits for consumers, industry as well as regulatory bodies.

Benefits for Consumers

- Lower risk of food borne illnesses
- Greater awareness of food safety
- Greater confidence in food supply
- Better quality of life through health and socioeconomic benefits

Benefits for Industry

- Greater consumer confidence on product
- Minimizes legal and insurance costs
- Increases market access
- Lower wastage, fewer no recalls, minimum or no reprocessing, and corrective action
- A consistent product
- Enhanced staff commitment to food safety
- Lower business risk

Benefits for Regulatory Bodies

- Improved health among the community
- More efficient food control
- Lower public health costs
- Trade promotion
- Greater confidence of the community in the food supply

9.11 APPLICATIONS OF HACCP

While the application of HACCP to all segments and sectors of the food chain is possible, it is assumed that all sectors should be operating according to good manufacturing practices (GMPs) and the Codex General Principles of Food Hygiene. The ability of an industry segment or sector to support or implement the HACCP system depends on the degree of its adherence to these practices. The successful application of HACCP requires the full commitment and involvement of management and the workforce. It requires a multidisciplinary approach which should include as appropriate, expertise in agronomy, veterinary health, microbiology, public health, food technology, environmental health, chemistry, engineering, etc. according to the particular situation. The application of the HACCP system is compatible with the implementation of TQM systems such as the ISO 9000 series. However, HACCP is the system of choice in the management of food safety within such systems.

9.12 SUM UP

In this unit we got acquainted with concept of food safety and food hazards. It introduced you to the principles of Food Safety Management System. The unit gave you an understanding about principles of GMP, GHP, HACCP and Codex Alimentarius. The unit also explained the need of HACCP and terminology associated with HACCP. The seven principles of HACCP were discussed in detail. The implementation of HACCP, benefits and applications of HACCP were also described.

9.13 CHECK YOUR PROGRESS

1. Define:

   (a) Food Hazard

   .....

   .....

   (b) Toxicity

   .....

   .....

   (c) Chemical hazards

   .....

   .....

   (d) Critical limit

   .....

   .....

   (e) Decision tree

   .....

   .....

2. Write full form of :

(a) GMP

.....

(b) GHP

.....

(c) HACCP

.....

(d) FSSA

.....

(e) CCP

.....

3. What are the principles of HACCP?

.....

.....

.....

4. Explain the step identification of critical control points in HACCP.

.....

.....

.....

5. What are the benefits of implementing the HACCP?

.....

.....

.....

Unit-X ROLE OF MICROBES IN FERMENTED FOODS AND GENETICALLY MODIFIED FOODS

Structure

10.1 INTRODUCTION

This unit will give you an understanding of fermentationprocess and history of fermentation. It will introduce you to different types of fermented foods and the microorganisms involved in preparation of these foods. The unit gives you an understanding about fermented milks, meat, fish, vegetables, vinegar, cheese as well as probiotics. The unit

will also introduce you to the concept of GM foods. The advantages and risks associated with use of GM foods are also discussed.

Objectives

After studying this unit, you will be able to:

- understand basics of fermentation
- explain history of fermentation
- identify the microorganisms involved in preparation of fermented foods
- understand the term probiotics
- explain the concept of GM foods
- understand the advantages and limitations of use of GM foods

10.2 FERMENTATION

Fermentation is the process that is used in converting sugar to gases or to alcohol. It is alsoused to refer to the growth of microorganisms with the aim of producing a specific chemicalproduct.

Fermentation is a metabolic process that converts sugar to acids, gases, or alcohol. It occurs in yeast and bacteria, and also in oxygen-starved muscle cells, as in the case of lactic acid fermentation. Fermentation is also used more broadly to refer to the bulk growth of microorganisms on a growth medium, often with the goal of producing a specific chemical product. The science of fermentation is known as zymology. Fermentation takes place when the electron transport chain is unusable (often due to lack of a final electron receptor, such as oxygen). In this case it becomes the cell's primary means of ATP (energy) production. It turns NADH and pyruvate produced in glycolysis into NAD+ and an organic molecule (which varies depending on the type of fermentation). In the presence of O2, NADH and pyruvate are used to generate ATP in respiration. This is called oxidative phosphorylation, and it generates much more ATP than glycolysis alone. For that reason, cells generally benefit from avoiding fermentation when oxygen is available, the exception being obligate anaerobes which cannot tolerate oxygen. Fermentation does not necessarily have to be carried out in an anaerobic environment. For example, even in the presence of abundant oxygen, yeast cells greatly prefer fermentation to aerobic respiration, as long as sugars are readily available for consumption (a phenomenon known as the Crabtree effect). The antibiotic activity of hops also inhibits aerobic metabolism in yeast. Fermentation reacts NADH with an

endogenous, organic electron acceptor. Usually this is pyruvate formed from the sugar during the glycolysis step. During fermentation, pyruvate is metabolized to various compounds through several processes:

- ethanol fermentation, aka alcoholic fermentation, is the production of ethanol and carbon dioxide
- lactic acid fermentation refers to two means of producing lactic acid:
    - homolactic fermentation is the production of lactic acid exclusively
    - heterolactic fermentation is the production of lactic acid as well as other acids and alcohols.

Sugars are the most common substrate of fermentation, and typical examples of fermentation products are ethanol, lactic acid, carbon dioxide, and hydrogen gas (H2). However, more exotic compounds can be produced by fermentation, such as butyric acid and acetone. Yeast carries out fermentation in the production of ethanol in beers, wines, and other alcoholic drinks, along with the production of large quantities of carbon dioxide. Fermentation occurs in mammalian muscle during periods of intense exercise where oxygen supply becomes limited, resulting in the creation of lactic acid.

10.3 HISTORY OF FERMENTATION

Natural fermentation precedes human history. Since ancient times, humans have exploited the fermentation process. The earliest evidence of an alcoholic drink, made from fruit, rice, and honey, dates from 7000 to 6600 BC, in the Neolithic Chinese village of Jiahu, and winemaking dates from 6000 BC, in Georgia, in the Caucasus area. Seven-thousand-year-old jars containing the remains of wine, now on display at the University of Pennsylvania, were excavated in the Zagros Mountains in Iran. There is strong evidence that people were fermenting alcoholic drinks in Babylon c. 3000 BC, ancient Egypt c. 3150 BC, pre-Hispanic Mexico c. 2000 BC, and Sudanc. 1500 BC. Fermented foods have a religious significance in Judaism and Christianity. The Baltic god Rugutis was worshiped as the agent of fermentation. The first solid evidence of the living nature of yeast appeared between 1837 and 1838 when three publications independently concluded as a result of microscopic investigations that yeast is a living organism that reproduces by budding. The focus on yeasts in early studies on fermentation in Europe was likely due to their importance in making basic

foods such as wine, beer, and bread. However, researchers soon discovered the presence of bacteria as well. The term "bacteria" was first used in English during the late 1840s, but it did not become commonly used until the 1870s, particularly in relation to the emerging germ theory of disease. Louis Pasteur (1822–1895), during the 1850s and 1860s, showed that fermentation is initiated by living organisms in a series of investigations. In 1857, Pasteur showed that lactic acid fermentation is caused by living organisms. In 1860, he conducted an experiment that demonstrated how bacteria cause souring in milk, which was previously thought to be a chemical change. This discovery led to the process of pasteurization, which involves heating milk to kill harmful bacteria and extend its shelf life.

Pasteur also investigated the role of microorganisms in food spoilage and correctly showed that specific types of microorganisms cause specific types of fermentations and specific end-products. He defined fermentation as "Life without air," although this definition was later proven to be incorrect. Nonetheless, his work paved the way for a better understanding of the role of microorganisms in food processing, preservation, and spoilage.

Although showing fermentation to be the result of the action of living microorganisms was a breakthrough, it did not explain the basic nature of the fermentation process, or prove that it is caused by the microorganisms that appear to be always present. In 1897, Eduard Buechner, a German chemist, successfully extracted a juice from yeast that was able to ferment a sugar solution, even though the yeast cells were dead. This discovery marked the birth of biochemistry and showed that fermentation is caused by enzymes produced by microorganisms. Buechner's findings also led to the term "enzyme" being applied to all ferments. In 1907, Buechner was awarded the Nobel Prize in chemistry for his work.

Since then, advances in microbiology and fermentation technology have continued to develop. In the late 1970s, it was discovered that microorganisms could be mutated with physical and chemical treatments to be higher-yielding, faster-growing, and more tolerant of less oxygen, and able to use a more concentrated medium. Strain selection and hybridization were also developed, which have had a significant impact on modern food fermentations. Other approaches to advancing the fermentation industry has been done by companies such as BioTork, a biotechnology company that naturally evolves microorganisms to improve fermentation processes. This approach differs from the more popular genetic modification, which has become the current industry standard.

10.4 FERMENTED FOODS

10.4.1 Fermented milks

Fermented milk is a dairy product that has undergone a fermentation process, which converts lactose into lactic acid. This results in a tangy taste, thicker consistency, and longer shelf life compared to regular milk. The fermentation process of fermented milks involves the conversion of lactose, the main sugar in milk, into lactic acid by lactic acid bacteria (LAB) or other microorganisms. This process gives fermented milks their characteristic tangy taste and creamy texture. The fermentation process can be carried out either naturally or through the addition of starter cultures. Natural fermentation occurs when raw milk is left at room temperature, allowing naturally present bacteria to ferment the milk. This process, however, can lead to inconsistent results and potential safety issues. The use of starter cultures, which contain specific strains of LAB, is the preferred method for fermenting milk in a controlled and consistent manner. The most commonly used LAB for milk fermentation are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, which are often used together as a starter culture.

The fermentation process usually takes place at a temperature of around 40-45°C for several hours, depending on the desired texture and flavor of the final product. During fermentation, the LAB metabolize the lactose in the milk and produce lactic acid, which lowers the pH of the milk, causing it to thicken and form a gel-like texture. After fermentation, the fermented milk can be further processed through homogenization, flavoring, and sweetening to produce various types of fermented milk products, such as yogurt, kefir, and buttermilk. There are several types of fermented milk products that are popular around the world, including:

- Yogurt: It is the most well-known fermented milk product. It is made by adding a starter culture of Lactobacillus bulgaricus and Streptococcus thermophilus to heated milk. The culture ferments the lactose in the milk, producing lactic acid and thickening the milk. Yogurt can be consumed plain or flavored with fruit, honey, or other sweeteners.
- Kefir: It is a fermented milk drink that originated in the Caucasus Mountains. It is made by adding kefir grains, a combination of yeast and bacteria cultures, to milk. The grains ferment the lactose in the milk, producing lactic acid and carbon dioxide, which gives kefir its characteristic fizzy texture. Kefir can be consumed plain or flavored with fruit or honey.

- Buttermilk: Buttermilk is a fermented milk product that is made by adding lactic acid bacteria to skim milk. It has a tangy taste and a thicker consistency than regular milk. It is commonly used in baking to add moisture and acidity to recipes.
- Labneh: Labneh is a Middle Eastern yogurt cheese that is made by straining yogurt to remove the whey. The resulting product is thick and creamy, similar in texture to cream cheese. It is often flavored with herbs, spices, or olive oil and used as a spread or dip.
- Ayran: It is a Turkish fermented yogurt drink that is made by mixing yogurt with water and salt. It has a thin consistency and a salty, tangy taste. It is often consumed as a refreshing drink in hot weather and is sometimes flavored with mint or other herbs.
- Filmjölk: Filmjölk is a Swedish fermented milk product that is made by adding a starter culture of Lactococcus lactis and Leuconostoc mesenteroides to milk. It has a tangy taste and a thinner consistency than yogurt. It is often consumed plain or with fruit and granola for breakfast.
- Amasi: It is a South African fermented milk product that is made by adding a starter culture of lactic acid bacteria to milk. It has a sour taste and a thick, creamy texture. It is often consumed as a snack or used in cooking.

Consumption of these fermented milks provide a variety of health benefits, such as improved digestion, strengthened immune system, and better absorption of nutrients. the health benefits of fermented milk products are likely due to a combination of probiotic bacteria and other bioactive compounds.

10.4.2 Cheese

Cheese is a dairy product made from milk that has undergone a process of coagulation and separation of the curd from the whey. One of the key processes in cheese production is fermentation, which is carried out by microorganisms such as bacteria and fungi that are added to the milk or naturally present in the milk. Fermentation plays several important roles in cheese production, including:

- Acidification: The primary function of fermentation in cheese production is to acidify the milk. During fermentation, the microorganisms convert lactose, the primary sugar in milk, into lactic acid. This lowers the pH of the milk, which helps to coagulate the proteins and separate the curd from the whey.
- Flavor development: Fermentation also plays a critical role in developing the flavor and aroma of cheese. Different microorganisms produce different compounds that contribute

to the unique flavor and aroma of each type of cheese. For example, some bacteria produce diacetyl, which gives buttery and nutty flavors, while others produce compounds that contribute to the sharp and tangy flavor of cheddar cheese.

- Texture and structure: Fermentation also helps to develop the texture and structure of cheese. The acid produced during fermentation can cause the proteins in the milk to denature and coagulate, forming a solid curd. The curd can then be cut and pressed to remove excess whey, resulting in a firmer cheese.

There are two main types of fermentation used in cheese production: lactic acid fermentation and ripening or maturation fermentation. Lactic acid fermentation is the first step in cheese production and involves adding lactic acid bacteria to the milk. The bacteria consume lactose and produce lactic acid, which lowers the pH of the milk and causes the proteins to coagulate. This results in the formation of a soft curd, which is then cut and separated from the whey.

Ripening or maturation fermentation, on the other hand, is a slower process that occurs after the cheese has been formed. During ripening, a variety of microorganisms such as bacteria, yeasts, and molds are allowed to grow on the surface of the cheese. These microorganisms break down the proteins and fats in the cheese, producing a variety of flavor compounds and contributing to the unique texture and structure of the cheese. There are many different types of fermented cheese, each with its own unique flavor, aroma, and texture. Some examples of fermented cheese include:

- Blue cheese: It is made by adding the mold Penicillium roqueforti to the milk during the cheese-making process. The mold grows throughout the cheese, forming blue-green veins and producing a sharp, tangy flavor.
- Brie: Brie is a soft cheese that is made by allowing the mold Penicillium camemberti to grow on the surface of the cheese. This mold forms a white rind around the cheese and produces a mild, creamy flavor.
- Cheddar: Cheddar is a hard cheese that is aged for several months or even years. During the aging process, bacteria break down the proteins and fats in the cheese, producing a sharp, tangy flavor.
- Gouda: It is a semi-hard cheese that is aged for several months. During the aging process, bacteria break down the proteins and fats in the cheese, producing a nutty, caramel-like flavor.

- Feta: Feta is a crumbly cheese that is made by allowing bacteria to grow on the surface of the cheese. This produces a tangy, salty flavor and a crumbly texture.

Fermentation is a critical process in cheese production that contributes to the flavor, texture, and structure of the final product. By carefully controlling the types of microorganisms used and the conditions in which they grow, cheesemakers can create a wide variety of unique and delicious cheeses.

10.4.3 Alcoholic Beverages

An alcoholic beverage is a drink that contains ethanol, which is commonly known as alcohol. The consumption of beer has been a cultural practice for over 8,000 years. Drinking beer and other alcoholic beverages in local bars or pubs is a cultural norm in Germany, Ireland, the United Kingdom, and many other European countries. Non-alcoholic beverages, on the other hand, typically contain less than 0.5% alcohol by volume, such as low-alcohol beer, non-alcoholic wine, and apple cider.

Wine fermentation is a crucial step in winemaking that involves the conversion of grape juice into alcohol and carbon dioxide by microorganisms, specifically yeast. The process starts with the harvesting of ripe grapes, which are then crushed and destemmed to remove stems and leaves. Afterward, yeast is added to the grape juice to initiate fermentation, during which yeast consumes the sugars in the juice and converts them into alcohol and carbon dioxide. The duration of fermentation and the type of yeast used greatly influence the flavor, aroma, and alcohol content of the wine. After fermentation is complete, the wine is aged and clarified before being bottled and consumed. The quality of the grapes, the fermentation process, and the aging process all play a significant role in the final product of the wine.

Preparation of beer involves the conversion of sugars present in malted grains into alcohol and carbon dioxide by yeast. The process starts with mashing, where malted grains are mixed with hot water to extract sugars and create wort, a sweet liquid. The wort is then boiled with hops to add flavor and bitterness, and the liquid is cooled and transferred to a fermentation vessel. Yeast is added to the vessel, and fermentation begins as the yeast consumes the sugars in the wort and converts them into alcohol and carbon dioxide. The process can take several days to complete, depending on the type of beer and the desired alcohol content. After fermentation, the beer is transferred to a conditioning vessel, where it undergoes a maturation process to develop its flavor and aroma. The beer is then filtered, carbonated, and bottled or kegged for consumption. The fermentation process plays a

significant role in determining the final flavor and alcohol content of the beer, making it a crucial step in beer production.

Cider fermentation is a natural process that involves converting apple juice into alcoholic cider through the action of yeast. The process begins with the harvesting of ripe apples, which are then washed, crushed, and pressed to extract their juice. The juice is strained to remove any pulp or debris before adding yeast to initiate fermentation. The type of yeast used greatly influences the flavor, aroma, and alcohol content of the final product. The fermentation process can take anywhere from one to three months, depending on the desired alcohol content and the temperature and humidity of the fermentation environment. During fermentation, yeast consumes the natural sugars present in the juice and produces alcohol and carbon dioxide as byproducts. After fermentation is complete, the cider is transferred to a storage vessel, where it is aged and conditioned to improve its flavor and clarity. Cider fermentation is a vital step in producing high-quality cider, and proper fermentation techniques are essential to achieving the desired taste and alcohol content.

There are numerous other types of alcoholic drinks besides wine, beer, and cider, including spirits which are distilled alcoholic drinks made from fermented grains, fruits, or vegetables eg. whiskey, vodka, gin, rum, and tequila; Liqueurs are sweetened alcoholic drinks that are often flavored with fruits, herbs, spices, or nuts eg. amaretto, Bailey's Irish Cream, and Grand Marnier; Mead is a fermented alcoholic beverage made from honey, water, and yeast. It is one of the oldest alcoholic beverages in the world and has been enjoyed for thousands of years; Sake is a traditional Japanese alcoholic beverage made from fermented rice with a distinct flavor and aroma and is often enjoyed with Japanese cuisine; Brandy is a distilled spirit made from fermented fruit juice or wine often aged in oak barrels to develop its flavor and aroma.

10.4.4 Fermented Vegetables

Most horticultural products can be preserved by lactic acid fermentation. In the West, the most important commercial vegeatables are cabbage, cucumbers and olives, although smaller amounts of others such as carrots, cauliflower, celery, okra, onions, sweet and hot peppers, and green tomatoes are also fermented. In Korea, fermented vegetables known as kimchi are an almost ubiquitous accompaniment to meals. More than 65 different types of kimchi have been identified on the basis of differences in raw materials and processing. Cabbages and radishes are the main substrates but garlic, peppers, onions and ginger are often also used. Surveys have shown its importance in the Korean diet, variously reporting kimchi

to comprise 12.5% of the total daily food intake or a daily adult consumption of 50–100 g in summer increasing to 150–200 g in winter. Sauerkraut production is thought to have been brought to Europe from China by the Tartars. Like a number of other traditional fermentations, the commercial process is technologically simple, but involves some interesting and complex chemistry and microbiology. Usually where sauerkraut is produced commercially special cabbage cultivars are grown. These tend to have a higher solids content than normal and so minimize production of liquid waste during processing.

10.4.5 Fermented Meat

Fermented meat products are defined as meats that are deliberately inoculated during processing to ensure sufficient control of microbial activity to alter the product's characteristics. If fresh meat is not preserved or cured in some manner, it spoils rapidly owing to the growth of indigenous gram-negative bacteria and subsequent putrefaction resulting from their metabolic activities. Although some manufacturers still depend upon naturally occurring microflora to ferment meat, most use starter cultures consisting of a single species or multiple species combinations of lactic acid bacteria (LAB) and/or staphylococci that have been selected for metabolic activities especially suited for fermentation in meat ecosystems. Understanding the technological, microbiological, and biochemical processes that occur during meat, poultry, and fish fermentation is essential for ensuring safe, palatable products. Dry and semidry sausages represent the largest category of fermented meat products, with many presentday processing practices having their origin in the Mediterranean region. Traditionally, dry sausages acquired their particular sensory characteristics from exposure to salt; indigenous gram-positive microorganisms such as LAB, coagulase-negative cocci, including staphylococci and kocuria (*Micrococcus* spp. that were mostly used for fermented meat were reclassified as *Kocuria* spp. in 1995), and yeasts residing on the meat; and the rapid drying conditions that exist in the warm, dry Mediterranean climate. These products were heavily seasoned and stuffed into sausage casings that excluded air, which favored the growth of certain gram-positive bacteria such as LAB. Typically, they were not smoked and preserved after fermentation while the accumulation of lactic acid, other organic acids, carbon dioxide, and alcohols served as the preservatives. The name "sausage" was derived from the Latin term salsus, meaning "salted." Sausage processing practices later spread to northern Europe, and by the Middle Ages, hundreds of varieties of dry and semidry sausages were manufactured across the continent.

10.4.6 Fermented Fish

Fermented fish products include a variety of fish sauces, fish pastes, and fish-vegetable blends that have been salted, packed whole in layers, or ground into small particles and then fermented in their own “pickle.” These products are eaten as a proteinaceous staple or condiment in Southeast Asia but are consumed as a condiment in northern Europe. Fish fermentation involves minimal bacterial conversion of carbohydrates to lactic acid but entails extensive tissue degradation by proteolytic and lipolytic enzymes derived from viscera and muscle tissues. Low-molecular-weight compounds from fish tissue degradation are the primary contributors to aroma and flavor characteristics of sauces. Indigenous microorganisms, however, do contribute to aroma and flavor but are limited to species tolerant of high salt concentrations (10 to 20%) in the curing brine. Partial tissue hydrolysis is responsible for the unique textural attributes of pastes and fish-vegetable blends.

10.4.7 Vinegar

Vinegar is the product of a two-stage fermentation process. In the first stage, yeasts convert sugars into ethanol anaerobically, while in the second ethanol is oxidized to acetic (ethanoic) acid aerobically by bacteria of the genera Acetobacter and Gluconobacter. This second process is a common mechanism of spoilage in alcoholic beverages and the discovery of vinegar was doubtless due to the observation that this product of spoilage could be put to some good use as a flavouring and preservative. The name vinegar is in fact derived from the French *vin aigre* for ‘sour wine’ and even today the most popular types of vinegar in a region usually reflect the local alcoholic beverage; for example, malt vinegar in the UK, wine vinegar in France, and rice vinegar in Japan. In vinegar brewing, the alcoholic substrate, known as vinegar stock, is produced using the same or very similar processes to those used in alcoholic beverage production. In the production of malt vinegar for example, hops are not used and the wort is not boiled so the activity of starch-degrading enzymes continues into the fermentation. Acetification, the oxidation of ethanol to acetic acid is performed by members of the genera Acetobacter and Gluconobacter. These are Gram negative, catalase-positive, oxidase-negative, strictly aerobic bacteria. Acetobacter spp. are the better acid producers and are more common in commercial vinegar production, but their ability to oxidize acetic acid to carbon dioxide and water, a property which distinguishes them from Gluconobacter, can cause problems in some circumstances when the vinegar brewer will see his key component disappearing into the air as CO_2. Fortunately over-oxidation, as it is known, is repressed by ethanol and can be controlled by careful monitoring to ensure that ethanol is not completely

exhausted during acetification. Most acetifications are run on a semi-continuous basis; when acetification is nearly complete and acetic acid levels are typically around 10–14% w/v, a proportion of the fermenter's contents is removed and replaced with an equal volume of fresh alcoholic vinegar stock. Since a substantial amount of finished vinegar is retained in the fermenter, this conserves the culture and means that a relatively high level of acidity is maintained throughout the fermentation, protecting against contamination. It also protects against over-oxidation as it has been found that *Acetobacter europaeus*, a species commonly found in commercial vinegar fermenters, will not over-oxidize when the acetic acid concentration is more than 6%.

10.4.8 Probiotics

Probiotics, or live microorganisms, are frequently utilized in various products such as food, drugs, and dietary supplements. The most commonly used probiotics are *Lactobacillus* and *Bifidobacterium* species, but other species such as yeast *Saccharomyces cerevisiae*, *E. coli*, and *Bacillus* species are also utilized. Lactic acid bacteria, including Lactobacillus species, have been utilized for thousands of years in food preservation through fermentation, offering dual benefits by acting as agents for food fermentation while also providing potential health benefits. Fermentation is a widely used process for preserving various raw agricultural materials such as cereals, tubers, fruits, vegetables, milk, and fish.

Lactic acid bacteria are Gram-positive, catalase-negative bacterial species that produce lactic acid as the primary end product of carbohydrate fermentation. Lactobacillus is a type of bacterium with many different species that typically live in our digestive, urinary, and genital systems without causing disease. Lactobacillus is also utilized to prevent and treat diarrhea associated with antibiotic use. Some people utilize lactobacillus for general digestion problems, irritable bowel syndrome (IBS), colic in babies, Crohn's disease, inflammation of the colon, and a severe gut problem called necrotizing enterocolitis (NEC) in premature babies.

Probiotics alter the microflora, secrete microbicidal substances, compete with pathogens to prevent their adhesion to the intestinal epithelium, compete for nutrients essential for pathogen survival, generate an antitoxin effect, and reverse some of the consequences of disease on the intestinal epithelium, such as secretory changes and neutrophil migration. Probiotics stimulate T-cells, B-cells, macrophages, and NK-cells with messages that promote specific immune responses. They also activate cytokines and phagocytic cells directly to coordinate their intelligent immune response.

Probiotics also produce various substances such as acetic acids, formic acids, lipopolysaccharides, peptidoglycans, superantigens, heat shock proteins, and bacterial DNA, in precise proportions to nourish each other, inhibit challengers, and/or benefit the host. Probiotics also produce antibacterial molecules called bacteriocins, such as lactolin produced by *Lactobacillus plantarum*, bulgarican secreted by *Lactobacillus bulgaricus*, and acidophilin, acidolin, bacterlocin, and lactocidin produced by *Lactobacillus acidophilus*. These and other antibacterial substances equip probiotic species with territorial mechanisms to combat and reduce pathologies related to *Shigella, Coliform, Pseudomonas, Klebsiella, Staphylococcus, Clostridium, Escherichia*, and other infectious genera.

10.5 GENETICALLY MODIFIED FOODS

Genetic modification is a special set of gene technology that alters the genetic machinery of such living organisms as animals, plants or microorganisms. The enhancement of desired traits has traditionally been undertaken through breeding, but conventional plant breeding methods can be very time consuming and are often not very accurate. Genetic engineering, on the other hand, can create plants with the exact desired trait very rapidly and with great accuracy. For example, plant geneticists can isolate a gene responsible for drought tolerance and insert that gene into a different plant. The new genetically-modified plant will gain drought tolerance as well. Not only can genes be transferred from one plant to another, but genes from non-plant organisms also can be used. Scientists first discovered in 1946 that DNA can be transferred between organisms. It is now known that there are several mechanisms for DNA transfer and that these occur in nature on a large scale, for example, it is a major mechanism for antibiotic resistance in pathogenic bacteria.

The first genetically modified (GM) plant was produced in 1983, using an antibiotic-resistant tobacco plant. China became the first countryin the world to commercialize a transgenic crop in the early 1990s with the introduction of virus resistant tobacco. In 1994, the transgenic ‘Flavour Saver tomato’ was approved by the Food and Drug Administration (FDA) for marketing in the USA. The best known example of this is the use of B.t. genes in corn and other crops. B.t., or *Bacillus thuringiensis*, is a naturally occurring bacterium that produces crystal proteins that are lethal to insect larvae. B.t. crystal protein genes have been transferred into corn, enabling the corn to produce its own pesticides against insects such as the European corn borer. While GM foods have potential benefits, such as increased yield, reduced pesticide use, and improved nutritional content, they also have potential risks, such as negative impacts on the environment and human health. It's important to continue

researching and evaluating the safety and effectiveness of GM foods to ensure that they are safe and beneficial for consumers and the environment.

10.5.1 GM Crops

Genetic modification is a special set of gene technology that alters the genetic machinery of living organisms as animals, plants or microorganisms. The enhancement of desired traits has traditionally been undertaken through breeding, but conventional plant breeding methods can be very time consuming and are often not very accurate. Genetic engineering, on the other hand, can create plants with the exact desired trait very rapidly and with great accuracy. For example, plant geneticists can isolate a gene responsible for drought tolerance and insert that gene into a different plant. The new genetically-modified plant will gain drought tolerance as well. Not only can genes be transferred from one plant to another, but genes from non-plant organisms also can be used. Scientists first discovered in 1946 that DNA can be transferred between organisms. It is now known that there are several mechanisms for DNA transfer and that these occur in nature on a large scale, for example, it is a major mechanism for antibiotic resistance in pathogenic bacteria. The first genetically modified (GM) plant was produced in 1983, using an antibiotic-resistant tobacco plant. China was the first country to commercialize a transgenic crop in the early 1990s with the introduction of virus resistant tobacco. In 1994, the transgenic 'Flavour Saver tomato' was approved by the Food and Drug Administration (FDA) for marketing in the USA. The best known example of this is the use of B.t. genes in corn and other crops. B.t., or *Bacillus thuringiensis*, is a naturally occurring bacterium that produces crystal proteins that are lethal to insect larvae. B.t. crystal protein genes have been transferred into corn, enabling the corn to produce its own pesticides against insects such as the European corn borer.

GM foods have been on the market for several decades, and many crops have been genetically modified, including corn, soybeans, cotton, canola, and sugar beets. These crops are widely used in processed foods, such as breakfast cereals, snack foods, and baked goods. In addition to crops, GM foods can also include animal products, such as salmon that has been genetically engineered to grow faster and larger. Some examples are discussed below:

- Corn: GM corn has been genetically modified to be resistant to pests and herbicides. This is done by introducing a gene from a bacterium called *Bacillus thuringiensis* (Bt) that produces a protein toxic to insects. When pests try to eat the GM corn, the protein is toxic

to them, killing or deterring them from feeding. This can reduce the amount of pesticides needed to grow the crop and reduce damage from pests.

- Soybean: Soybeans have been genetically modified to be herbicide-tolerant, meaning they can withstand exposure to herbicides without being damaged. This is done by introducing a gene that produces an enzyme that breaks down the herbicide, allowing the soybean plant to survive while weeds are killed. This can make weed control easier and more effective, as farmers can use herbicides to kill weeds without harming the crop.
- Tomatoes: GM tomatoes have been engineered to have a longer shelf life. This is done by introducing a gene that slows down the ripening process, reducing the likelihood of spoilage and waste. This can have environmental benefits, as it can reduce food waste.
- Canola: Canola has been genetically modified to be herbicide-tolerant and produce higher yields than non-GM varieties. This is done by introducing genes that produce enzymes that break down the herbicide, allowing the canola plant to survive while weeds are killed. This can make weed control easier and more effective, as farmers can use herbicides to kill weeds without harming the crop. The increased yield can also help address global food security issues.
- Papaya: GM papaya varieties have been engineered to be resistant to the papaya ringspot virus, a disease that can devastate papaya crops. This is done by introducing a gene that produces a protein that is toxic to the virus, allowing the papaya plant to survive and produce fruit.
- Potatoes: GM potatoes have been engineered to be resistant to bruising and produce less acrylamide, a potentially harmful chemical that forms when potatoes are cooked at high temperatures. This is done by introducing genes that produce enzymes that break down the starch in the potato, reducing the amount of acrylamide that forms during cooking.
- Rice: Rice has been developed to contain higher levels of vitamin A, which can help address vitamin A deficiency in developing countries. This is done by introducing a gene from a bacterium that produces beta-carotene, a precursor to vitamin A, which is then converted into vitamin A in the body.
- Salmon: GM salmon has been genetically engineered to grow faster and larger than non-GM salmon. This is done by introducing a gene from another fish species that controls growth hormone production, allowing the salmon to grow year-round instead of only during the warmer months.

10.5.2 Advantages of GM foods

- Pest resistance: Crop losses from insect pests can result in huge financial loss for farmers and starvation in developing countries. Farmers conventionally use large quantities of chemical pesticides annually. Consumers today are aware of potential health hazards associated withpesticides treated crops, moreover run-off of agricultural wastes from excessive use of pesticides and fertilizers can poison the water supply and cause harm to the environment. Growing GM foods can help eliminate the application of chemical pesticides and reduce the cost of production.
- Herbicide tolerance: Farmers typically use tilling for removal of weeds but for some crops, it is not cost-effective to remove weeds by these physical means so farmers often spray large quantities of different herbicides to destroy weeds, a time-consuming and expensive process, which requires care so that the herbicide doesn't harm the crop plant or the environment. Genetically-engineered crops to be resistant to one very powerful herbicide that could help prevent environmental damage by reducing the amount of herbicides needed.
- Disease resistance: There are many viruses, fungi and bacteria that cause plant diseases. Plant biologists are working to create plants with genetically-engineered resistanceto these diseases.
- Cold tolerance: Unexpected frost can destroy sensitive seedlings. An antifreeze gene from cold water fish has been introduced into plants such as tobacco and potato. With this antifreeze gene, these plants are able to tolerate cold temperatures that normally would kill unmodified seedlings.
- Drought tolerance/salinity tolerance: As the world population grows and productive land isreducing farmers will need to grow crops that can survive in adverse conditions. Creating plants that can withstand long periods of drought or high salt content in soil and groundwater will help people to grow crops in formerly inhospitable places.
- Nutrition: Malnutrition is common in third world countries where impoverished peoples rely on a single crop such as rice for the main staple of their diet which does not contain adequate amounts of all necessary nutrients to prevent malnutrition. For example, blindness due to vitamin A deficiency is a common problem in third world countries. Researchers at the Swiss Federal Institute of Technology Institute for Plant Sciences have created a strain of "golden" rice containing an unusually high content of beta-carotene (vitamin A).

- Pharmaceuticals: Medicines and vaccines often are costly to produce and sometimes require special storage conditions not readily available in third world countries. Researchers are working to develop edible vaccines in tomatoes and potatoes. These vaccines will be much easier to ship, store and administer than traditional injectable vaccines.

10.5.3 Risks associated with GM Foods

There are many controversies around GM foods, including whether food produced with it is safe, whether it should be labelled and if so how, whether it is needed to address world hunger now or in the future, and more specifically with respect to intellectual property and market dynamics, environmental effects of GM crops and GM crops' role in industrial agricultural more generally. Many issues like the risk of unintended harm to the environment, the health concerns that consumers should be aware of and the benefits of recombinant technology, also arise with pest-resistant and herbicide-resistant plants. The evolution of resistant pests and weeds termed superbugs and super weeds is another problem. Health risks associated with GM foods are concerned with toxins, allergens, or genetic hazards. The mechanisms of food hazards fall into three main categories i.e.they are inserted genes and their expression products, secondary and pleiotropic effects of gene expression and the insertional mutagenesis resulting from gene integration. With regards to the first category, it is not the transferred gene itself that would pose a health risk. It should be the expression of the gene and the effects of the gene product that are considered. New proteins can be synthesized that can produce unpredictable allergenic effects. For example, bean plants that were genetically modified to increase cysteine and methionine content were discarded after the discovery that the expressed protein of the transgene was highly allergenic. Due attention should be taken for foods engineered with genes from foods that commonly cause allergies, such as milk, eggs, nuts, wheat, legumes, fish, molluscs and crustaceans.

10.6 SUM UP

This unit gave an understanding of fermentation process and outlined the history of fermentation. It introduced you to different types of fermented foods and explained about the microorganisms involved in preparation of these foods. The unit gave you an understanding about fermented milks, meat, fish, vegetables, vinegar, cheese as well as probiotics. The unit also introduced you to the concept of GM foods. The advantages and risks associated with use of GM foods were also explained in detail.

10.7 CHECK YOUR PROGRESS

1. Explain fermentation process.

.....

.....

.....

2. Write a short note on history of fermentation.

.....

.....

.....

3. Explain the role of microorganisms in preparation of alcoholic beverages.

.....

.....

.....

4. What is vinegar? How is it prepared?

.....

.....

.....

3 Write short notes on:

a) Probiotics

.....

.....

a) Fermented Vegetables

.....

.....

6. Explain role of fermentation process in cheese preparation.

.....

.....

.....

.....

7. What are GM foods? What are advantages of GM crops?

.....

.....

.....

8. What are the risks associated with GM foods?

.....

.....

.....

SUPPLEMENTARY READING

- Frazier, William C and Westhoff, Dennis C. Food Microbiology (2008). New Delhi: Tata McGraw-Hill Publishing Co. Ltd. P. 401-439.
- Adams, M R and Moss, M O. Food microbiology (2000). Cambridge: The Royal Society of Chemistry. P. 182-268.
- Ramesh, K Vijaya (2009). Food Microbiology. Chennai: MJP Publishers. P. 417-480
- Caroline Smith De Wall, (2003). Safe Food from a Consumer Perspective. Food Control. (14); 75-79.
- Shafiur Rahman, M. (2007). GMP and HACCP. Hand book of Food Preservation (Second Edition). CRC Press,,U.S.A.
- Sivasankar, B. (2005). Food Processing and Preservation. Food Quality. Published by Prentice-Hall of Indian Pvt Ltd, New Delhi.
- Prescott L.M., Harley J.P and Klein D.A (2005). Microbiology. 6th ed. McGraw Hill Publishers, USA. Pp. 296-299.
- R.C. Dubey and D.K. Maheshwari. A textbook of Microbiology, S Chand & Company P Ltd, New Delhi.
- Kathleen Park Talaro and Barry Chess, Foundations in Microbiology, 10^{th} edition
- Gerard J. Tortora, Berdell R. Funke and Christine L. Case. Microbiology: An Introduction, 13th Edition.
- Food microbiology by V. Ramesh, MJP publishing.
- Food microbiology by W.C. Frazier, Ist Edition by Mcgraw Hill Pub. Co. New York.
- Modern Food Microbiology, J.M. Jay. CBS publisher.
- Chandrashekar, U. (2002). Food Science and Applications in Indian Cookery. Phoenix Publishing House Pvt. Ltd.
- Insel, P., Turner, E.R., Rross, D. Nutrition, 3rd edition. American Dietetic Association.
- Jood, S. and N. Khetarpaul, 1991. Food Preservation. Agrotech Publishing Academy. Jaipur.
- Joshi, S. A. (2010). Nutrition and Dietetics with Indian Case Studies. Tata McGraw Hill Education Pvt. Ltd., Mumbai.

- Manay, S. N. AND M. Shadaksharawamy, (2001). Foods, Facts and Principles. 3rd edition, New Age International. New Delhi.
- Mudambi, S. R. AND M. V. Rajgopal (2006). Fundamentals of Foods and Nutrition. Wiley Eastern Ltd.
- Raheena Begum. (1989). A Textbook of Foods, Nutrition and Dietetics. Sterling Publishers Pvt. Ltd. New Delhi.
- Rao, J. P. (2006). Nutrition and Food Science. Aavishkar Publishers, Distributors, Jaipur.
- Satarkar, A. (2008). Food Science and Nutrition. ABD Publishers. Jaipur.
- Sethi, M. (2008). Institutional Food Management. New Age Publishers. New Delhi.
- Subbulakshmi, G. and S. A. Udipi, (2006). Food Processing and Preservation. New Age Publishers, New Delhi.
- Wadhwa, A. and S. Sharma, (2003). Nutrition in the Community. A Textbook. Elite Publishing House Pvt. Ltd.
- Black, J.G. (2008). Microbiology: Principles and Explorations (7th ed.). Hoboken, NJ: J. Wiley & Sons.
- Nester E.W, Anderson D.G, Roberts C.E and Nester M.T (2009). Microbiology: A Human Perspective. Sixth edition. McGraw-Hill Companies, Inc, New York, USA.
- Singleton P and Sainsbury D (1995). Dictionary of microbiology and molecular biology, 3d ed. New York: John Wiley and Sons.
- Slonczewski J.L, Foster J.W and Gillen K.M (2011). Microbiology: An Evolving Science. Second edition. W.W. Norton and Company, Inc, New York, USA.
- Salyers A.A and Whitt D.D (2001). Microbiology: diversity, disease, and the environment. Fitzgerald Science Press Inc. Maryland, USA.

SUGGESTED ONLINE LINKS

- www.FDA/CFSAN FDA 2001- Food Code.
- www.FAO. Org
- Microbial diversity and systematic: 1075X_CH03_025.qxd (jblearning.com
- Microbiological Laboratory Techniques: Microbiological Laboratory Techniques (mowr.gov.in)
- Antibiotics and chemotherapeutic agents: Micro 260 Antibiotic agents and Modes of Action.pdf (spokane.edu)
- http://www.who.int/foodsafety/publications/fs_management/en/surface_decon.pdf
- http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5009a1.htm
- http://www.medicinenet.com/botulism/article.htm
- http://www.cdc.gov/listeria/
- http://www.cdc.gov/ncidod/dbmd/diseaseinfo/yersinia_g.htm
- http://www.nlm.nih.gov/medlineplus/ency/article/000224.htm
- Environmental Toxicology: Environmental Toxicology 3rd edition.pdf (unp.ac.id)
- WHO website www. whoindia.org, www.who.int
- UNICEF website www.unicef.org
- Websites of Government of India's Ministry of Food Processing, Ministry of Health and Family Welfare, Women and Child Development