Uttar Pradesh Rajarshi Tandon Open University

UGHN 105- Elementary Food Microbiology

Unit I: Brief History of Microbiology: Introduction important microorganism in foods.-

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Content

1.0 Objectives

After reading this unit, students will be able to:

i. Describe the practical applications of microbiology.
ii. Recognise the value and application of food microbiology.
iii. List the significant genera of microbes connected to food.
iv. Describe the typical micro biota in several common foods..

1.1 Introduction

Microorganisms are microscopic living things, such as bacteria, viruses, fungus (a collective term for moulds, yeasts, and algae), protozoa, and bacteria. Virens do not have regular cell structures and are categorised independently. While bacteria are prokaryotes (cells without distinct nuclei), fungi, algae, and protozoa are eukaryotes (cells with nuclei). The soil, water, atmosphere, animals, plants, and other living things all contain microorganisms, and all of these places are conducive to their growth, with the exception of the atmosphere. They collectively outnumber all other living things on the planet by a large margin. Over 3 billion years ago, they were the first living cells to occupy the planet. Since then, they have played significant functions, many of which are advantageous to other living systems. Some moulds, yeasts, bacteria, and viruses are microorganisms that can play both beneficial and harmful roles in our food. This unit has covered the range of food microbiology, the significance of food microbes, and the most common food-related bacteria.

1.2 The science of microbiology

The field of biology known as microbiology studies microorganisms such as bacteria, fungus, certain algae, protozoa, viruses, viroids, and prions. The majority of microbes exhibit the following traits:-

1. Since they are typically too small to be observed by the unaided eye, some form of microscopy is necessary to analyse their structure.
2. Cells or other structures are relatively simple and less specialized than those of higher plants and animals.
3. In the laboratory, they are handled and cultivated in ways that are typically relatively similar.

The science of microbiology has evolved in such a way that may be approached from various perspectives. Each of the distinct groups that gave rise to the following disciplines might be the subject of a specialised study:

- Mycology - the study of fungi
- Protozoology - the study of protozoa
- Bacteriology - the study of bacteria
- Virology - the study of viruses
- Phycology (algology) - the study of algae

Microorganisms can also be studied from an applied perspective, i.e., how they interact with the environment, human activity, and other microorganisms. This again results in a number of fields of specialised research:

- **Medical microbiology** includes some aspects of pathology (the study ofdiseases), immunology (how the immune system operates to preventinvasion by micro-organisms) and epidemiology (how diseases aredistributed and spread).
- **Agricultural microbiology:** The study of micro-organisms for crop/plant health and related areas.
- **Industrial microbiology / biotechnology:** The study of the use of Microorganismsin large scale industrial processes.
- **Food microbiology:** The study of the role that micro-organisms play infood spoilage, food production, food preservation and food-borne disease.

Each of these specialised fields of study cannot function independently. For instance, food microbiology incorporates many facets of industrial microbiology and biotechnology in the production of fermented foods and single-cell proteins. Aspects of both medical microbiology and agricultural microbiology are involved in the study of food-borne illness. Expertise must be supported by a solid grasp of fundamental concepts. For instance, the food microbiologist must comprehend microbial structure, microorganism classification and identification, how microorganisms grow, factors that affect growth, and how growth can be controlled, as well as microorganism death, nutrition, and laboratory culture techniques.

1.3 Origin and scope of food microbiology

Although food deterioration mechanisms, food preservation techniques, and food fermentation have been understood since ancient times, the relationship between food and microorganisms was not established until the 1800s. Between 1857 and 1876, Pasteur demonstrated that microorganisms are responsible for the chemical changes that occur in foods and beverages. In 1837, Schwann hypothesised that the yeast that formed during alcoholic fermentation was a microscopic plant. Their findings served as the cornerstone for the growth of food microbiology as it is known today. Soon after these early discoveries, studies on the function that microbes play in food preservation, food spoilage, and food

poisoning developed quickly to the point where food microbiology gradually became recognised as a separate field of study. Food microbiology is now a highly developed area of knowledge with the main areas of interest highlighted in Fig. 1.1.

Fig. 1.1 Major topics of study in food microbiology

The food microbiologist is not equally invested in all microbial groups. The group of organisms is heavily dominated by bacteria, followed by moulds and yeasts, which are also extremely significant, and viruses, which are less so. The relationship that these organisms have with the production and consumption of foods are summed up in Fig. 1.2.

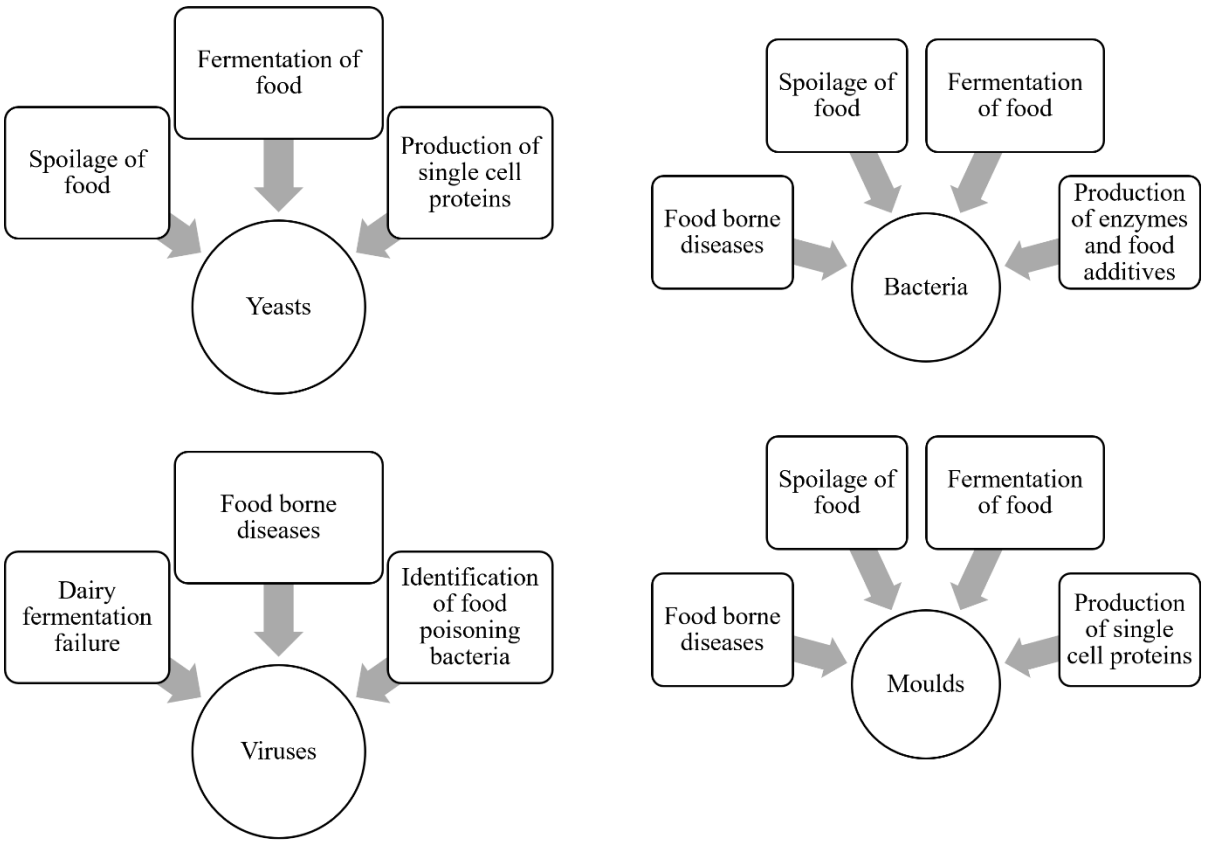

Fig. 1.2 Different group of microorganisms and their relationship with food

Protozoa and algae seldom directly affect how food is grown, processed, and consumed. Some protozoa can cause food-borne illness, and others in this group are crucial for the proper waste management. Algae are used to make alginates; some could be utilised to make single-cell proteins; and some marine species make poisons that could end up in our food supply along with seafood.

1.4 Important micro-organisms in food

Among the most important genera and species typically found in food products are the microbes described below. Moulds and yeasts are the two main types of microorganisms that constitute the fungi, which are less prevalent than bacteria. In addition to this, food may also include parasites like worms, protozoans, and viruses.

1.4.1 Bacteria

Unicellular bacteria have a diameter of around one micrometre (10^{-3} mm) and come in a variety of shapes, including short, elongated rods (bacilli), spherical or ovoid forms (cocci), vibrio (comma-shaped), and even spirals. Cocci are spherical bacteria with the name "berry" in their name. According to their genera, individual bacteria closely unite to generate distinct forms. Some sphere-shaped bacteria, such as staphylococci, are found in clusters resembling a bunch of grapes. Streptococci are joined together to form chains with other bacteria that are rod- or sphere-shaped, such as cocci. While some genera of sphere-shaped bacteria appear as a single bacterium, others are found in pairs (diplococci, such as Pneumococci) or in groups of four (Square or cubical packages formation, such as *Sarcinia*). The majority of other bacteria are rod-shaped, have flagella, and can move. Bacteria create a wide range of colours, from light yellow colours to dark pigments like brown or black. Some bacteria have intermediate colour pigmentation, including red, pink, orange, blue, green, and purple. These bacteria specifically induce food discoloration in foods like meat that contain unstable colour pigments. Slime formation is another way that some bacteria produce discolouration.

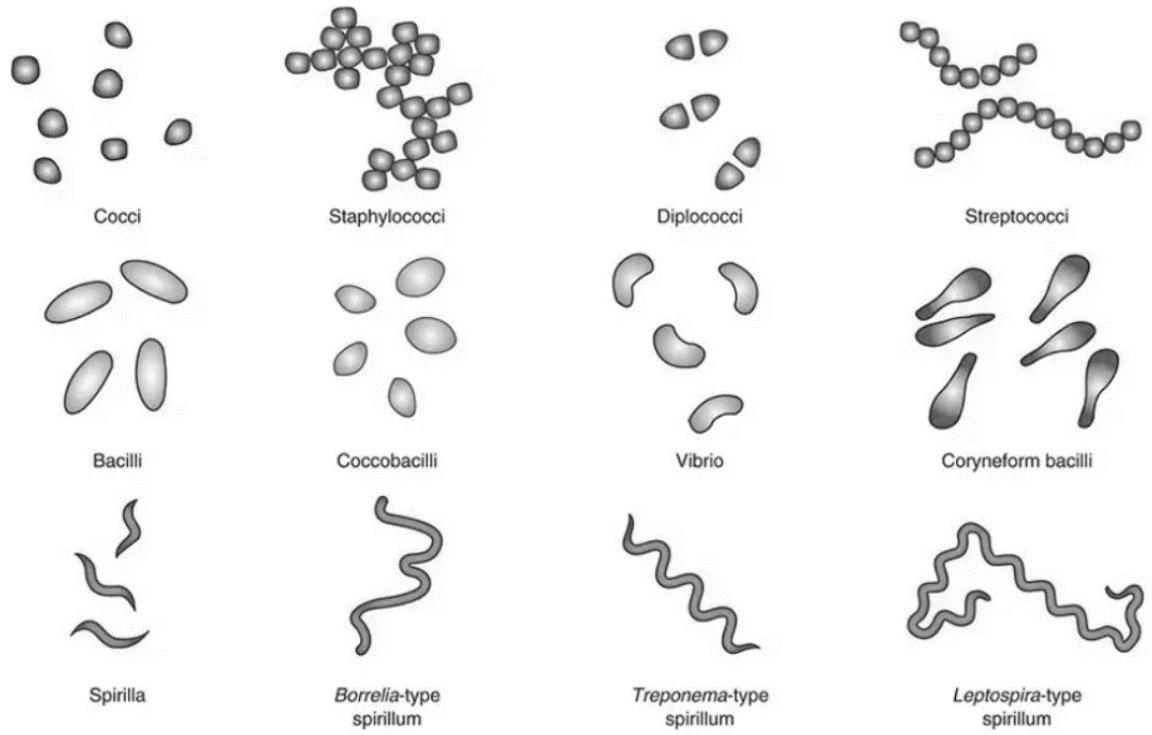

Fig.1.3 Different shapes of bacteria

Common Bacterial Groups in Foods

One of the most significant groups of microorganisms present in food is bacteria. This is due to a variety of factors, including their rapid growth rates, capacities for utilising food nutrients, resistance to a wide range of environmental conditions, including aerobiosis, pH, and water activity, as well as their ability to endure challenging conditions, such as the survival of spores at high temperatures. Food-related bacteria that are significant have been arbitrarily categorised into numerous groups based on shared traits for the sake of convenience.There is no taxonomic importance to this category. Here is a summary of some of these groups and the significance of foods in each.

1)Lactic Acid Bacteria

Those microorganisms that convert carbohydrates into lactic acid in quite significant amounts. Include species primarily from the genera *Streptococcus thermophilus, Lactococcus, Leuconostoc, Pediococcus.*

2) Acetic Acid Bacteria

Those bacteria that produce acetic acid, such as *Acetobacter aceti.*

3) Propionic Acid Bacteria

Those bacteria that are used in dairy fermentation produce propionic acid. Include species such as *Propionibacterium freudenreichii.*

4) Butyric Acid Bacteria

Those microorganisms that generate a substantial amount of butyric acid. Some Clostridium spp., such as *Clostridium butyricum.*

5) Proteolytic Bacteria

These are bacteria that produce extracellular proteinases, which enable them to hydrolyze proteins. Species in genera *Micrococcus, Staphylocccus, Bacillus, Clostridium, Pseudomonas, Alteromonas, Flavobacerium,* and *Alcaligenes;* some in *Enterobacteriaceae* and *Brevibacterium*are also included in this group.

6) Lipolytic Bacteria

Bacteria which are able tohydrolyze triglycerides due to production of extracellular lipases. Species in genera *Micrococcus, Staphylococcus, Serration, Pseudomonas, Alteromonas, Alcaligenes* and *Flavobacterium* are included in this group.

7*)* Saccharolytic Bacteria

Bacteria which are able tohydrolyze complex carbohydrates. Include some species in genera *Bacillus, Clostridium, Aeromonas, Pseudomonas,* and *Enterobacter*.

8) Thermophillic Bacteria

Those bacteria which are able to grow at 50˚C and above. Include some species from genera *Bacillus, Clostridium, Pediococcus, Streptococcus,* and *Lactobacillus.*

9) Psychrotrophic Bacteria

Able to grow at refrigerated temperature (<5˚C). Include some species of *Pseudomonas, Alteromonas, Alcaligenes, Flavobacterium, Serratia, Bacillus, Clostridium, Lactobacillus, Leuconostoc, Listeria, Yersinia* and *Aeromonas.*

10) Thermoduric Bacteria

Able to survive pasteurization temperature. Include some species of *Micrococcus, Enterococcus, Lactobacillus, Pediococcus, Bacillus* (spores) and *Clostridium* (spores).

Some of the most common genera of bacteria found in food are listed here.

a) ***Acinetobacter***

Acinetobacter is a genus of Gammaproteobacteria, which includes Gram-negative bacteria. Under magnification, it can be seen that *Acinetobacter* species are non-motile, oxidase-negative, and usually found in pairs. Young cultures have morphologies that resemble rods. They do not lower nitrates since they are stringent aerobes. They are significant water and soil organisms and are also present on many foods, particularly fresh items that have been refrigerated. *A. baumannii* is a prevalent cause of nosocomial pneumonia, particularly

pneumonia caused by late beginning ventilator use. It can also result in bacterial meningitis, skin and wound infections, and bacteremia.

b) ***Bacillus cereus***

The organism, known as *B. cereus*, is a thick, long, rod-shaped, aerobic, Gram-positive, catalase-positive spore producer that plays a significant role in food-borne disease. It is a typical soil resident that stays away from a variety of foods. Because it is thermoduric, eating desserts, meat, dishes, dairy products, rice, pasta, and other foods that are cooked and held at room temperature is frequently a cause of diarrheal disease. Some *B. cereus* strains are psychrotrophic, meaning they can grow at cold temperatures.

c) ***Bacillus subtilis***

The gram-positive, catalase-positive bacteria *Bacillus subtilis*, usually referred to as the hay bacillus or grass bacillus, is frequently found in soil. *Bacillus subtilis* is a slim, short rod-shaped member of the genus with the capacity to create a hard, protective endospore, which enables the organism to withstand harsh environmental conditions. The proteolytic enzyme subtilisin is produced by B. subtilis. Spores of *B. subtilis* can endure the high heat of cooking. Ropiness, a sticky, stringy texture brought on by bacterial synthesis of long-chain polysaccharides in ruined bread dough, is induced by *B. subtilis*.

d) ***Clostridium perfringens***

C. perfringens is a Gram-positive encapsulated anaerobic non-motile bacterium commonly found on meat and meat products. It has the potential to spread food-borne illness. It is a toxin-producing organism which produces enterotoxin and -toxin, both of which have effects on the human GI system.

In food, it multiplies very quickly (doubling time less than10 min). Spores can live in foods that have been partially or insufficiently cooked because they are resistant to radiation, desiccation, and heat.

However, it tolerates moderate exposure to air. Vegetative cells of *C. perfringens* are also somewhat heat tolerant as they have relatively high growth temperature (43°C -45°C) and can often grow at 50°C. They are not tolerant to refrigeration and freezing. No growth occurs at 6 °C. *C. perfringens* is present in soil and the other natural environment.

e) ***Clostridium botulinum***

The most lethal toxin currently known is produced by *C. botulinum*. It is a rod-shaped, Gram-positive anaerobic bacterium. In cultures in the stationary phase, oval endospores form. Based on the serological specificity of the neurotoxin generated, there are seven different kinds of *C. botulinum* (A to G). Botulism is a rare but deadly illness. Neurotoxin generated

by the organism and ingested through food can be fatal. Heat, on the other hand, can quickly deactivate the toxin (a protein).The organism can grow at temperature ranging from 10-48 °C with optimum growth temperature at 37°C. Spores are highly heat resistant. The outgrowth of spores is inhibited at pH < 4.6, NaCl> 10% or water activity< 0.94. The radiation-resistant spores that pose the greatest threat to public health are likely botulinum spores. Foods are contaminated through the soil and sediments, which frequently contain them. The bacteria thrives in obligatory anaerobic environments and creates toxins in improperly canned, low acid foods that have not been sufficiently processed.

f) ***Escherichia coli***

Food-borne gastroenteritis has an association with some *E. coli* strains. These lactose-fermenting asprogeneous rods on Endo agar generate dark colonies with a metallic sheen and are gram-negative. The organism thrives in a variety of diets and on a wide range of mediums. They grow over a wide range of temperature (4 to 46 °C) and pH (4.4 to 9.0).
However, they grow very slowly in foods held at refrigerator temp. (5 °C). They are members of the *Enterobacteriaceae* family. The bacteria also serve as a marker for faecal contamination. Additionally, the bacterium has the ability to produce gas, acid, and off odours in meals. Enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC), and facultatively enteropathogenic (FEEC) strains of E. coli are those that cause foodborne sickness.

g) ***Salmonella* (*S. typhimurium, S. typhi, S.enteritidis*)**

According to reports, *Salmonella* spp. are a major factor in human food-borne infections. Salmonellosis is the most common foodborne bacterial infection in people. Serious human illness known as enteric fever is linked to typhoid and paratyphoid strains. The optimum growth temperature is 37-45 °C. The organism can also grow at about 7°C in foods. It ferments carbohydrates with its production of acid and gas. Salmonella are oxidase negative, catalase positive and grow on citrate as a sole carbon source and produce H_2S. Some *Salmonella* strains can grow at higher temperatures (54°C) while others exhibit psychrotrophic properties. The organism has the ability to grow at pH values ranging from 4.5 to 9.5, with an optimum pH growth at 6.5 to 7.5. They are facultatively anaerobic, small Gram-negative, non-spore forming, rod-shaped (2-4 mm) bacteria belonging to the familyMilk, meat and poultry are principal vehicles of human foodborne salmonellosis. Ingestion of only a few salmonella cells can be infectious. Low levels of salmonellae in a finished food products may, therefore, be of serious public health consequence.

h) ***Staphylococcus aureus***

Humans and *Staphylococcus aureus* are often associated. It is a catalase-positive, gram-positive cocci. Foodborne gastroenteritis, often known as staphylococcal food poisoning, is frequently caused by *Staphylococcus aureus*. The consumption of food containing one or more enterotoxins, which are produced by some strains of *S. aureus*, results in staphylococcal gastroenteritis.

Although numerous species of *Staphylococcus* that do not produce coagulase or TNase are known to also create enterotoxin, it is widely thought that enterotoxin production is related with *S. aureus* strains that produce these two enzymes. Human nasal cavities are the primary source of S. aureus, from which it spreads to the skin and wounds.Mastitis in animals due to *S. aureus* is quite common and from the infected udder the organism finds its way to the milk.

1.4.2 Moulds

Moulds are multicellular, filamentous microbes having a mycelial morphology. These microorganisms can also display a range of colours, and they can be identified by their fuzzy, mildewed look that resembles cotton. Moulds are able to produce a large number of the minute spores that are present in the air and are distributed by air currents. If these spores are moved to an area with favourable germination conditions, they may result in fresh mould growth. Moulds can frequently survive more temperature variation and can generally withstand greater pH variation than bacteria and yeasts. Even though moulds prefer an acidic to neutral pH, they can survive a pH range of 2.0 to 8.0. Moulds thrive best at or around a pH of 7.0. Although they can develop below 0°C, moulds thrive better at room temperature than in a colder environment. Although water activity (A_w) of about 0.85 is the ideal level for mould growth, it can and can happen below 0.80. At an A_w of 0.90 or higher, yeasts and bacteria often develop more quickly and take advantage of the available nutrients at the expense of moulds. Moulds might grow more quickly when the Aw falls below 0.90. Foods with little moisture content, such pastries, cheeses, and nuts, are therefore more susceptible to spoiling due to the growth of mould. Some of the most common genera of moulds found in food are listed here.

1) ***Aspergillus*:** They are widely distributed and contain many species that are important in food. They generate conidia or a sexual spore (black in colour) and have septate hyphae. Many may grow in low A_w (xerophilic) environments and can grow in grains, spoiling them. Additionally, they contribute to the rotting of foods including jams, cured ham, nuts, fruits, and vegetables. Some species and strains (like *Aspergillus flavus*, which makes aflatoxin) produce mycotoxin. In the preparation of food and food additives, numerous

species and strains are also utilised. In order to make sake, *Aspergillus oryzae* is employed to hydrolyze starch using alpha-amylase. Citric acid is produced by *Aspergillus niger* from sucrose, and it also makes galactosidase and other enzymes.

2) ***Alternaria*:** They are also septate and form dark-brown colored many celled conidia on the conidiophere. They cause rot in tomatoes and rancid flavor in dairy products. Species: *Alternaria tenuis.*

3) ***Geotrichum*:** The hyphae are septate and form rectangular asexual arthrospores (oidia). They grow forming a yeast like, cottony, creamy colony. They establish easily in equipment and often grow on dairyproducts **(**also known as dairy mold**).** Species: *Geotrichumcandidum.*

4) ***Mucor*:** They are widely distributed. They have nonseptate hyphae andproduce sporangiophores. They produce cottony colonies. Some speciesare used in food fermentation and production of enzymes. They causespoilage of vegetables. Species: *Mucor rouxii.*

5) ***Penicillium*:** They have several different species and are widely dispersed. They have septate hyphae and produce conidiophore on the heads of blue-green conidia that resemble brushes. Some species, such *Penicillium roquefortii* and *Penicillium camembertii* in cheese, are employed in the manufacturing of food. There are numerous species that cause fruit and vegetable rot.

6) **Rhizopus:** The aseptate hyphae produce sporangiophores in the sporangium. Many fruits and vegetables get spoiled as a result of their involvement. Black bread mould, or *Rhizopus stolonifer*, is quite prevalent.

1.4.3 Yeasts

Yeasts are typically unicellular and distinct from bacteria due to their huge cell size, distinctive shape, and ability to generate buds during divisional reproduction. Similarto moulds, yeasts can spread by the air or by other ways and settle on food surfaces. Yeast colonies typically have a slimy or damp appearance and are creamy white in colour. Although they can grow below 0.90, yeasts prefer an A_w of 0.90 to 0.94. These microorganisms thrive in the pH 4.0–4.5 range of intermediate acidity. Food with a high yeast contamination rate typically smells slightly fruity. Several important genera of yeast are briefly described below:

1) ***Saccharomyces*:** Cells might be elongated, oval, or spherical. It is the most significant genus and is made up of various groups. Different strains of *Saccharomyces cerevisiae* are employed in baking to leaven bread and ferment alcohol. Additionally, they contribute to food degradation by producing alcohol and CO_2.

2) ***Pichia*:** They are pellicle-forming cells that range in shape from oval to cylindrical and ruin beer, wine, and brine. Others are employed in the fermentation of Asian foods. Species: *Pichia membranaefaciens.*

3) ***Rhodotorula*:** They are pigment-forming yeasts that can turn foods red, pink, or yellow, which can affect meat, fish, and sauerkraut, among other things.Species *Rhodotorulaglutinis.*

*4)****Torulopsis*:** They have spherical to oval structure. They cause spoilageof milk due to the ability to ferment lactose (*Torulopsissphaerica*).They also spoil fruit juice concentrates and acid foods.

5) ***Candida*:** Many contaminate food by adding excessive salt, sugar, or acid to it, or by forming pellicle on the surface of liquids. Some may cause butter and dairy products to go rancid. *(Candida lipolytica).*

6) ***Zygosaccharomyces*:** Involved in spoilage of foods, containing high sugar/salt levels ex. honey, sirups, molasses, soy sauce. (*Zygosaccharomycesnussbaumeri).* These yeasts are termed osmophilic, because they cangrow in high concentrations of solutes.

1.4.4 Viruses

Viruses can infect plants, animals, and microorganisms and range in size from 10 to 450 nm. They can also cause specific diseases in particular hosts and cannot multiply without a living host. Foods, water, and air can all spread disease. Bacteriophages are the name for viruses that infect bacteria. The order **Virales**includes viruses. Because of their small size, viruses cannot be seen under a standard compound microscope. Direct observation of viruses was only possible following the invention of the electron microscope. A protein coat surrounds the DNA or RNA core of viruses. They employ the cellular machinery of the host cell to grow and divide because they lack all the components for typical cellular metabolism. Once they invade a host cell, however, viruses can multiply very rapidly.

Table 1.1: Human Intestinal Viruses with High Potential as Food Contaminants

Picornaviruses	Reoviruses	Parvoviruses	Papovaviruses	Adenoviruses
• Polioviruses • Coxsackievirus A • Coxsackievirus B • Echovirus • Enterovirus	• Reovirus • Rotavirus	• Human gastrointestional viruses	• Human BK and JC viruses	• Human adenoviruses

1.3.5 Parasitic Organisms

Many parasitic worms can infect humans and spread diseases through food. Animals' hearts, lungs, and gastrointestinal tracts inhabits home to cestodes, which are flatworms. Fish, dogs, bears, swine, beef, and other canine animals can all carry tapeworms and flatworms that can spread to humans and infect them. Trematodes are non-segmented flatworms with a mouth and an oral sucker that need a snail as an intermediary host before they may infect people when they consume aquatic vegetation or drinking water. Examples of parasites that are spread through food include intestinal flukes, pyriform worms from fish, sheep and Chinese liver flukes, and oriental lung flukes. True roundworms or nematodes can also be transferred from animals to people. Humans are contaminated by cockroach and dung beetle eggs found in the faeces of cattle, sheep, and hogs. Trichinella spiralis, a worm, can cause trichinosis, an inflammation of the muscles. The most prevalent vector is pork. Pinworms, whipworms, and capillary worms are other nematode parasite types. Protozoa are microscopic single-celled animals, which can be taken in withfood or water to cause human illness. *Entamoeba histolytica, Toxoplasmagondii, Balantidium coli,* and *Giardia lamblia* are the most common foodborne protozoan parasites.

1.5 Summary

In this unit,the definition of microbiology as a science and its practical applications are briefly covered. The scope of food microbiology has been highlighted. The unit's contents place a strong emphasis on the different kinds and significance of microorganisms in food. In terms of morphology, occurrence, and importance in food, various microorganisms (such as bacteria, fungi, moulds, and yeasts), viruses, and parasitic creatures are discussed. It has been addressed how they affect food production, food preservation, food deterioration, and food-borne illnesses. The typical micro flora of various popular food groups is also addressed because bacteria are connected to the majority of the meals we consume. As a result, the entire course focuses on the most common microorganisms found in food and highlights both of their roles in foods—both beneficial and detrimental.

1.6Suggested reading

Banwart, G.J. (1979), *Basic Food Microbiology,* AVI Publishing Co. Inc., Westport, Connecticut.

Frazier, W.C. and Westoff, D.C. (1996), *Food Microbiology,* Tata McGraw Hill Publishing Co. Ltd., New Delhi.

Pelczar, M. Jr.; Chan, E.C.S. and Kreig, N.R. (1993), *Microbiology,* Tata McGraw Hill Inc., New York.

Garbutt, J. (1998), *Essentials of Food Microbiology,* Arnold International Student's Edition, London.

Check your knowledge

Fill in the blanks:

1. _____ multicellular, filamentous microbes having a mycelial morphology.
2. Viruses are _____ in size; cannot reproduce without a living host.
3. Lactic Acid Bacteria convert carbohydrates into _____.
4. *Salmonella*has the ability to grow at pH values ranging from _____, with an optimum pH growth at _____.
5. The most lethal toxin currently known is produced by _____.

Write short note on the following:

1. What is food Microbiology?

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2. Classify bacteria based on their morphology.

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3. Discuss about parasitic organisms which infect food.

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Unit II: Cultivation of Microorganisms: Nutritional requirement of microorganisms, Types of media used and methods of isolation

Content

2.1 Introduction

From germs to people, all life forms have basic dietary needs to thrive and function normally. The following observations support this assertion and demonstrate the wide variety of nutritional forms that are present in bacteria.

1. All organisms need a source of energy. Some rely on chemical compounds fortheir energy and are regarded as chemotrophs. Others can utilize radiant energy(light) and are called phototrophs. Both chemotrophs and phototrophs exist amongbacteria.
2. All organisms require a sourceof electrons for their metabolism. Some organismscan use reduced inorganic compounds as electron donors and are termed lithotrophs (some may be chemolithotrophs, others photolithotrophs). Other organismsuse organic compounds as

electron donors and are called organotrophs (some arechemo organotrophs, others photoorganotrophs).

3. All organisms require carbon in some form for use in synthesizing cell components.However, some can use CO_2 as their major, or even sole, source of carbon; such organisms are termed autotrophs. Others require organic compounds as their carbon source and are termed heterotrophs.
4. All organisms require nitrogen in some form for cell components.
5. All organisms require oxygen,sulphur, and phosphorus for cell components. Oxygen is provided in various forms, such as water; component atoms of various nutrients; or molecular oxygen. sulphur is needed for synthesis of certain amino acids (cysteine, cystine, and methionine). Some bacteria require organic sulphur compounds, some are capable of utilizing inorganic sulphur compounds, and some can even use elemental sulphur. Phosphorus, usually supplied in the form of phosphate, is an essential component of nucleotides, nucleic acids, phospholipids, teichoic acids, and other compounds.
6. All microorganisms require metal ions, such as K_+, Ca^{2+}, Mg^{2+}, and Fe^{2+} and other metals ions for normal growth.
7. All microorganisms contain vitamins and vitamin like compounds. These function either as coenzymes for several enzymes or as the building blocks for coenzymes.
8. All living organisms require water, and in the case of bacteria all nutrients must be in aqueous solution before they can enter the cells.

2.2 Nutritional types of microorganisms

Microorganisms can be divided into many groups based on their nutritional requirements. The major separation is into two groups, phototrophs and chemotrophs.

2.2.1 Phototrophs

Among the phototrophic bacteria are species that use inorganic compounds as their source of electrons (i.e., photolithotrophs). For example, *Chromatiumokenii* uses H_2S as its electron donor, oxidizing it to elemental sulphur.Some phototrophic bacteria use organic compounds such as fatty acids and alcohols as electron donors and are therefore photoorganotrophs. For example, *Rhodospirillum rubrum* can use succinate as an electron donor.

2.2.2 Chemotrophs

Among the chemotrophic bacteria are species that use inorganic compounds astheir source of electrons (i.e., chemolithotrophs). For example, bacteria of the genus *Nitrosomonas* use ammonia as their electron source, obtaining energy by oxidizing ammonia to nitrite.Many other chemotrophic bacteria use organic compounds, such as sugars and amino acids, at electron donors and are therefore chemoorganotrophs. Certain bacteria can grow as either chemolithotrophs or chemoorganotrophs. For example, *Pseudomonas pseudoflovo* can use either the organic compound glucose or the inorganic compound H_2 as its source of electrons.In terms of chemical complexity of nutrient substances

required for growth, the autotrophic bacteria exhibit the simplest requirements.The fact that an autotrophic organism can grow and reproduce in a mixture of inorganic compounds indicates that it has an elaborate capacity to transform these compounds into the carbohydrates, proteins, nucleic acids, lipids, vitamins, and other complex organic substances that constitute the living cell.The heterotrophic bacteria, although they constitute one major nutritional group, vary considerably in the specific nutrients required for growth, particularly with respect to their organic carbon sources, nitrogen sources, and vitamin requirements.

2.2.3 Autotrophs and Heterotrophs

Organisms that can use CO_2 as their sole source of carbon for assimilation are termed autotrophs.Some autotrophs are facultative autotrophs; i.e., they can either live as autotrophs, deriving their carbon from CO_2, or they can live as heterotrophs, deriving their carbon from organic compounds. For example, *P. pseudoflava* can live as a heterotroph, using glucose as a source of carbon for assimilation however, if H_2 is provided as the electron source, then it can use CO_2 as its sole carbon source and can grow as an autotroph.

2.2.4 Obligate Parasites

Some bacteria have not yet been successfully cultivated on an artificial medium, and their nutritional and physical requirements are not understood. At present, such bacteria can be propagated only in association with a living host which, in a sense, serves as the medium. One example is the bacterium that causes leprosy, *Mycobacterium ieprae*, which can be cultivated by infecting mice or armadillos.

2.3 Bacterial media

Chemically defined media are needed for the cultivation of autotrophs and are also useful for defining the nutritional requirements of heterotrophs. However, for the routine cultivation of heterotrophs, chemically defined media are not generally used. Instead, certain complex raw materials such as peptones, meat extract, and yeast extract are used, and the resulting media support the growth of a wide variety of heterotrophic bacteria. Agar is included as a non-nutritive solidifying agent when a solid medium is desired.A description of these raw materials is given in Table 2.1. Examples of relatively simple liquid and solid media that support the growth of many common heterotrophs are nutrient broth and nutrient agar (Table 2.2). The addition of yeast extract to each of these formulas improves the nutrient quality, since yeast extract contains several of the B vitamins and other growth-promoting substances. Other complex supplements such as bovine rumen fluid, animal blood, blood serum, or extracts of plant and animal tissues may be required for the cultivation of certain fastidious heterotrophs.

Table 2.1 Characteristics of several Complex Materials Used as Ingredients of Media

Raw Material	Characteristics	Nutritional importance
Beef extract	An aqueous extract of lean beef tissue concentrated to a paste	Contains the water-soluble substances of animal tissue, which include carbohydrates, organic nitrogen compounds, water-soluble vitamins, and

		salts
Peptone	The product resulting from the digestion of proteinaceous materials, e.g., meat, casein, and gelatin, digestion of the protein material is accomplished with acids or enzymes; many different peptones (depending upon the protein used and the method of digestion) are available for use in bacteriological media; peptones differ in their ability to support growth of bacteria	Principal source of organic nitrogen; may also contain some vita. mins and sometimes carbohydrates, depending upon the kind of proteinaceous material digested
Agar	A complex carbohydrate obtained from certain marine algae; processed to remove extraneous substances	Used as a solidification agent for media; agar, dissolved in aqueous solutions, gels when the temperature is reduced below 45°C; agar not considered a source of nutrient to the bacteria
Yeast extract	An aqueous extract of yeast cells, commercially available as a powder	A very rich source of the B vitamins; also contains organic nitrogen and carbon compounds

Table 2.2Composition of Nutrient, Broth and Nutrient Agar

Nutrient broth	**Quantity**
Beef extract	3g
Peptone	5g
Water	1,000 ml
Nutrient agar	
Beef extract	3g
Peptone	5 g
Agar	15 g
Water	1,000 ml

2.3.1 Types of Media

Many special-purpose media are needed to facilitate recognition, enumeration, and isolation of certain types of bacteria.To meet these needs, the microbiologisthas availablenumerous media which, on thebasis of their application or function, may be classified as follows:

2.3.1.1 Selective Media

These media provide nutrients that enhance the growth and predominance of a particular type of bacterium and do not enhance (and may even inhibit) other types of organisms that may be present. For instance, a medium in which cellulose is the only carbon source will specifically select for or enrich the growth of cellulose-utilizing organisms when it is inoculated with a soil sample containing many kinds of bacteria.

2.3.1.2 Differential Media

Certain reagents or supplements, when incorporated into culture media, may allow differentiation of various kinds of bacteria. For example, if a mixture of bacteria is inoculated onto a blood-containing agar medium- (blood agar), some of the bacteria may hemolyze (destroy) the red blood cells; others do not. Thus, one can distinguish between hemotyticand non-hemolytic bacteria on the same medium.

2.3.1.3 Assay Media

Media of prescribed compositions are used for the assay of vitamins, amino acids, and antibiotics. Media of special composition are also available for testing disinfectants.

2.3.1.4 Media for Enumeration of bacteria

Specific kinds of media are used for determining the bacterial content of suchmaterials as milk and water. Their composition must adhere to prescribed specifications.

2.3.1.5 Media for Characterization of bacteria

A wide variety of media are conventionally used to determine the type of growthproduced by bacteria, as well as to determine their ability to produce certain chemical changes.

2.3.1.6 Maintenance Media

Satisfactory maintenance of the viability and physiological characteristics of a culture over time may require a medium different from that which is optimum for growth. Prolific, rapid growth may also be associated with rapid death of the cells at the end of the growth phase. For example, glucose in a medium frequently enhances growth, but acid harmful to the cells is likely to be produced. Therefore, omission of the glucose is preferable in a maintenance medium.

2.3.1.7 Solid and Semisolid media

In addition to liquid media, solid and semisolid media are widely used for bacteria. Solid media are useful for isolating bacteria or for determining the characteristics of colonies. The solidifying agent is usually agar, which at concentrations of 1.5 to 2.0 percent forms firm, transparent gels that are not degraded by most bacteria. Silica gel is sometimes used as an inorganic solidifying agent for autotrophic bacteria. Semisolid media, prepared with agar at concentrations of 0.5 percent or less, have a soft, custardlike consistency and are useful for the cultivation of microaerophilic bacteria or for determination of bacterial motility.

2.4 Preparation of Media

Some naturally occurring substances are used for the cultivation of bacteria. Notable among these is milk, usually skimmed rather than whole. Such natural materials are merely dispensed into tubes or flasks and sterilized before use. Media of the nutrient broth or nutrient agar type are prepared by compounding the required individual ingredients or, more conveniently, by adding water to a

dehydrated product which contains all the ingredients. Practically all media are available commercially in powdered form. The preparation of bacteriological media usually involves the following steps:

1. Each ingredient, or the complete dehydrated medium, is dissolved in the appropriate volume of distilled water,
2. The pH of the fluid medium is determined with a pH meter and adjusted if necessary.
3. If a solid medium is desired, agar is added and the medium is boiled to dissolve the agar.
4. The medium is dispensed into tubes or flasks.
5. The medium is sterilized, generally by autoclaving. Some media (or specific ingredients) that are heat-labile are sterilized by filtration

2.5 Physical conditionsrequired for growth

In addition to knowing the proper nutrients for the cultivation of bacteria, it is also necessary to know the physical environment in which the organisms willgrow best. Just as bacteria vary greatly in their nutritional requirements, so do they exhibit diverse responses to physical conditions such as temperature, gaseous conditions, and pH.

2.5.1 Temperature

The temperature that allows for most rapid growth during a short period of time (12 to 24 h) is known as the optimum growth temperature. Table 2.3 shows the optimum temperature for several bacteria and also the range of temperatures within which they will grow.

Table 2.3Characteristics of several species of bacteria with regard to temperatures at which they grow

	Temperature of Growth, °C		
	Minimum	Optimum	Maximum
Vibrio marinus strain MP-1	-1	15	20
Vibrio psychroerythrus	0	15	19
Pseudomonas fluorescens	4	25–30	40
Staphylococcus aureus	6.5	30–37	46
Corynebacterium diphtheriae	15	37	40
Neisseria gonorrhoeae	30	35–36	38.5
Streptococcus thermophilus	20	40–45	50
Thermoactinomyces vulgaris	27–30	60	65–70
Thermus aquaticus	40	70–72	79

Source: *Data from R. Y. Morita, Bacterial Rev, 39:144, 1975, and horn Bergey's Manual of Determinative Bacteriology, 8th ad, Williams & Wilkins. Baltimore, 1974.*

On the basis of their temperature relationships, bacteria are divided into three main groups:

i. **Psychrophiles**can grow at 0°C or lower, though they grow best at higher temperatures. Many microbiologists restrict the term psychrophile to organisms that can grow at 0°C but have an optimum temperature of 15°C or lower and a maximum temperature of about 20°C; the term psychrotroph or facultative psychrophile is used for those organisms able to grow at 0°C but which grow best at temperatures in the range of about 20 to 30°C

ii. **Mesophiles** grow best within a temperature range of approximately 25 to 40°C. For example, all bacteria that are pathogenic for humans and warm-blooded animals are mesophiles, most growing best at about body temperature (37°C).

iii. **Thermophiles** grow best at temperatures above 45°C. The growth range of many thermophiles extends into the mesophilic region; these species are designated facultative thermophiles.

2.5.2 Gaseous requirement

The principal gases that affect bacterial growth are oxygen and carbon dioxide. Requirements Bacteria display such a wide variety of responses to free oxygen that it is convenient to divide them into four groups on the following bases:

a) **Aerobic bacteria** require oxygen for growth and can grow when incubated in an air atmosphere (i.e., 21 percent oxygen).

b) **Anaerobic bacteria** do not use oxygen to obtain energy; moreover, oxygen is toxic for them and they cannot grow when incubated in an air atmosphere. Some can tolerate low levels of oxygen non-stringent or tolerant anaerobes), but others stringent orstrict anaerobes) cannot tolerate even low levels and may die upon brief exposure to air.

c) **Facultatively anaerobic** bacteria do not require oxygen for growth, although they may use it for energy production if it is available. They are not inhibited by oxygen and usually grow as well under an air atmosphere as they do in the absence of oxygen.

d) **Microaerophilic** bacteria require low levels of oxygen for growth but cannot tolerate the level of oxygen present in an air atmosphere.

2.5.3 Acidity or Alkalinity (pH)

For most bacteria the optimum pH for growth lies between 6.5 and 7.5, and the limit somewhere lie between 5 and 9. However, a few bacteria prefer more extreme pH values for growth. For example, *Thiobocillusthiooxidans* has an optimum pH of 2.0 to 3.5 and can grow in a range between pH 0.5 and 6.0. Radical shifts in pH can be prevented by incorporating a buffer (i.e. a substance that resists change in pH) into the medium.

2.5.4 Miscellaneous Physical Requirement

Some bacteria have additional requirements. For example. phototrophic bacteria must be exposed to a source of illumination, since light is their source of energy. Bacterial growth may also be influenced by hydrostatic pressure. Bacteria have been isolated from the deepest ocean trenches where the pressure is measured in tons per square inch, and many of these organisms will not grow in the laboratory unless the medium is subjected to a similar pressure.

2.6 Methods of Isolation and cultivation of microorganism

Microorganisms can also be used as indicators. Microorganisms like *E. coil* that are usually associated with the presence of pathogens are called "indicators". Most *E. coli* do not cause illness but they are indicators for fecal contamination. Therefore, there is a need for food safety control hazards to the consumer. Microorganism can also cause foodborne hazard that may cause a food to be unsafe for human consumption. Therefore, food quality controls deterioration of food. The development of microbial isolation techniques makes it possible to use pure culture in food production, isolation of these bacteria and identify them. It is essential to maintain the originality of know microorganisms. The study of pure cultures opened the possibility of classifying microorganisms, on the basis of the characteristics they display in pure culture. The first step in the cultivation of microorganisms is therefore the creation of a pure culture. A key development for the production of pure culture was the ability to grow microorganisms on a solid medium. Koch had noticed that when a nutrient surface such as cut potato was exposed to air, individual microbial colonies grew up, and he inferred from this that these had each arisen from the numerous divisions of single cells. The use of agar was first suggested by Koch's colleague's wife, who had used it as a setting agent in jam making. Agar does not melt until near boiling point hence an ideal setting agent, resisting both thermal and microbial breakdown, shortly after wards, Richard Petri developed the two-part culture dish that wash named after him as Petri plate. Petri plates can be sterilized separately from the medium and provide protection from contamination by mean of its lid. This is still a standard equipment today, although the original glass has been largely replaced by pre-sterilized, disposable plastic.

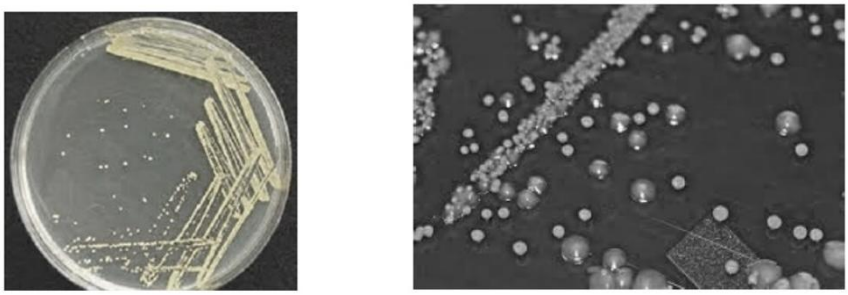
2.6.1Isolation technique

One of the first requirements to study specific microorganisms is to separate them from the mixed microbial populations in which they are found in the environment. To achieve this goal microbiologists use culture media and aseptic transfer techniques.

a) **Inoculation:** It is an introduction of a very small sample of cells (the inoculum) into a receptacle containing nutrient or culture medium.The aseptic (sterile) technique is a technique designed to keep the working environment as free of contaminants as possible. This is achieved first, by sterilizing all equipment and media that will be in contact with the microorganisms. This includes minimizing the air movement on the working area. Usually,

the work is done within the vicinity of a flame. Aseptic technique is required for the maintenance of pure cultures and the successful isolation of specific types of microorganisms. In the present-day Laminar Air flow (LAF) devices are used for all microbiological works which are stationed in clean rooms.

b) **Pure and mixed culture:** A pure culture is a culture that contains only one species of bacteria.A mixed culture encompasses more than one species. When isolating bacteria from the environment the microbiologist always starts with a mixed culture. The samples are diluted and plated in any one of the techniques listed below to obtain a mixed culture and also to count the total population in the given sample. A pure culture can be obtained from the mixed culture by sub-culturing and streaking for isolation.



Pure Culture **Mixed Culture**

Fig. 2.1Pure culture and mixed culture

c) **Use of the Loop/Stab Inoculator:** Two different types of inoculators can be used depending on the purpose of the work. i.e a loop and a needle. The loop is used to a) transfer cultures from one medium to another, b) to prepare bacterial smears, and c) to streak plates. The loop is the tool of choice for working with a liquid inoculum culture. The stab is used to prepare stab cultures and to pick single colonies from a plate. Stab is usually carried out with inoculation needle.

d) **Inoculation of an agar slant:** Rest the inoculum gently at the lower end of the slant and withdraw it slowly upwards moving it from side to side (the surface of the agar should not be broken). This should leave a streak on the surface of the slant.

e) **Inoculation of an agar stab**: Using aseptic technique pick a single well isolated colony with a sterile inoculating stab needle and stab the needle several times through the agar to the bottom of the vial or tube. Replace and tighten the cap. Make sure the tube and cap are well labelled.

f) **Inoculation of an agar plate:** In case of agar plates there is a greater surface area of sterile media that can be exposed to contaminations in the atmosphere. The key is to keep as much of the lid over (covering) the open agar plate as possible. Incubate the plates at appropriate temperature and in a secluded container. This is normally carried out in an

incubator. Never open the lid on the lab bench when in an open contaminating environment.

2.7 Methods of isolating pure cultures

To study microorganisms in food samples, it is necessary to isolate them as mixed population and further from mixed into individual species. For isolation and cultivation of these microorganisms basic laboratory apparatus are required. Firstly, samples are taken from the food source which needs to be tested. These samples if are not going to be analysed immediately are then stored at preferably at 0-4 ℃ in sterile containers.

a) **Serial dilution**: For analysis, samples are then diluted by serial dilution method to get the microbial count. Serial dilution involves taking a sample and diluting it through a series of standard volumes of sterile diluent, *e.g.* distilled water or 0.9 % saline. Then a small measured volume of each dilution is used to make a series of pour or spread. By diluting the sample in this controlled way it is possible to obtain an incubated plate with an easily countable number of colonies (30–100) and then calculate the number of microbes present in the sample.

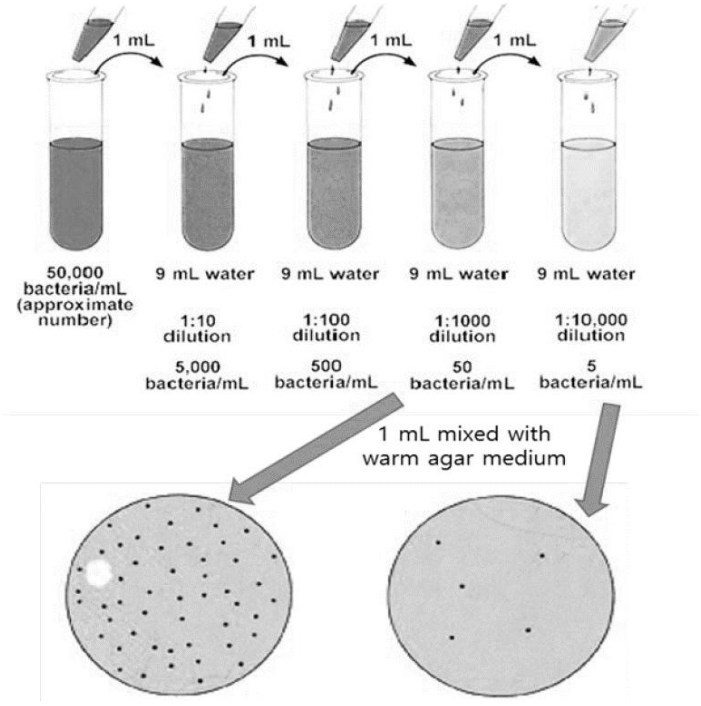


Fig. 2.2 Serial dilution process for pure culture isolation

b) **Pour plate method:** A pour plate is one in which a small amount of inoculum from broth culture is added by pipette to the center of a Petri dish. Molten, cooled agar medium in a test tube or bottle, is then poured into the Petri dish containing the inoculum. The dish is gently rotated clock wise for three times and anticlock wise once. This ensure that the

culture and medium are thoroughly mixed and the medium covers the plate evenly. Pour plates allow micro-organisms to grow both on the surface and within the medium.

c) **Spread plate method:** Spread plates, also known as lawn plates, should result in a culture spread evenly over the surface of the growth medium. This means that they can be used to test the sensitivity of bacteria to many antimicrobial substances, for example mouthwashes, garlic, disinfectants and antibiotics. The spread plate can be used for quantitative work (colony counts) if the inoculum is a measured volume, usually 0.1 mL, of each of a dilution is delivered by pipette. The sample is then spread using a spreader.

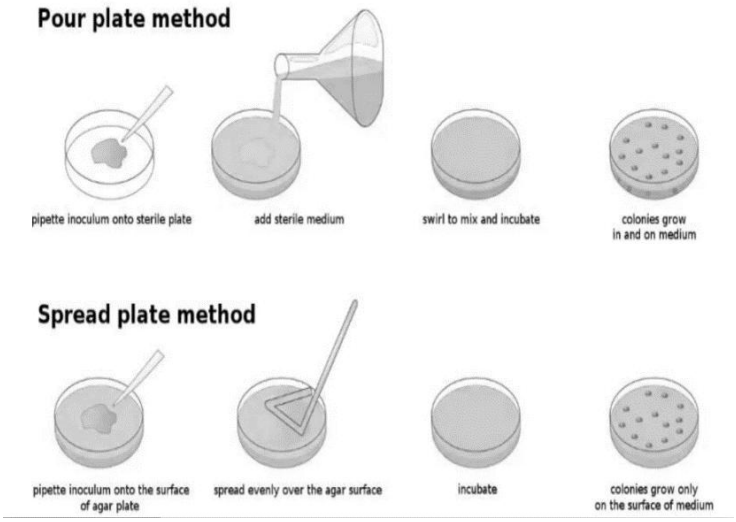


Fig. 2.3 Pour plate vs spread plate method

d) **Streak plate:** A sterile inoculation loop is used for preparing a streak plate as show in fig 2.4. This involves the progressive dilution of an inoculum of bacteria or yeast over the surface of solidified agar medium in a Petri dish. The progress of streaking should be in such a way that colonies grow well separated from each other. The aim of the procedure is to obtain single isolated pure colonies. A culture developed from single unaltered colony is called as pure culture. A loop is flamed and then touched to the first area to retrieve a sample of bacteria. This sample is then streaked several times in the second area of the medium. The loop is then re-flamed, touched to the second area, and streaked once again in the third area. The process can be repeated in a fourth and fifth area if desired. During incubation, the bacteria will multiply rapidly and form colonies.

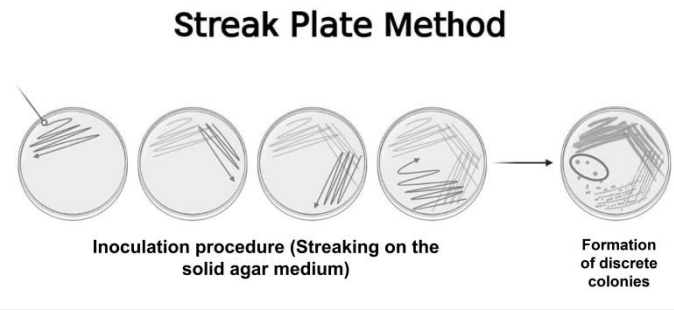


Fig 2.4 Streak Plate method

2.8 Suggested Reading

Banwart, G.J. (1979). *Basic Food Microbiology,* AVI Publishing Co. Inc.,Westport, Connecticut.

Frazier, W.C. and Westoff, D.C. (1996). *Food Microbiology,* Tata McGrawHill Publishing Co. Ltd., New Delhi.

Pelczar, M. Jr.O, Chan, E.C.S. and Kreig, N.R. (1993). *Microbiology,* TataMcGraw Hill Inc., New York.

Garbutt, J. (1998). *Essentials of Food Microbiology,* Arnold InternationalStudent's Edition, London.

2.9 Exercise

Q.1 What nutritional requirements in terms of chemicals are needed by all forms of life for growth and cellular maintenance?

Q.2 Distinguish between (a) phototrophs and chemotrophs, (b) lithotrophs and organotrophs, and (c) autotrophs and heterotrophs.

Q.3 Discuss different kinds of media used for the cultivation of microorganisms.

Q.4 What are the different methods for the isolation of microorganisms?

Q.5 Discuss the importance of different components which are used in media.

Fill in the blanks:

i. **……**grow best at temperatures above 45°C.

ii. The temperature that allows for most rapid growth during a short period of time (12 to 24 h) is known as the……

iii. Organisms that can use CO_2 as their sole source of carbon for assimilation are termed …….

iv. Mesophiles grow best within a temperature range of approximately …….

v. A ……. culture is a culture that contains only one species of bacteria.

vi. ……involves taking a sample and diluting it through a series of standard volumes of distilled water or 0.9 % saline.

Unit III: Primary Sources of Microorganisms in Foods: Physical and chemical methods used in the destruction of microorganisms (sterilization and disinfection).

Content

3.1 Primary source of microorganisms found in food

- **3.1.1 Soil**
- **3.1.2 Sewage**
- **3.1.3 Water**
- **3.1.4 Air**
- **3.1.5 Food handlers**
- **3.1.6 Equipment**
- **3.1.7 Miscellaneous**

3.2 Control of Microorganisms

- **3.2.1 Physical methods**
  - **3.2.1.1 High temperature**
    - **3.2.1.1.1 Moist Heat**
    - **3.2.1.1.2Dry Heat**
  - **3.2.1.2 Low temperature**
  - **3.2.1.3 Desiccation**

3.1 Primary source of microorganisms found in food

The foods of plants and animal origin carry several microorganisms associated with their natural habitat. Plants carry typical micro-flora on their surface and also get contaminated from outside sources. Animals carry microorganisms on their surface and intestine, and also contain contaminants from surrounding environment. Through their excretions and secretions animals release microorganisms in to surrounding environment. Besides, both plants and animals carry pathogenic microorganisms capable of causing human illness. The food associated microorganisms are influenced by the availability of specific nutritional requirements and the environmental parameters. The primary sources of entry of microorganisms in to foods are from the soil, water, air, during handling, processing transportation and storage of foods.The primary sources of microorganisms in food include: Soil and water, sewage, plant and plant products, food utensils, intestinal tract of man and animals, food handlers, animal hides and skins, air and dust (Fig. 3.1).

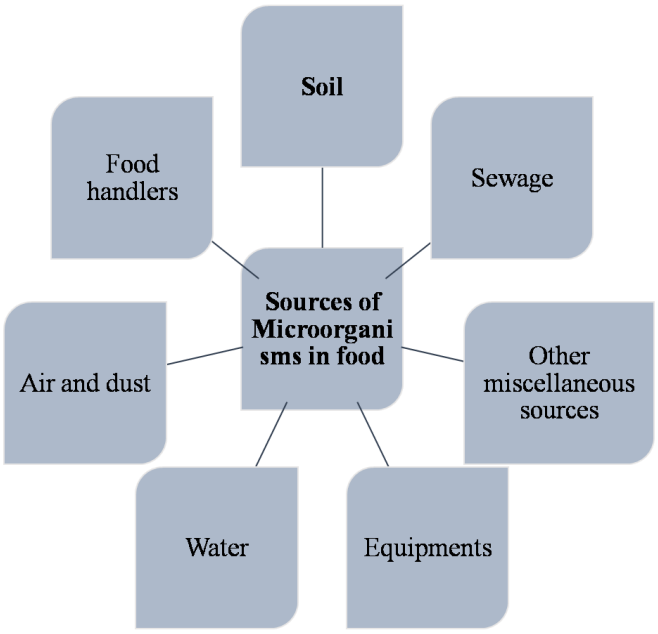


Fig.3.1 various sources from which microorganisms enters into our food

3.1.1 Soil

Soil being the rich source of several kinds of microorganisms immediately contaminates the plants and edible plant parts, and the surface of animals with the soil associated microorganisms. As the soil particles are carried in to aquatic environment through wind, rain and other means contamination of water takes place with several soil micro-flora. Therefore, it is not uncommon to find several microorganisms both in soil and water environment. These soil derived microorganisms form part of the the microbial flora involved in spoilage of foods of plant and animal source. Thus, there is a need to reduce the load of soil microorganisms in foods which can be achieved by removing the soil by washing the surface of foods with good quality water, and by avoiding contact with soil/ dust.

3.1.2 Sewage

Sewage,especially when used as fertilizer in crops, can contaminate food with microorganisms, the most significant of which are different entero pathogenic bacteria and viruses. This can be a great concern with organically grown food and many imported fruits and vegetables, in whichuntreated sewage and manure might be used as fertilizer. pathogenic parasites can also get infood from sewage. To reduce incidence of microbial contamination of foods from sewage, it is better not to use sewage as fertilizer. If used, it should be efficiently treated to 'ill the pathogens. Also, effective washing of foods following harvesting is important.

3.1.3 Water

Natural waters not only contain several microorganisms native to the aquatic environment but also from soil, raw/treated sewage and pollutants entering the water body. The microbial numbers and types vary in different water bodies depending on the nutrient status. Thus, all kinds of microorganisms found in water are likely to be associated with the aquatic organisms as surface flora. Use of such water for food processing will add microorganisms from water

to food. Sewage waters containing human pathogenic microorganisms contaminate foods when such waters are used without proper treatment. The water used in food processing should meet agreeable chemical and bacteriological characteristics.

3.1.4 Air

Air contains several microorganisms which may get deposited on the food being processed and handled. Though the air does not contain natural flora of microorganisms, whatever microorganisms encountered are those associated with the suspended solid material and water droplets. The sources of microorganisms to air are from dust, dry soil, and water spray from natural surface waters, droplets of moisture from coughing, sneezing and talking by food handlers, from sporulating moulds growing on walls, ceilings, floor, foods and food ingredients. Thus, it is likely that the microorganisms persisting in air get deposited on the food being processed and contribute for microbial load and subsequent spoilage of food.The number of microorganisms present in air depends on factors such as extent of movement of air, sunshine, humidity, location, and amount of suspended dust in air. Quiet air allows settling of microorganisms but the moving air brings in microorganisms and keeps them suspended. Thus, the number of microorganisms in air is increased by air currents caused by movement of people, by ventilation and by breeze. The rain or snow removes microorganisms from the air.

3.1.5 Food handlers

Between production and consumption, foods come in contact with different people handling thefoods. They include not only people working in farms and food processing plants, but also thosehandling foods at restaurants, catering services, retail stores, and at home. Human carriers have been the source of pathogenic microorganisms in foods that later caused food borne diseases, especially with ready to eat foods. Improperly cleaned hands, lack of aesthetic sense and personal hygiene, and dirty clothes and hair can be major sources of microbial contamination in foods. The presence of minor cuts and infection in hands and face and mild

generalised diseases (e.g., flu, strep throat, or hepatitis A in an early stage) can amplify the situation. In addition tospoilage bacteria, pathogens such as *S. aureus, Salmonella*, *Shigella* spp. pathogenic *E. Coli.* and hepatitis A can be introduced into foods from human sources. Proper training of personnel in personal hygiene, regular checking of health, and maintaining efficient sanitary and aesthetic standards are necessary to reduce contamination from this source.

3.1.6 Equipment

A wide variety of equipment is used in harvesting, slaughtering, transporting, processing, andstoring foods. Many types of microorganisms from air, raw foods, water, and personnel can getinto the equipment and contaminate foods. Depending on the environment (moisture, nutrients,and temperature) and time, microorganisms can multiply and, even from a low initial population,reach a high level and contaminate large volumes of foods. Also, when processing equipment is used continuously for a long period of time, microorganisms initially present can multiply and act as a continuous source of contamination in the product produced subsequently. In some equipment, small parts, inaccessible sections, and certain materials might not be efficiently cleaned and sanitized. These dead spots can serve as sources of both pathogenic and spoilage microorganisms in food. Small equipment, such as cutting boards, knives, spoons, and similar articles, because of improper cleaning, can be sources of cross contamination.

3.1.7 Miscellaneous

Foods might be contaminated with microorganisms from several other sources, namely packaging and wrapping materials, containers, flies, vermin’s, birds, house pets, and rodents. Many types of packaging materials are used in food. Since they are used in products ready for consumption and in some cases without further heating, proper microbiological standards (or specifications) for packaging materials are necessary. House pets can also harbour pathogens, proper care should be taken not tocontaminate food from this source.

3.2 Control of Microorganisms

The term control as used here refers to the reduction in numbers and/or activity of the total microbial flora. The principal reasons for controlling microorganisms are: to prevent transmission of disease and infection, to prevent contamination by or growth of undesirable microorganisms, and to prevent deterioration and spoilage of materials by

microorganisms.Microorganisms can be -removed, inhibited, or killed by various physical agents. physical processes, or chemical agents.

3.2.1 Physical methods

The major physical agents or processes used for the control of microorganisms are temperature (high and low), desiccation, osmotic pressure, radiation, and filtration.

3.2.1.1 High temperature

Microorganisms can grow over a wide range of temperatures, from very low temperatures characteristic of psychrophiles to the very high growth temperatures characteristic of thermophiles. Every type has an optimum, minimum, and maximum growth temperature. Temperatures above the maximum generally kill, while those below the minimum usually produce stasis (inhibition of metabolism) and may even be considered preservative.High temperatures combined with high moisture is one of the most effective methods of killing microorganisms. It is important to distinguish between dry heat and moist heat in any procedure for microbial control. Moist heat kills microorganisms by coagulating their proteins and is much more rapid and effective than dry heat, which destroys microorganisms by oxidizing their chemical constituents. For example, Spores of *Clostridium botulinum* are killed in 4 to 20 min by moist heat at 120°C, whereas a 2-h exposure to dry heat at the same temperature is required.

Vegetative cells are much more sensitive to heat than are spores; the higher level of 'water activity" in the vegetative cells accounts for this. Cells of most bacteria are killed in 5 to 10 min at 60 to 70°C (moist heat). Vegetative cells of yeasts and other fungi are usually killed in 5 to 10 min by moist heat at 50 to60°C; their spores are killed in the same time but at temperatures of 70 to 80°C.The susceptibility of viruses to heat is generally similar to that of mesophilic vegetative bacterial cells.Thermal death time refers to the shortest period of time to kill a suspension of bacteria (or spores) at a prescribed temperature and under specific conditions. Another unit of measurement of the destruction of microorganisms by heat is thedecimal reduction time.This is the time in minutes to reduce the population by 90 percent, or stated differently, it is the time in minutes for the thermaldeath-time curve to pass through one log cycle. Thermal-death-time data and decimal-reduction-time data are extremely important in many applications of microbiology, The canning industry, for example, carries out extensive studies on this subject to establish satisfactory processing temperatures for the

preservation of canned foods.Practical procedures by which heat is employed are conveniently divided into two categories moist heat and dry heat.

3.2.1.1.1 Moist Heat

Steam Under Pressure: Heat in the form of saturated steam under pressure is the most practical and dependable agent for sterilization. Steam under pressure provides temperatures above those obtainable by boiling (Table 3.1). It has the advantages of rapid heating, penetration, and moisture in abundance, which facilitates the coagulation of proteins.The laboratory apparatus designed to use steam under regulated pressure is called an autoclave (Fig. 3.2). It is essentially a double-jacketed steamchamber equipped with devices which permit the chamber to be filled withsaturated steam and maintained at a designated temperature and pressure forany period of time.The autoclave is an essential unit of equipment in every microbiology laboratory. Many media, solutions, discarded cultures, and contaminated materialsare routinely sterilized with this apparatus.Generally, but not always, the autoclave is operated at a pressure of approximately 15 lb/in^2 (at 121°C). The time of operation to achieve sterility depends on the nature of the material being sterilized, the type of the container, and the volume.

Table 3.1 Temperature of steam under pressure

Steam Pressure (lb/in^2)	**Temperature (°C)**
0	100.00
5	109.00
10	115.00
15	121.5
20	126.5

Fig. 3.2 Autoclave

Fractional Sterilization (tyndallization). Some microbiological media, solutions of chemicals, and biological materials cannot be heated above 100°C without being damaged. If, however, they can withstand the temperature of free-flowing steam(100°C), it is possible to sterilize them by fractional sterilization (tyndallization).This method involves heating the material at 100°C on three successive days with incubation periods in between. Resistant spores germinate during the incubationperiods; on subsequent exposure to heat, the vegetative cells will be destroyed.

Boiling Water: Contaminated materials or objects exposed to boiling water cannot be sterilized with certainty. It is true that all vegetative cells will be destroyed within minutes by exposure to boiling water, but some bacterial spores can withstand this condition for many hours. The practice of exposing instruments forshort periods of time in boiling water is more likely to bring about disinfection (destruction of vegetative cells of disease-producing microorganisms) rather than sterilization.

Pasteurization: Milk, cream, and certain alcoholic beverages (beer and wine) are subjected to a controlled heat treatment (called pasteurization) which kills microorganisms of certain types but does not destroy all organisms. Pasteurized milk is not sterile milk.

3.2.1.1.2 Dry Heat

Hot-Air Sterilization: Dry-heat, or hot-air, sterilization is recommended where it is either undesirable or unlikely that steam under pressure will make direct and complete contact with the materials to be sterilized. This is true of certain items of laboratory glassware, such as Petri dishes and pipettes, as well as oils, powders, and similar substances. The apparatus employed for this type of sterilization may be a special electric or gas oven or even the kitchen stove oven. For laboratory glassware, a 2-h exposure to a temperature of 161°C is sufficient for sterilization.

Incineration: Destruction of microorganisms by burning is practiced routinely in the laboratory when the transfer needle is introduced into the flame of the Bunsen burner.

3.2.1.2 Low temperature

Temperatures below the optimum for growth depress the rate of metabolism, and if the temperature is sufficiently low, growth and metabolism cease. Low temperatures are useful for preservation of cultures, since microorganisms have a unique capacity for surviving extreme cold. Agar-slant cultures of some bacteria, yeasts, and molds are customarily stored for long periods of time at refrigeration temperatures of about 4 to 7°C. Many bacteria and viruses can be maintained in a deep-freeze unit at temperatures. from —20 to - 70°C. Liquid nitrogen, at a temperature of —196°C. is used for preserving cultures of many viruses and microorganisms, as well as stocks of mammalian tissue cells. From these facts it is immediately apparent that low temperatures, however extreme, cannot be depended upon for disinfection or sterilization. Microorganisms maintained at freezing or subfreezing temperatures may be considered dormant; they perform no detectable metabolic activity. This static condition is the basis of successful application of low temperatures for the preservation of foods, Thus, from a practical standpoint, high temperatures may be considered as microbicidal and low temperatures (freezing or lower) as microbistatic.

3.2.1.3 Desiccation

Desiccation of the microbial cell causes a cessation of metabolic activity, followed by a decline in the total viable population. In general, the time of survival of microorganisms after desiccation varies, depending on the kind of microorganism, the material in or on which the organisms are dried, the completeness of the drying process and the physical conditions to which the dried organisms are exposed, e.g., light, temperature, and humidity.In the process of lyophilization, organisms are subjected to extreme dehydration in the frozen state and then

sealed in a vacuum. In this condition, desiccated (lyophilized) cultures of microorganisms remain viable for many years.

3.2.1.4 Osmotic Pressure

When two solutions with differing concentrations of solute are separated by a semipermeable membrane, there will occur a passage of water, through the membrane, in the direction of the higher concentration. The trend is toward equalizing the concentration of solute on both sides of a membrane. The solute concentration within microbial cells is approximately 0.95 percent. Thus, if cells are exposed to solutions with higher solute concentration, water will be drawn out of the cell. The process is called plasmolysis. The reverse process, that is, passage of water from a low solute concentration into the cell, is termed plasmoptysis. The pressure built up within the cell as a result of this water intake is termed osmotic pressure.

3.2.1.5 Radiation

Energy transmitted through space in a variety of forms is generally called radiation. For our purposes, the most significant type of radiation is probably electromagnetic radiation, of which light and x-rays are examples. Electromagnetic radiation has the dual properties of a continuous wave phenomenon and a discontinuous particle phenomenon: the particles are packets, or quanta. of energy, sometimes called photons, which vibrate at different frequencies. Radiation of a given frequency can also be described by its wavelength, λ; it is measured in angstroms, where 10,000 Å = 1μm, and the energy of the radiation in electron volts (ev) is given by 12,350/ λ.The various parts of the electromagnetic spectrum, distinguished by their wavelengths, are shown in Fig. 3.1.

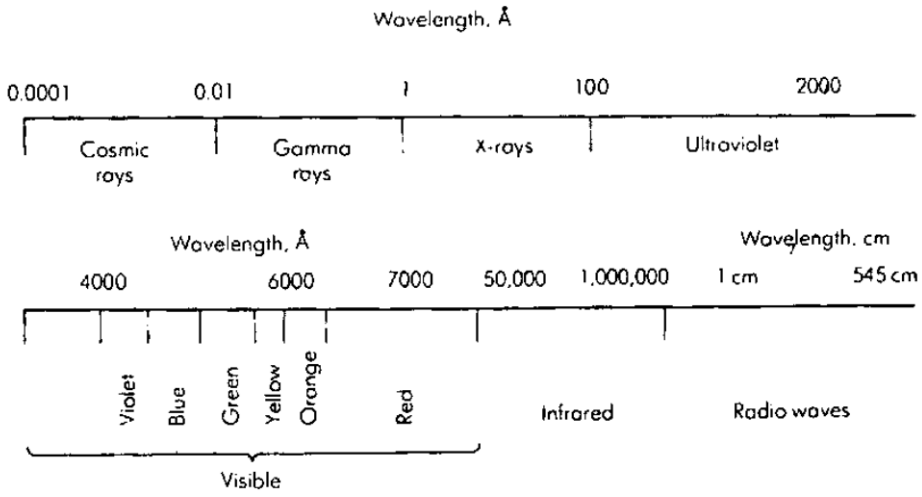


Fig. 3.1 Spectrum of radiant energy

Gamma rays and x-rays, which have energies of more than about 10 eV, are called ionizing radiations because they have enough energy to knock electrons away from molecules and ionize them. When such radiations pass through cells, they create free hydrogen radicals, hydroxyl radicals, and some peroxides, which in turn can cause different kinds of intracellular damage. Moreover, since this damage is produced in a variety of materials, ionizing radiations are rather nonspecific in their effects. Less energetic radiation, particularly ultraviolet light, does not ionize; it is absorbed quite specifically by different compounds because it excites electrons and raises them to higher energy levels, thus creating different chemical species that can engage in a variety of chemical reactions not possible for unexcited molecules.

Besides the fundamental research in radiation microbiology, there have been many developments in the application of ionizing radiation to sterilize biological materials. This method is called **cold sterilization** because ionizing radiations produce relatively little heat in the material being irradiated. Thus, it is possible to sterilize heat-sensitive substances, and such techniques are being developed in the food and pharmaceutical industries.

Ultraviolet Light: The ultraviolet portion of the spectrum includes all radiations from 150 to 3900 Å. Wavelengths around 2650 Å have the highest bactericidal efficiency. Many lamps are available which emit a high concentration of ultraviolet light in the most effective region, 2600 to 2700 Å. Germicidal lamps, which emit ultraviolet radiations, are widely used to reduce microbial populations. An important practical consideration in using this means of destroying microorganisms is that ultraviolet light has very little ability to penetrate matter. Even a thin layer of glass filters off a large percentage of the light. Thus, only the microorganisms on the surface of an object where they are exposed directly to the ultraviolet light are susceptible to destruction.Ultraviolet light is absorbed by many cellular materials but most significantly by the nucleic acids, where it does the most damage. The absorption and subsequent reactions are predominantly in the pyrimidines of the nucleic acid. One important alteration is the formation of a pyrimidine dimer in which two adjacent pyrimidines become bonded. Unless dimers are removed by specific intracellular enzymes, DNA replication can be inhibited and mutations can result.

X-rays:rays are lethal to microorganisms and higher forms of life. Unlike ultraviolet radiations, they have considerable energy and penetration ability. However, they are impractical for purposes of controlling microbial populations because (1) they are very

expensive to produce in quantity and (2) they are difficult to utilize efficiently, since radiations are given off in all directions from their point of origin.

Gamma rays: Gamma radiations are high-energy radiations emitted from certain radioactive isotopes such as ^{60}Co. Because of their great penetrating power and their microbicidal effect, gamma rays are attractive for use in commercial sterilization of materials of considerable thickness or volume, e.g. packaged foods and medical devices.

Cathode rays: When a high-voltage potential is established between a cathode and an anodeevacuated tube, the cathode emits beams of electrons, called cathode rays or electron beams. Special types of equipment have been designed which produce electrons of very high intensities (millions of volts), and these electrons are accelerated to extremely high velocities. These intense beams of accelerated electrons are microbicidal as well as having other effects on biological and nonbiological materials. One of the unique features of the process is that the material can be sterilized after it has been packaged (the radiations penetrate the wrappings) and at room temperature. Electron-beam radiation has limited power of penetration; but within its limits of penetration, sterilization is accomplished on very brief exposure.

3.2.1.6 Filtration

For many years a variety of filters have been available to the microbiologist which can remove microorganisms from liquids or gases. These filters are madeof different materials—an asbestos pad in the Seitz filter, diatomaceous earth in the Berkefeld filter, porcelain in the Chamberland-Pasteur filter, and sintered glass disks in other filters.The mean pore diameter in these bacteriological filters ranges from approximately one to several micrometers; most filters are available in several grades, based on the average size of the pores. However, these filters do not act as mere mechanical sieves; porosity alone is not the only factor preventing the passage of organisms. Other factors, such as the electric charge of the filter, the electric charge carried by the organisms, and the nature of the fluid being filtered, can influence the efficiency of filtration.In recent years a new type of filter termed the membrane or molecular filter has been developed whose pores are of a uniform and specific predetermined size.They are prepared as circular membranes of about 150 µm thickness and contain millions of microscopic pores of very uniform diameter. Filters of this type can be produced with known porosities ranging from approximately 0.01 to 10 µm. Membrane filters are used extensively in the laboratory and in industry to sterilize fluid materials.

The development of high-efficiency particulate air (HEPA) filters has made it possible to deliver clean air to an enclosure such as a cubicle or a room. This type of air filtration together with a system of laminar airflow is now used extensively to produce dust- and bacteria-free air (Fig. 3.3).

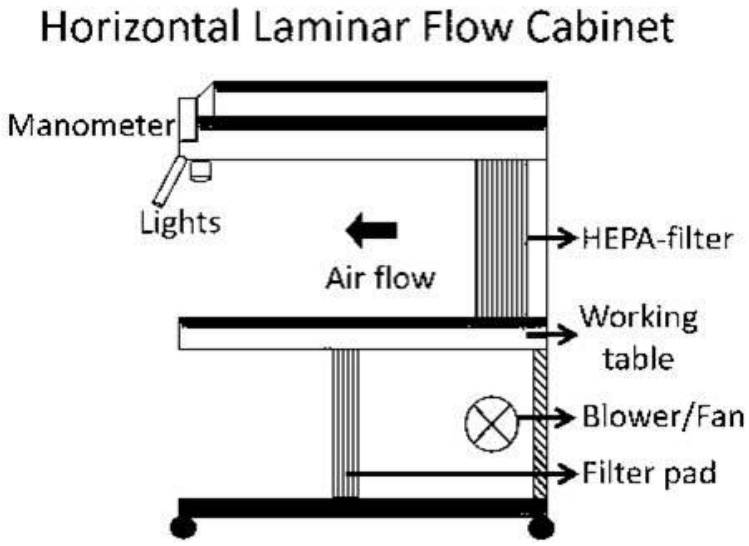


Fig. 3.3 Laminar Air Flow

A summary of the application of physical agents for the control of microorganisms is provided in Table 3.2.

Table 3.2 Applications of physical agents for the control of microorganisms

Method	Recommended Uses	Limitations
Moist heat		
Autoclave	Sterilizing instruments, linens, utensils, and treatment trays, media and other liquids	Ineffective against organisms in materials impervious to steam; cannot be used for heat-sensitive articles
Free-flowing steam or boiling water	Destruction of nonsporeforming pathogens; sanitizes bedding, clothing, and dishes	Cannot be guaranteed to produce sterilization on one exposure
Dry heat		
Hot-air oven	Sterilizing materials impermeable to or damaged by moisture, e.g., oils, glass, sharp instruments, metals	Destructive to materials which cannot withstand high temperatures for long periods
Incineration	Disposal of contaminated objects that cannot be reused.	Size of incinerator must be adequate to burn largest load promptly and completely; potential of air pollution
Radiation		
Ultraviolet light	Control of airborne infection: disinfection of surfaces	Must be absorbed to be effective (does not pass through transparent glass or opaque objects); irritating to eyes and skin; low penetration
X-ray, gamma, and cathode radiation	Sterilization of heat-sensitive surgical materials and other medical devices	Expensive and requires special facilities for use
Filtration		
Membrane filters	Sterilization of heat-sensitive biological fluids	Fluid must be relatiely free of suspended particulate matter
Fiberglass filters (HEPA)	Air disinfection	Expensive
Physical cleaning		
Ultrasonics	Effective in decontaminating delicate cleaning instruments	Not effective alone, but as adjunct procedure enhances effectiveness of other methods
Washing	Hands, skin, objects	Sanitizes; reduces microbial flora

3.2.2 Chemical methods

A large number of chemical compounds have the ability to inhibit the growth and metabolism of microorganisms or to kill them.there is not a single chemical agent which is best for thecontrol of microorganisms for any and all purposes. If there were an idealgeneral-purpose chemical antimicrobial agent, it would have to possess anextremely elaborate array of characteristics, the specifications for such an ideal compound, as they are described below:

1. **Antimicrobial activity:** The capacity of the substance to kill or inhibit microorganisms is the first requirement. The chemical, at a low concentration, should have a broad spectrum of antimicrobial activity.
2. **Solubility**. The substance must be soluble in water or other solvents to the extent necessary for effective use.
3. **Stability.** Changes in the substance upon standing should be minimal and should not result in significant loss of germicidal action.
4. **Nontoxicity to humans and other animals**. Ideally, the compound should be lethal to microorganisms and non-injurious to humans and other animals.
5. **Homogeneity.** The preparation must be uniform in composition so that active ingredients are present in each application. Pure chemicals are uniform, but mixtures of materials may lack homogeneity.
6. **Noncombination with extraneous organic material**. Many disinfectants have an affinity for proteins or other organic material. When such disinfectants are used in situations where there is considerable organic material besides that of the microbial cells, little, if any, of the disinfectant will be available for action against the microorganisms.
7. **Toxicity to microorganisms at room or body temperatures**. In using the compound, it should not be necessary to raise the temperature beyond that normally found in the environment where it is to be used.
8. **Capacity to penetrate**. Unless the substance can penetrate through surfaces, its germicidal action is limited solely to the site of application. Sometimes, of course, surface action is all that is required.
9. **Noncorroding and non-staining**. It should not rust or otherwise disfigure metals nor stain or damage fabrics.
10. **Deodorizing ability**. Deodorizing while disinfecting is a desirable attribute, Ideally the disinfectant itself should either be odorless or have a pleasant smell.
11. **Detergent capacities**. A disinfectant which is also a detergent (cleaning agent) accomplishes two objectives, and the cleansing action improves the effectiveness of the disinfectant.
12. **Availability.** The compound must be available in large quantities at a reasonable price.

3.2.2.1 Some important terminology

The following terms are used to describe the processes and chemical, agents employed in controlling microorganisms.

Sterilization. The process of destroying all forms of microbial life. A sterile object, in the microbiological sense, is free of living microorganisms. The terms sterile, sterilize, and sterilization therefore refer to the complete absence or destruction of all microorganisms and should not be used in a relative sense.

Disinfectant. An agent, usually a chemical, that kills the growing forms but not necessarily the resistant spore forms of disease-producing microorganisms.**Disinfection** is the process of destroying infectious agents.

Antiseptic. A substance that opposes sepsis, i.e., prevents the growth or action of microorganisms either by destroying microorganisms or by inhibiting their growth and metabolism.

Sanitizer. An agent that reduces the microbial population to safe levels as judged by public health requirements. Usually, it is a chemical agent that kills 99.9 percent of the growing bacteria.

Germicide (Microbicide). An agent that kills the growing forms but not necessarily the resistant spore forms of germs; in practice a germicide is almost the same thing as a disinfectant, but germicides are commonly used for all kinds of germs (microbes) for any application.

Bactericide. An agent that kills bacteria (adjective, bactericidal). Similarly, the terms fungicide, virucide, and sporicide refer to agents that kill fungi, viruses, and spores, respectively.

Bacteriostasis. A condition in which the growth of bacteria is prevented (adjective, bacteriostatic). Similarly, fungistatic describes an agent that stops the growth of fungi. Agents that have in common the ability to inhibit growth of microorganisms are collectively designated microbistatic agents.

Antimicrobial Agent. One that interferes with the growth and metabolism of microbes. In common usage the term denotes inhibition of growth, and with reference to specific groups of organisms such terms as antibacterial or antifungal are frequently employed. Some antimicrobial agents are used to treat infections, and they are called chemotherapeutic agents.

3.2.2.2 Selection of chemical agent

The major factors that need to be assessed in the process of selecting the most chemical agent appropriate chemical agent are:

1. **Nature of the material to be treated**: The substance selected must be compatible with the material to which it is applied as the chemical agent used to disinfect utensils cannot be applied to disinfect skin.
2. **Types of microorganisms**. Chemical agents are not all equally effective againstbacteria, fungi, viruses, and other microorganisms.*E.g.*Spores are more resistant than vegetative cells.
3. **Environmental conditions**: The factors like temperature, pH, time, concentration, and presence of extraneous organic material, may all have a bearing on the rate and efficiency of antimicrobial action.

3.2.2.3 Major group of chemical agents

The major antimicrobial chemical agents are described as follows:

3.2.2.3.1 Phenol and Phenolic compounds

Phenol has the distinction of being used successfully in the 1880s by JosephLister, a surgeon, to reduce infection of surgical incisions and surgical wounds. Lister instituted the practice of applying a solution of phenol (carbolic acid) to surgical incisions and wounds.Phenol has the additional distinction of being the standard against which other disinfectants of a similar chemical structure are compared to determine their antimicrobial activity. The procedure used is called the phenol-coefficient technique.Phenol and phenolic compounds are very effective disinfectants. A 5% aqueous solution of phenol rapidly kills the vegetative cells of microorganisms; spores are much more resistant. Many derivatives of phenol have been prepared and evaluated for their antimicrobial activity. *E.g.*Hexylresorcinol, a derivative of phenol, is marketed in a solution of glycerine and water. It is a strong surface-tension reductant, which may account in part for its high bactericidal activity.

Phenolic substances may be either bactericidal or bacteriostatic, depending upon the concentration used. Bacterial spores and viruses are more resistant than are vegetative cells. Some phenolics are highly fungicidal. The antimicrobial activity of phenolics is reduced at an alkaline pH and by organic material. Low temperatures and the presence of soap also reduce antimicrobial activity. Pure crystalline phenol is colorless. Aqueous solutions of from 2 to 5% can be employed to disinfect such materials as sputum, urine,

feces, and contaminated instruments or utensils.One of the widely used phenolic derivatives is o-phenylphenol.

Mode of Action. Exposure of microbial cells to phenolic compounds produces a variety of effects. Depending upon the concentration of the phenolic compound to which microbial cells were exposed, effects such as disruption of cells, precipitation of cell protein, inactivation of enzymes, and leakage of amino acids from the cells has been reported. Although the specific mode of action is not clear, there is a consensus that the lethal effect is associated with physical damage to the membrane structures in the cell surface, which initiates further deterioration.

3.2.2.3.2 Alcohols

Ethyl alcohol, CH_3CH_2OH, in concentrations between 50 and 90%, is effective against vegetative or non-sporeforming cells, For practical application a 70% concentration of alcohol is generally used. Ethyl alcohol cannot be relied upon to produce a sterile condition. Concentrations which are effective against vegetative cells are practically inert against bacterial spores.

Methyl alcohol is less bactericidal than ethyl alcohol: furthermore, it is highly poisonous. Even the fumes of this compound may produce permanent injury to the eyes, and is not generally employed for the destruction of microorganisms. The higher alcohols—propyl, butyl, amyl, and others—are more germicidal than ethyl alcohol. In fact, there is a progressive increase In germicidal power as the molecular weight of alcohols increases (as shown in Table 3.3).

Table 3.3 Phenol coefficient of different alcohols

	Phenol Coefficient	
Alcohol	Against *Salmonella typhi*	Against *Staphylococcus aureus*
Methyl, CH_3OH	0.026	0.03
Ethyl, CH_3CH_2OH	0.04	0.039
n-Propyl, $CH_3CH_2CH_2OH$	0.102	0.082
Isopropyl, $(CH_3)_2CHOH$	0.064	0.054
n-Butyl, $CH_3(CH_2)_2CH_2OH$	0.273	0.22
n-Amyl, $CH_3(CH_2)_3CH_2OH$	0.78	0.63
n-Hexyl, $CH_3(CH_2)_4CH_2OH$	2.3	
n-Heptyl, $CH_3(CH_2)_5CH_2OH$	6.8	
n-Octyl, $CH_3(CH_2)_6CH_2OH$	21.0	0.63

Source: C. Sykes, Disinfection and Sterilization, ad ed., Lippincott, Philadelphia, 1965

Alcohol is effective in reducing the microbial flora of skin and for the disinfection of clinical oral thermometers. Alcohol concentrations above 60% are effective against viruses; however, the effectiveness is influenced considerably by the amount of extraneous protein material in the mixture, The extraneous protein reacts with the alcohol and thus protects the virus.

Mode of Action. Alcohols are protein denaturants, and this property may, to a large extent, account for their antimicrobial activity. Alcohols are also solvents for lipids, and hence they may damage lipid complexes in the cell membrane. They are also dehydrating agents.

3.2.2.3.3 Halogens (Iodine)

Iodine is one of the oldest and most effective germicidal agents. It has been in usefor more than a century. Pure iodine is a bluish-black crystalline element having a metallic lustre. It is only slightly soluble in water but readily soluble in alcohol and aqueous solutions of potassium or sodium iodide. The element is traditionally used as a germicidal agent in a form referred to as tincture of iodine. There are several preparations available, such as 2% iodine plus 2% sodium iodide diluted in alcohol, 7% iodine plus 5% potassium iodide in 63% alcohol, and 5% iodine and 10% potassium iodide in aqueous solution. Iodine is also used in the form of substances known as iodophors, Iodophors are mixtures of iodine with surface-active agents which act as carriers and solubilizers for the iodine. One of these agents is polyvinyl pyrrolidone (PVP); the complex can be expressed asPVP-l. Iodine is released slowly from this complex. Iodophors possess the germicidal characteristics of iodine and have the additional advantages of non-staining and low irritant properties.Iodine solutions are chiefly used for the disinfection of skin, and for this purpose they rank among the best disinfectants. Iodine preparations are effective for other purposes, such as disinfection of water, disinfection of air (iodine vapours), and sanitization of food utensils.

Mode of Action. The mechanism by which iodine exerts its antimicrobial activity is not specifically understood. Iodine is an oxidizing agent, and this fact may account for its antimicrobial action. Oxidizing agents can irreversibly oxidize and thus inactivate essential metabolic compounds such as proteins with sulfhydryl groups. It has also been suggested that the action may involve the halogenation of tyrosine units of enzymes and other cellular proteins requiring tyrosine for activity.

3.2.2.3.4 Chlorine and its compounds

Chlorine, either in the form of gas or in certain chemical combinations, represents the most widely used disinfectants. The compressed gas in liquid form is almost universally employed for the purification of municipal water supplies. Chlorine gas is difficult to handle unless special equipment is available to dispense it. Hence, its usefulness in the gaseous state is limited to large-scale operations such as water-purification plants, where it is feasible for installing suitable equipment for safe handling. There are available many compounds of chlorine which can be handled more conveniently than free chlorine and which, under proper conditions of use, are equally effective as disinfectants. One class of compounds in this category is the hypochlorites. Calcium hypochlorite, $Ca(OCl)_2$ (also known as chlorinated lime), and sodium hypochlorite, NaOCl, are popular compounds. The chloramines represent another category of chlorine compounds used as disinfectants, sanitizing agents, or antiseptics. The simplest of these is monochloramine, NH_2Cl. Chloramine-T and azochloramide, two of several germicidal compounds in this category.

Mode of Action. The antimicrobial action of chlorine and its compounds comes through the hypochlorous acid formed when free chlorine is added to water. Similarly, hypochlorites and chloramines undergo hydrolysis, with the formation of hypochlorous acid. The hypochlorous acid formed in each instance is further decomposed. The oxygen released in this reaction (nascent oxygen) is a strong oxidizing agent, and through its action on cellular constituents, microorganisms are destroyed. The killing of microorganisms by chlorine and its compounds is also due in part to the direct combination of chlorine with proteins of the cell membranes and enzymes.

3.2.2.3.5 Heavy Metals and Their Compounds

Most of the heavy metals, either alone or in certain compounds, exert a detrimental effect upon microorganisms. Table 3.4 summarizes the applications and compounds of some common heavy metals.

Table 3.4 Applications of some of the most common heavy metals

Heavy Metal	Examples of Compounds	Applications
Mercury	Inorganic compounds: Mercuric chloride Mercurous chloride	Bactericidal in dilutions of 1:1,000; limited use because of corrosive action, high toxicity to animals, and reduction of effectiveness in presence of organic material; insoluble compounds, used in ointments as antiseptics

	Mercuric oxide Organic compounds: Mercurochrome Metaphen Merthiolate Mercresin	Less irritating and less toxic than the inorganic mercury compounds; employed as antiseptics on cutaneous and mucosal surfaces; maybe bactericidal or bacteriostatic
Silver	Colloidal silver compounds: Silver nitrate Silver lactate Silver picrate	Consist of protein in combination with metallic silver or silver oxide (colloidal solution); bacteriostatic or bactericidal effect is a function of the free silver ions released from the combination; used as antiseptics, silver nitrate is the most widely used of these compounds, all of which are germicidal and employed as antiseptics in specific conditions; silver nitrate is bactericidal for most organism at a dilution of 1:1,000; many states require that the eyes of newborns be treated with a few drops of 1% silver nitrate solution to prevent ophthalmia neonatorum, a gonococcal infection of eyes
Copper	Copper sulphate	Much more effective against algae and molds than bacteria; 2 ppm in water sufficient to prevent algal growth; used in swimming pools and open water reservoirs; used in the form of Bordeaux mixture as a fungicide for prevention of certain plant diseases.

Mode of Action. Heavy metals and their compounds act antimicrobially by combining with cellular proteins and inactivating them. For example, in the case of mercuric chloride the inhibition is directed at enzymes which contain the sulfhydrvl grouping. High concentrations of salts of heavy metals like mercury, copper, and silver coagulate cytoplasmic proteins, resulting in damage or death to the cell.

3.2.2.3.6 Dyes

Two classes of dye compounds which have antimicrobial properties are of special interest to microbiologists. These are triphenylmethane and acridine dyes. uded in this category are malachite green, brilliant green, and crystal violet. As a rule Cram-positive organisms are more susceptible to lower concentrations of these compounds than are Gram-negative ones. Crystal violet will inhibit Gram-positive cocci at a dilution of 1:200,000 to 1:300,000; 10 times this concentration is required to inhibit *Eschetichia coli*. Two examples of dyes derived from acridine are acriflavine and tryptoflavine. These compounds exhibit selective inhibition against bacteria, particularly staphylococci and gonococci.

Mode of Action. The mode of action of triphenylmethane dyes is uncertain, but there is speculation that they exert their inhibitory effect by interfering with cellular oxidation processes.

3.2.2.3.7 Gaseous Agents

Sterilization by means of gaseous agents is very effective. The main agents currently used for gaseous sterilization are ethylene oxide, 3-propiolactone and formaldehyde.

Ethylene Oxide: It is a liquid at temperatures below 10.8°C (51.4°F). Above this temperature it vaporizes rapidly. Vapours of this compound in air are highly flammable even in low concentrations. Ethylene oxide is a unique and powerful sterilizing agent. Its use for sterilizing heat- or moisture-sensitive materials in hospitals, industry, and laboratories has become universal. Bacterial spores, which are many times more resistant than vegetative cells as measured by other antimicrobial agents, show little resistance to destruction by this agent. An outstanding and desirable feature of ethylene oxide is its power to penetrate. It will pass through and sterilize large packages of materials, bundles of cloth, and even certain plastics.

Mode of Action. The mode of action of ethylene oxide is believed to be alkylation reactions with organic compounds such as enzymes and other proteins.

β-Propiolactone: This compound is a colorless liquid at room temperature with a high boiling point (155°C). It is not flammable like ethylene oxide but is a vesicant and lachrymator and consequently must be handled with care. It lacks the penetrating power of ethylene oxide but is considerably more active against microorganisms, it is sporicidal, fungicidal, and virucidal. Whereas the usual concentration of ethylene oxide for sterilization purposes is 400 to 800 mg/liter, only 2 to 5 mg of β-propiolactone is required. β-propiolactone is very effective in destroying microorganisms on surfaces. However, the fact that it has a low power of penetration coupled with its alleged carcinogenic properties has restricted its use as a practical sterilizing agent.

Table 3.5 Summary of various chemical compounds used as controlling agents for microorganisms

Chemical Agent	Recommended Use	Limitations
Phenol and phenolic compounds	General disinfectant	Microbial effectiveness limited; irritating and corrosive
Alcohols: ethyl and	Skin and thermometer	Antiseptic

isopropyl	antiseptic	
Iodines	Disinfect skin	Irritating to mucous membranes
Chlorine	Water disinfection	Inactivated by organic material; pH dependent for effectiveness; objectionable taste and odor unless strictly controlled
Silver nitrate	Treating burns	Possible irritation
Mercurials	Skin disinfection	Slow-acting; toxic
Quaternaries	Skin disinfection	Not sporicidal
Formaldehyde	Sterilizing instruments; fumigation	Permeation poor; corrosive
Glularaldehyde	Sterilizing instruments; fumigation	Stability limited
Ethylene oxide	Sterilizing heat-sensitive materials, instruments, and large equipment	Flammable; potentially explosive in pure form
β-propiolactone	Sterilizing Instruments and heat-sensitive materials	Lacks penetrating power

3.3 Exercise

Answer the following:

Q.1 Describe the process of fractional sterilization, or tyndallization.

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Q.2 List several physical agents (or processes) that produce a microbistatic condition.

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Q.3 How are microorganisms affected by subzero temperatures?

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Q.4 The mechanism of antimicrobial action caused by desiccation is similar to that caused by plasmolysis. Explain why.

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Q.5 List several different kinds of radiations that are destructive to microorganisms. Comment on the practical application of each.

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Q.6 What is a membrane or molecular filter? How does it differ from older types of bacteriological filters in terms of how it removes microorganisms?

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Q.7 What is the distinction between the following terms?

a) Bactericidal and bacteriostatic

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b) Sterile and disinfected

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c) Virucidal and fungicidal

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d) Germicidal and bactericidal

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e) Sporicidal and bactericidal

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Q.8 List several halogens and compounds of halogens that are used to control microbial populations. Describe several practical applications for these agents. What is their mode of action upon microorganisms?

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Q.9 What are the attractive features of sterilization by ethylene oxide? What kinds of materials are sterilized with ethylene oxide? Why?

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Q.10 As a sterilizing agent, how does -propiolactone compare with ethylene oxide?

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3.4 Suggested Reading

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Unit IV: Fundamentals of control of microorganism in foods: Extrinsic and intrinsic parameters affecting growth and survival of microbes, use of high and low temperature dehydration, freeze drying, irradiation and preservatives in food preservation.

Contents

4.1 Microbial growth

Microbial growth is an autocatalytic process: no growth will occur without the presence of at least one viable cell and the rate of growth will increase with the amount of viable biomass present.This can be represented mathematically by the expression:

$$dx/dt = \mu x$$

where d*x*/dt is the rate of change of biomass, or numbers *x* with time t, and μ is a constant known as the specific growth rate.This can be simply illustrated by considering the case of a bacterial cell dividing by fission to produce two daughter cells. In time t, a single cell will divide to produce two cells; after a further doubling time has elapsed four cells will be present; after another, eight, and so on. Thus, the rate of increase as well as the total cell number is doubling with every doubling time that passes.

If, however, we perform the experiment measuring microbial numbers with time and then plot log *x* against time, we obtain the curve shown as Figure 4.1 in which exponential growth occurs for only a part of the time. A simple analysis of this curve can distinguish three major phases. In the first, the lag-phase, there is no apparent growth while the inoculum adjusts to the new environment, synthesizes the enzymes required for its exploitation and repairs any lesions resulting from earlier injury, *e.g.* freezing, drying, heating. The exponential or logarithmic phase which follows is characterized by an increase in cell numbers following the simple growth law equation.

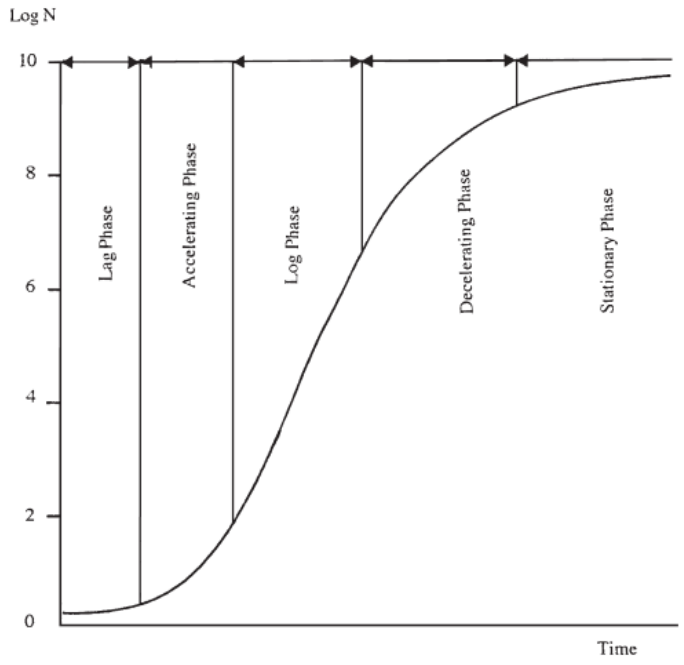

Fig.4.1 The growth curve of microbes

Finally, changes in the medium as a result of exponential growth bring this phase to an end as key nutrients become depleted, or inhibitory metabolites accumulate, and the culture moves into the stationary phase. The significance of exponential growth for food processing hardly needs emphasizing. A single bacterium with a doubling time of 20 minutes (μ=2.1hr^{-1}) growing in a food, or pockets of food trapped in equipment, can produce a population of greater than 10^7 cells over an 8-hour working day. It is therefore, a prime concern of the food microbiologist to understand what influences microbial growth in foods with a view to controlling it. The situation is complicated by the fact that the microflora is unlikely to consist of a single pure culture. During growth, harvesting/ slaughter, processing and storage, food is subject to contamination from a range of sources. Some of the micro-organisms introduced will be unable to grow under the conditions prevailing, while others will grow together in what is known as an association, the composition of which will change with time.

The factors that affect microbial growth in foods, and consequently the associations that develop, also determine the nature of spoilage and any health risks posed. For convenience they can be divided into four groups along the lines suggested more than 50 years ago in a seminal review by Mossel and Ingram (Fig 4.2) – physico-chemical properties of the food itself (intrinsic factors); conditions of the storage environment (extrinsic factors); properties and interactions of the microorganisms present (implicit factors); and processing factors.

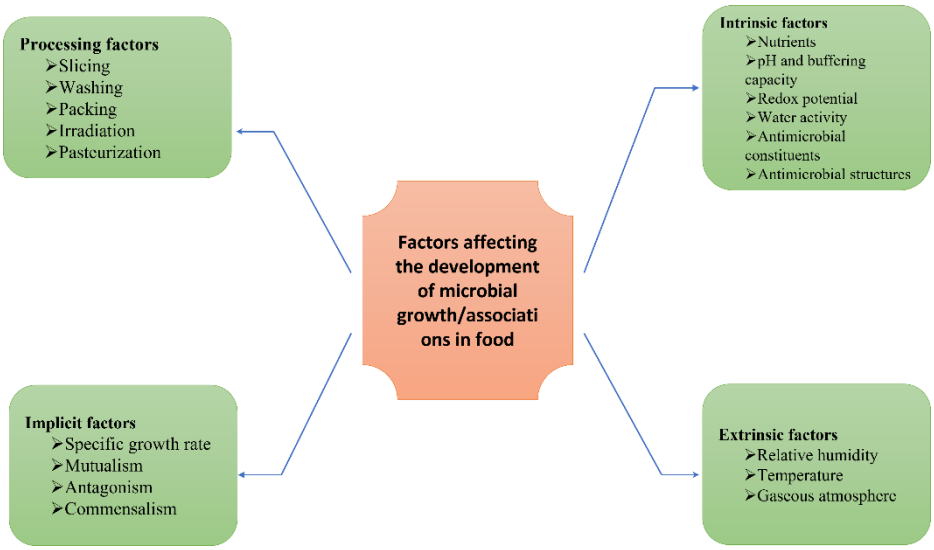

Fig 4.2 Various factors affecting growth of microorganisms

4.2 INTRINSIC FACTORS

4.2.1 Nutrient content

Microorganisms derive the chemical elements from food that constitute microbial biomass, those molecules essential for growth that the organism cannot synthesize, and a substrate that can be used as an energy source. Most suitable food products for microorganisms include meat or casein digests (peptone and tryptone), meat infusions, tomato juice, maltextract, sugar and starch in microbiological media.The inability of an organism to utilize a major component of a food material will limit its growth and put it at a competitive disadvantage compared with those that can. Thus, the ability to synthesize amylolytic (starch degrading) enzymes will favour the growth of an organism on cereals and other farinaceous products. The addition of fruits containing sucrose and other sugars to yoghurt increases the range of carbohydrates available and allows the development of a more diverse spoilage microflora of yeasts.

4.2.2 pH and Buffering Capacity

pH is equal to the negative logarithm of the hydrogen ion activity as measured by glass electrode. For aqueous solutions, pH 7 corresponds to neutrality (since $[H^+][OH^-] = 10^{-14}$ forwater), pH values below 7 are acidic and those above 7 indicate an alkaline environment.The acidity or alkalinity of an environment has a profound effect on the activity and stability of macromolecules such as enzymes, so it is not surprising that the growth and metabolism of micro-organisms are influenced by pH.In general, bacteria grow fastest in the pH range 6.0–8.0, yeasts 4.5– 6.0 and filamentous fungi 3.5–4.0.The pH of maximum growth rate is called the optimal growth pH. Based on optimal growth pH, microbes can be separated into three groups: acidophiles grow best at pH < 5, neutrophiles grow optimally at pH between 5 and 9, and alkaliphiles grow fastest above pH 9. For example, lactobacilli and acetic acid bacteria with optima usually between pH 5.0 and 6.0.The acidity of a product can have important implications for its microbial ecology and the rate and character of its spoilage. For example, plant products classed as vegetables generally have a moderately acid pH and soft-rot producing bacteria such as *Pectobacteriumcarotovorum* and pseudomonads play a significant role in their spoilage. In fruits, however, a lower pH prevents bacterial growth and spoilage is dominated by yeasts and moulds.

4.2.3 Redox potential (E_h)

Microbial growth in a food reduces its E_h. This is usually ascribed to a combination of oxygen depletion and the production of reducing compounds such as hydrogen by the micro-organisms. Oxygen depletion appears to be the principal mechanism; as the oxygen content of the medium decreases, so the redox potential declines from a value of around 400 mV at air saturation by about 60 mV for each tenfold reduction in the partial pressure of oxygen. The decrease in E_h as a result of microbial activity is the basis of some long-established rapid tests applied to foods, particularly dairy products. Redox dyes such as methylene blue or resazurin are sometimes used to indicate changes in E_h which are correlated with microbial levels. Methylene blue is also used to determine the proportion of viable cells in the yeast used in brewing.

Redox potential exerts an important elective effect on the microflora of a food. Although microbial growth can occur over a wide spectrum ofredox potential, individual micro-organisms are conveniently classified into one of several physiological groups based on the redox range over which they can grow and their response to oxygen.Obligate or strict aerobes are those organisms that are respiratory, generating most of their energy from oxidative phosphorylation using oxygen as the terminal electron acceptor in the process.For example, pseudomonads, such as *Pseudomonas fluorescens*, which grows at an E_h of +100 to +500 mV, and other oxidative Gram-negative rods produce slime and off-odours at meat surfaces.

Obligate anaerobes tend only to grow at low or negative redox potentials and often require oxygen to be absent. Anaerobic metabolism gives the organism a lower yield of utilizable energy than aerobic respiration, so a reducing environment that minimizes the loss of valuable reducing power from the microbial cell is favoured. The presence or absence of oxygen can naturally affect this, but for many anaerobes, oxygen exerts a specific toxic effect of its own. For example, it has been observed that *Clostridium acetobutylicum* can grow at an E_h as high as +370 mV maintained by ferricyanide, but would not grow at +110 mV in an aerated culture.Obligate anaerobes, such as clostridia, are of great importance in food microbiology. They have the potential to grow wherever conditions are anaerobic such as deep in meat tissues and stews, in vacuum packs and canned foods causing spoilage and, in the case of *C. botulinum*, the major public health concern: botulism.

4.2.4 Antimicrobial Barriers and Constituents

All foods have one or the other mechanism to prevent or limit potentially damaging effects by microorganisms through protective physical barriers to infection (*e.g.* skin, shell, and

husk) and antimicrobial components. Natural covering of some foods provides excellent protection against entry and subsequent damage by spoilage microorganisms. These include outer covering of fruits, outer shell of egg, skin covering of fish and meats. The outer covering is usually composed of macromolecules and these are resistant to degradation and create inhospitable environment for microorganisms due to low a_w and shortage of readily available nutrients.The antimicrobial substances such as short chain fatty acids in animal skin and essential oils in plant surfaces help to prevent entry of microorganisms.

Physical damage to outer barrier allows microbial invasion and cause spoilage. Some foods are resistant to attack by microorganisms and remain stable due to the presence of naturally occurring substances which have antimicrobial property. Many plant species possess essential oils which are antimicrobial. Antimicrobial agents in plants include isothiocyanates (mustard oils) and in Allium species (garlic, onions and leeks) to produce thiosulfinates such as allicin and phytoalexins produced by many plants in response to microbial invasion, for example the antifungal compound phaseollin produced in green beans. Many natural constituents of plant tissues such as pigments, alkaloids and resins have antimicrobial properties, but limited practical use is made of these. Benzoic and sorbic acids found in cranberries and mountain ash berries respectively are notable exceptions that are used in their pure forms as food preservatives. Antmicrobial properties of spices and herbs especially important e.g.allicin in garlic, eugenol from allspice (pimento), cloves and cinnamon, thymol from thyme and oregano, and cinnamic aldehyde from cinnamon and cassia resulting use of herbs and spices contributing to microbiological stability of foods.

Animal products too, have a range of non-specific antimicrobial constituents. Probably the supreme example of this is the white or albumen of the hen’s egg which possesses a whole battery of inhibitory components. Many of the same or similar factors can also be found in milk where they are present in lower concentrations and are thus less effective.Both products contain the enzyme lysozyme which catalyses the hydrolysis of glycosidic linkages in peptidoglycan, the structural polymer responsible for the strength and rigidity of the bacterial cell wall.

4.2.5 Water activity (a_w)

With a reduction of water activity in their environment the number of groups of micro-organisms capable of active growth decreases (Fig.4.3). The exact range of water activities allowing growth is influenced by other physico-chemical and nutritional conditions.

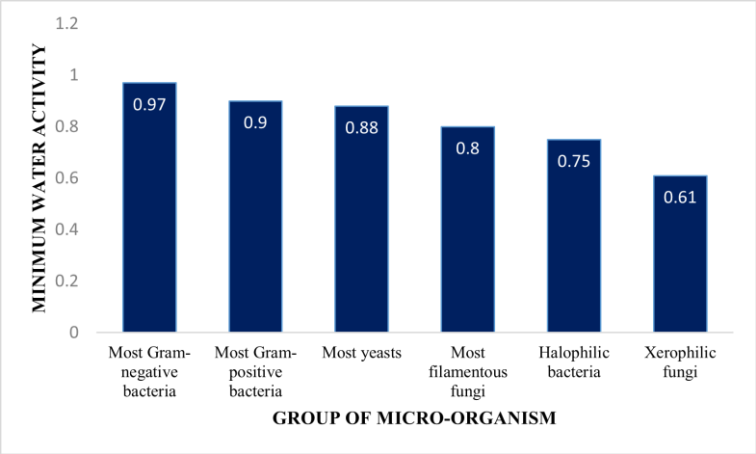

Fig. 4.3 Minimum water activities at which active growth can occur.

Because low water activities are associated with three distinct types of food three terms are used to describe the micro-organisms especially associated with these foods:

a) halotolerant – able to grow in the presence of high concentrations of salt
b) osmotolerant – able to grow in the presence of high concentrations of unionized organic compounds such as sugars
c) xerotolerant – able to grow on dry foods.

Some micro-organisms grow better at reduced a_w and may be described as halophilic, osmophilic or xerophilic, indeed the halobacteria are obligately halophilic and cannot grow in the absence of highconcentrations of salt. This group of bacteria, which includes such genera as *Halobacterium* and *Halococcus*, belong to the Archaebacteria and accumulate potassium chloride as their compatible solute. The limiting value of water activity for the growth of any microorganism is about 0.6 and below this value the spoilage of foods is not microbiological but may be due to insect damage or chemical reactions such as oxidation.

4.3 EXTRINSIC FACTORS

4.3.1 Relative humidity

Relative humidity and water activity are interrelated, thus relative humidity is essentially a measure of the water activity of the gas phase. When food commodities having a low water

activity are stored in an atmosphere of high relative humidity water will transfer from the gas phase to the food. It may take a very long time for the bulk of the commodity to increase in water activity, but condensation may occur on surfaces giving rise to localized regions of high water activity. It is in such regions that propagules which have remained viable, but unable to grow, may now germinate and grow. Once micro-organisms have started to grow and become physiologically active they usually produce water as an end product of respiration.Thus they increase the water activity of their own immediate environment so that eventually micro-organisms requiring a high a_w are able to grow and spoil a food which was initially considered to be microbiologically stable. Such a situation can occur in grain silos or in tanks in which concentrates and syrups are stored.The storage of fresh fruit and vegetables requires very careful control of relative humidity. If it is too low then many vegetables will lose water and become flaccid. If it is too high then condensation may occur and microbial spoilage may be initiated.

4.3.2 Temperature

Microbial growth can occur over a temperature range from about -8˚C up to 100˚C at atmospheric pressure. The most important requirement is that water should be present in the liquid state and thus available to support growth. No single organism is capable of growth over the whole of this range; bacteria are normally limited to a temperature span of around 35˚C and moulds rather less, about 30˚C. Each organism exhibits a minimum, optimum and maximum temperature at which growth can occur. These are known as cardinal temperatures and are, to a large extent, characteristic of an organism, although they are influenced by other environmental factors such as nutrient availability,pH and a_w. Micro-organisms can be classified into several physiological groups based on their cardinal temperatures (Fig. 4.4).In food microbiology mesophilic and psychrotrophic organisms are generally of greatest importance. Mesophiles, with temperature optima around 37˚C, are frequently of human or animal origin and include many of the more common foodborne pathogens such as *Salmonella, Staphylococcus aureus* and *Clostridium perfringens*. Mesophiles grow more quickly at their optima than psychrotrophs and so spoilage of perishable products stored in the mesophilic growth range is more rapid than spoilage under chill conditions.

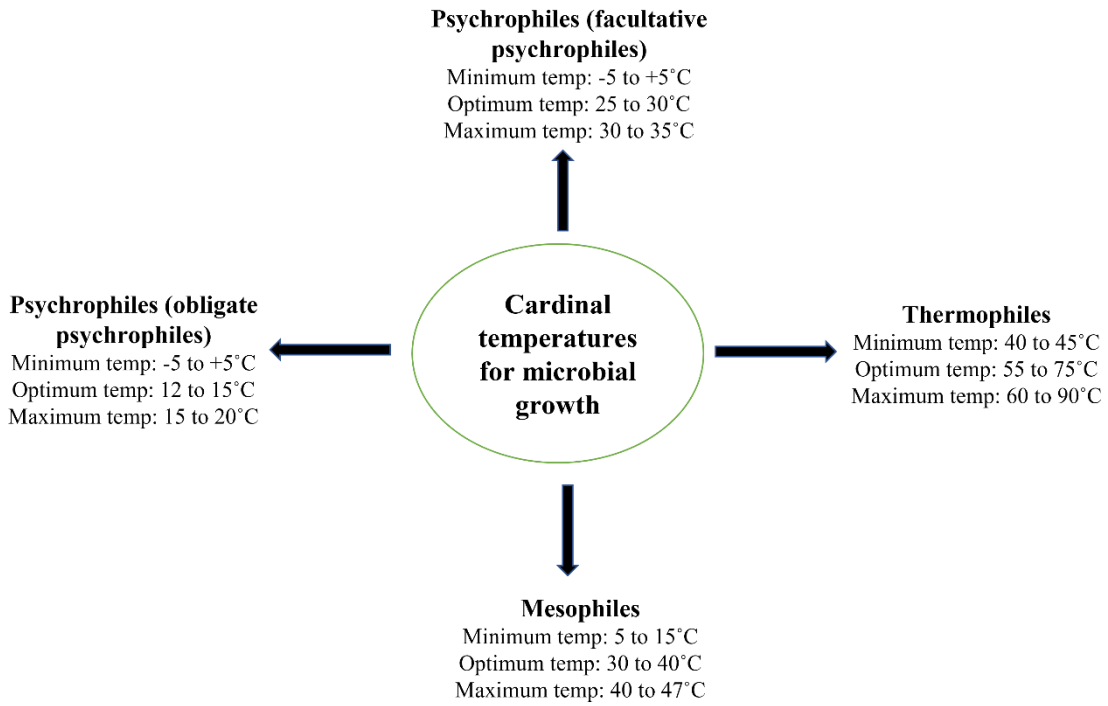

Fig. 4.4 Different physiological groups of microorganisms based on their cardinal temperatures

(Adapted from ICMSF 1980)

Among the organisms capable of growth at low temperatures, two groups can be distinguished: the true or strict psychrophiles ('cold loving') have optima of 12–15˚C and will not grow above about 20˚C.Thermophiles are generally of far less importance in food microbiology, although thermophilic spore formers such as certain *Bacillus* and *Clostridium* species do pose problems in a restricted number of situations.

4.3.3 Gaseous Atmosphere

The presence of O_2 and its influence on redox potential are important determinants of the microbial associations that develop and their rate of growth. The effect of O_2on the microbial growth has already discussed in section 4.2.3 hence in this section effects of other gases will be discussed only. The inhibitory effect of carbon dioxide (CO_2) on microbial growth is applied in modified-atmosphere packing of food and is an advantageous consequence of its use at elevated pressures (hyperbaric) in carbonated mineral waters and soft drinks.Carbon dioxide is not uniform in its effect on micro-organisms. Moulds and oxidative Gram-negative bacteria are most sensitive and the Gram-positive bacteria, particularly the lactobacilli, tend to be mostresistant. Some yeasts such as *Brettanomyces spp.* also show considerable

tolerance of high CO_2 levels and dominate the spoilage microflora of carbonated beverages.Some micro-organisms are killed by prolonged exposure to CO_2 but usually its effect is bacteriostatic.The mechanism of CO_2 inhibition is a combination of several processes.

Carbon dioxide dissolves in water to produce carbonic acid which partially dissociates into bicarbonate anions and protons. Carbonic acid is a weak dibasic acid (pK_a 6.37 and 10.25); in an unbuffered solution it can produce an appreciable drop in pH hence it acts as weak organic acids thus penetrating the plasma membrane and acidifying the cell's interior. Other contributory factors are thought to include changes in the physical properties of the plasma membrane adversely affecting solute transport; inhibition of key enzymes, particularly those involving carboxylation /decarboxylation reactions in which CO_2 is a reactant; and reaction with protein amino groups causing changes in their properties and activity.

4.4 Food Preservation

Food preservation is the process of treating and handling food in such a way as to stop or greatly slow down its spoilage and to prevent food borne illness while maintaining the food item's nutritional value, texture, and flavour.Today food preservation is associated with the refrigerator, the deep freeze, andthe canning process, all developments of the nineteenth and twentieth centuries. However, humans have dealt with the problem of food preservation for many centuries. The ancient Egyptians and Romans were aware of the preservative effects of salting, drying, and smoking. It has been suggested that the first salt preservation was accomplished by burying the food along the shore, where seawater effected the cure. The American Indians placed strips of fresh bison and venison at the top of a teepee or over a campfire, where preservation was accomplished through drying and smoking. Dried salt cod was a common food for colonial Americans. Perishable foods were stored in caves and springs, where the low temperature prolonged the preservation.

Modern methods of food preservation employ elaborate refinements of the primitive processes plus additional new techniques. The various practices used for food preservation may be summarized as Fig. 4.5:

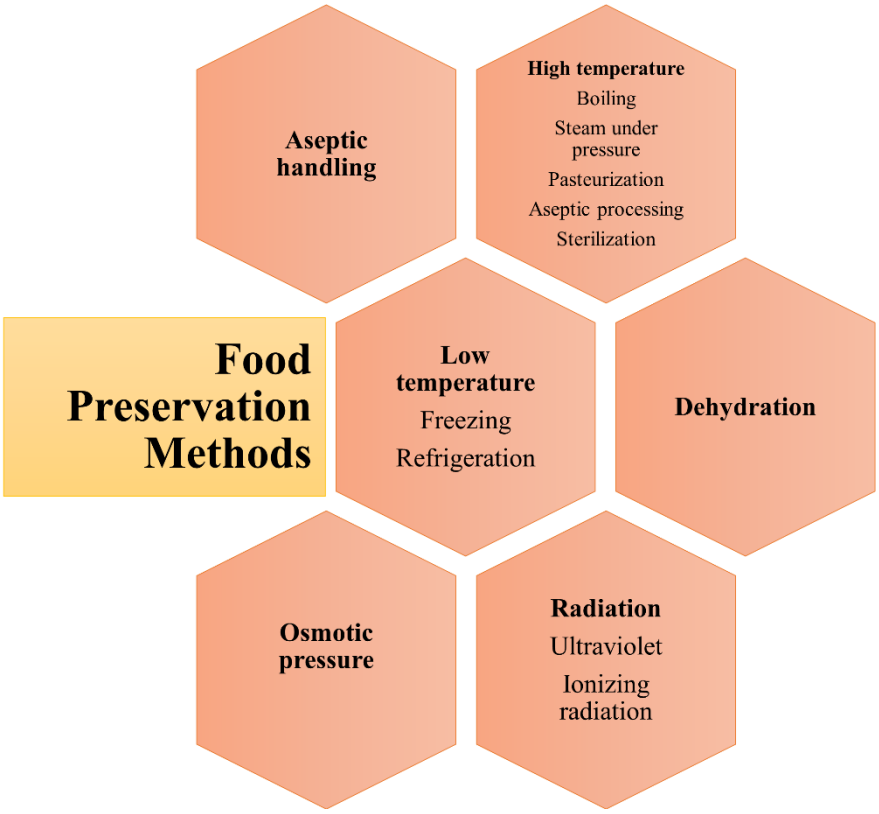

Fig. 4.5 Different food preservation methods

All methods of food preservation are based upon one or more of the following principles:

(1) prevention or removal of contamination,

(2) inhibition of microbial growth and metabolism (microbistatic action) and

(3) killing of microorganisms (microbicidal action).

4.4.1 High Temperature

High temperature is one of the safest and most reliable methods of food preservation (Table 4.1). Heat is widely used to destroy organisms in food products in cans, jars, or other types of containers that restrict the entrance of microorganisms after processing. Steam under pressure, such as in a pressure cooker, is the most effective method of high-temperature food preservation since it can kill all vegetative cells and spores. Food preservation by heat requires knowledge of the heat resistance of microorganisms, particularly spores. In addition, one must consider the rate at which heat penetrates through foods of different consistencies as well as the size of the containers in which they are packed.

Table 4.1 Various methods of heating in food preservation

Heat process	*Temperature*	*Objective*
Cooking baking boiling frying grilling	⩽100 °C	Improvement of digestibility, *e.g.* starch gelatinization, collagen breakdown during cooking of meat. Improvement of flavour. Destruction of pathogenic micro-organisms
Blanching	<100 °C	Expulsion of oxygen from tissues. Inactivation of enzymes
Drying/ Concentration	<100 °C	Removal of water to enhance keeping quality
Pasteurization	60–80 °C	Elimination of key pathogens and spoilage organisms
Appertization	>100 °C	Elimination of micro-organisms to achieve 'commercial sterility'

Canning has been the basic method of food preservation for approximately 175 yearsgiven by Nicholas Appert and Peter Durand in 1810.The temperatures used for canning foods ranges from 100°C for high-acid foods to 121°C for low-acid foods. The canning process does not guarantee a, sterile product. For example, spores of some bacterial species may survive these temperatures. The most important organism to be eliminated in canned foods is the sporeforming anaerobe *Cl. botulinum*, which can produce a very potent lethal toxin.

Pasteurization of Milk: Pasteurization, the term given to heat processes typically in the range 60–80˚C and applied for up to a few minutes, is used for two purposes. First is the elimination of a specific pathogen or pathogens associated with a product. This type of pasteurization is often a legal requirement introduced as a public health measure when a product has been frequently implicated as a vehicle of illness. Notable examples are milk, bulk liquid egg and ice cream mix, all of which have a much improved safety record as a result of pasteurization. The second reason for pasteurizing a product is to eliminate a large proportion of potential spoilage organisms, thus extending its shelf-life. This is normally the objective when acidic products such as beers, fruit juices, pickles, and sauces are pasteurized.

The original time-temperature relationships for pasteurization were worked out with Mycobacterium tuberculosis since this was regarded as the most heatresistant pathogen likely to occur in milk. This organism isdestroyed when exposed to a temperature of 140°F for 10 mm. The pasteurization temperature was set at 143°F for 30 min.

Pasteurization Processes. - Methods of pasteurization of milk used commercially include a low-temperature holding (LTH) method and a high-temperature shorttime (HTST) method. The holding method, or vat pasteurization, exposes milk to 145°F (62.8°C) for 30 min in

appropriately designed equipment. The HTST process employs equipment capable of exposing milk to a temperature of 161°F (71.7°C) for 15 s (seconds). In either method of pasteurization, it is essential that the equipment be designed and operated so that every particle of milk is heated to the required temperature and held for the specified time. Precautions must be taken to prevent recontamination after pasteurization. The finished product should be stored at a low temperature to retard growth of microorganisms which survived pasteurization. In addition to milk numerous other food products and some fermented beverages like beers and wines are commercially pasteurized.

Sterilization: Commercial milk-sterilization techniques have been developed which expose milk to ultrahigh temperatures for very short periods of time, for example, 300°F (148.9°C) for 1 to 2 sec. In addition, the sterilization process includes steps that eliminate any traces of cooked flavour. The final product is comparable in flavour and nutritional quality to pasteurized milk. The sterile milk product has several attractive features: it does not require refrigeration and it has an indefinite shelf life.

Appertization refers to processes where the only organisms that survive processing are non-pathogenic and incapable of developing within the product under normal conditions of storage. As a result, appertized products have a long shelf-life even when stored at ambienttemperatures. The term was coined as an alternative to the still widely used description commercially sterile which was objected to on the grounds that sterility is not a relative concept; a material is either sterile or it is not. An appertized or commercially sterile food is not necessarily sterile – completely free from viable organisms. It is however free from organisms capable of growing in the product under normal storage conditions.

4.4.2 Low Temperature

Temperatures approaching 0°C and lower retard the growth and metabolic activities of microorganisms. Modern refrigeration and freezing equipment has made it possible to transport and store perishable foods for long periods of time. Refrigerated trucks and railway cars, ships' storage vaults, and the home refrigerator and freezer have improved the quality of the human diet and increased the variety of foods available.Before freezing, the fresh produce is steamed (blanched) to inactivate enzymes that would alter the product even at low temperatures. Quick-freeze methods, using temperatures of - 32°C or lower, are considered most satisfactory; smaller crystals of ice are formed, and cell structures in the food are not disrupted. It should be emphasized that freezing foods, no matter how low the temperature,

cannot be relied upon to kill all microorganisms. The number and types of viable and nonviable microorganisms present in frozen foods reflect the degree of contamination of the raw product, the sanitation in the processing plant, and the speed and care with which the product was processed. The microbial count of most frozen foods decreases during storage; but many organisms, including pathogens, e.g., species of *Salmonella*, survive for long periods of time at —9 and - 17°C. Mechanical methods of refrigeration and ice making were first patented in the 1830s. These were based on the cooling produced by the vaporization of refrigerant liquids, originally ether but later liquid ammonia.

Chilled foods are those foods stored at temperatures near, but above their freezing point, typically 0–5˚C. This commodity area has shown a massive increase in recent years as traditional chilled products such as fresh meat and fish and dairy products have been joined by a hugevariety of new products including complete meals, prepared and delicatessen salads, dairy desserts and many others.Though psychrotrophs can grow in chilled foods they do so only relatively slowly so that the onset of spoilage is delayed.

Freezing is the most successful technique for long-term preservation of food since nutrient content is largely retained and the product resembles the fresh material more closely than in appertized foods.Foods begin to freeze somewhere in the range -0.5 to -3˚C, the freezing point being lower than that of pure water due to the solutes present. As water is converted to ice during freezing, the concentration of solutes in the unfrozen water increases, decreasing its freezing point still further so that even at very low temperatures, e.g.-60˚C, some water will remain unfrozen. The temperatures used in frozen storage are generally less than -18˚C. At these temperatures no microbial growth is possible, although residual microbial or endogenous enzyme activity such as lipases can persist and eventually spoil a product. This is reduced in the case of fruits and vegetables by blanching before freezing to inactivate endogenous polyphenol oxidases which would otherwise cause the product to discolour during storage.

4.4.3 Dehydration

Dried foods have been used for centuries, and they are more common throughout the world than frozen foods. The removal of water by drying in the sun and air or with applied heat causes dehydration- The preservative effect of dehydration is due mainly to microbistasis; the microorganisms are not necessarily killed. Growth of all microorganisms can be prevented by reducing the moisture content of their environment below a critical level. The critical level is

determined by the characteristics of the organism and the capacity of the food item to bind water so that it is not available as free moisture.

4.4.4 Irradiation

Ultraviolet light of sufficient intensity and time of exposure is microbicidal to exposed microorganisms. Because ultraviolet light has very limited penetration power, microorganisms that are embedded or covered are unlikely to be affected. Thus, ultraviolet irradiation is limited to control of microorganisms on surfaces or thin, clear layers of liquid. Examples of applications in the food industry include meat-processing plants, control of surface growth on bakery products, sanitation of equipment, and treatment of water used for the depuration (cleansing) of shellfish. UV radiation has wavelengths below 450 nm ($\upsilon=10^{15}$ Hz) and a quantum energy of 3–5 eV (10^{-12} ergs). The quanta contain energy sufficient to excite electrons in molecules from their ground state into higher energy orbitals making the molecules more reactive. Chemical reactions thus induced in micro-organisms can cause the failure of critical metabolic processes leading to injury or death.give us an indication of the sensitive chemical targets within the cell. The greatest lethality is shown by wavelengths around 260 nm which correspond to a strong absorption by nucleic acid bases. The pyrimidine bases appear particularly sensitive, and UV light at this wavelength will, among other things, induce the formation of covalently linked dimers between adjacent thymine bases in DNA. If left intact these will prevent transcription and DNA replication in affected cells.The resistance of micro-organisms to UV is largely determined by their ability to repair such damage, although some organisms such as micrococci also synthesize protective pigments.

Ionizing radiations are lethal to microorganisms. The fact that they are microbicidal at room temperature and can penetrate are characteristics that make them attractive candidates for control of microorganisms in foods. Ionizing radiation has frequencies greater than 10^{18} Hz and carries sufficient energy to eject electrons from molecules it encounters. Gamma rays and electron beams (beta and cathode rays) have been experimented with extensively for use in the food industry. Canned and packaged foods can be sterilized by an appropriate radiation dosage. This 'cold sterilization" producesa rise in temperature of the product of only a few degrees. Radiation pasteurization is a term describing the killing of over 98but not 100percent of the organisms by intermediate doses of ionizing radiation.Ionizing radiation can affect

micro-organisms directly by interacting with key molecules within the microbial cell, or indirectly through the inhibitory effects of free radicals produced by the radiolysis of water.

4.5 Exercise

Answer the following:

Q.1 List and describe various intrinsic factors affecting microbial growth.

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Q.2 List and describe various extrinsic factors affecting microbial growth

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Q.3 What is food preservation? Enlist various methods for food preservation.

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Q.4 Discuss different Antimicrobial Barriers and Constituents present in natural foods.

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Q.5List and describe the principles upon which methods of food preservation are based.

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Q.6 Compare the antimicrobial action of the following methods of food preservation: canning, refrigeration, dehydration, and radiation.

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Q.7 What is the lowest temperature range at which food-poisoning bacteria will grow?

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Q.8 What physiological types of bacteria are most likely to be present when canned food spoils?

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4.6 Suggested Reading

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CONTROL OF MICROORGANISM AND FOOD SPOILAGE OF MEAT,MILK AND INDICATORS OF FOOD SAFETY AND QUALITY

Unit VII: Meat and its products, Poultry.

Content

7.0 Objective

Upon completing this unit, we will have the ability to:

1. Recognize sources of Animal food products contamination.
2. Different spoilage occurred Animal food products, including its types, causative agents, and the changes associated with it.
3. List the factors that can impact the rate of spoilage.
4. Describe the principles and techniques of Animal food products preservation.

7.0 Introduction

Meat refers to the flesh of animals that are typically used for food, such as beef, pork, lamb, and game meats. Meat products can include processed meats like bacon, ham, sausages, and deli meats. Poultry refers to domesticated birds that are raised for their meat and eggs, such as chickens, turkeys, ducks, and geese. Poultry meat products can include processed meats like chicken nuggets, turkey bacon, and duck sausage. Meat is a highly perishable food product that is susceptible to spoilage by microorganisms, such as bacteria, yeasts, and molds. Spoilage can result in a change in the color, texture, odor, and flavor of the meat, making it unappetizing and potentially unsafe for consumption. Meat and meat products can be contaminated with a wide range of microorganisms, including bacteria, viruses, and parasites that can pose a health risk to humans. Common pathogens include Salmonella, Campylobacter, E. coli, and Listeria. Salmonella can infect certain types of hens, which can result in the contamination of the internal contents of an egg, even if it appears normal and healthy, before the formation of its shell. In addition, filter-feeding shellfish such as oysters can accumulate naturally occurring Vibrio bacteria in seawater.

During food processing, various foodborne microorganisms can be introduced to the food from infected individuals handling it, or through cross-contamination from other raw agricultural products. For instance, Shigella bacteria, hepatitis A virus, and Norwalk virus can be transmitted by food handlers with unwashed hands who are themselves infected. In the kitchen, microbes can be transferred from one food to another if the same utensil, such as a knife or cutting board, is used for both foods without proper washing in between. Even if a food is thoroughly cooked, it can still become recontaminated if it comes in contact with raw foods or drippings from raw foods that may contain harmful microorganisms that cause spoilage.

Contaminated food can lead to outbreaks, and how it is handled afterwards can make a difference. Some bacteria need time to multiply before they can cause disease. Under favorable conditions, one bacterium can produce millions of offspring in 12 hours. Refrigeration or freezing generally stops bacterial growth, but two types of bacteria, Listeria monocytogenes and Yersinia enterocolitica, can still grow at refrigerator temperatures.

7.1 Sources of Contamination in Meat and Meat products and Poultry: It helps to understand at which point our meat food might become contaminated, as this will provide us with a better impetus for taking personal responsibility to reduce the potential for further contamination. Meat and poultry products can become contaminated with harmful

microorganisms during various stages of production and processing. Here are some common sources of contamination:

Animal intestines: Intestinal contents can contain harmful bacteria such as Salmonella and Escherichia coli (E. coli). If the intestines are not properly removed and the meat is not thoroughly cleaned, these bacteria can contaminate the meat.

Feces: During slaughter, feces can contaminate the meat and equipment. Cross-contamination can also occur when contaminated hands, clothing, or equipment touch other meat products.

Processing equipment: Meat processing equipment that is not properly cleaned and sanitized can harbor bacteria and transfer them to the meat.

Water: Contaminated water used during processing or in the animal's drinking supply can spread harmful bacteria.

Workers: Employees who handle meat products can inadvertently transfer bacteria if they have poor personal hygiene or if they are sick.

Environment: Animals living in unsanitary conditions can have higher levels of harmful bacteria, which can then spread to the meat during processing.

Feed: Contaminated animal feed can be a source of bacteria such as Salmonella and E. coli. If the animals are not properly monitored and the contaminated feed is not removed, the bacteria can end up in the meat.

Insects and rodents: Insects and rodents can carry bacteria and contaminate meat and processing areas.

Improper storage: If meat products are not stored at the correct temperature, harmful bacteria can grow and multiply, increasing the risk of contamination.

Cross-contamination: Cross-contamination can occur when raw meat comes into contact with cooked meat, vegetables, or other food products. This can happen during preparation or in storage.

Packaging: Improper packaging can lead to contamination, especially if the packaging is damaged or not sealed properly.

Imported products: Imported meat and poultry products may not meet the same safety standards as domestic products, increasing the risk of contamination.

Antibiotic-resistant bacteria: The overuse of antibiotics in livestock can lead to the development of antibiotic-resistant bacteria, which can contaminate meat products.

7.2Meat and Meat products and Poultry Spoilage

The concept of spoilage encompasses the idea of edibility, implying that the food is unfit to eat. Spoilage refers to the decomposition of food, where it can harbor harmful bacteria or toxins in quantities that render the food poisonous and unsafe for human consumption. This highlights the importance of proper handling, storage, and hygiene practices to minimize the risk of spoilage and ensure food safety.

The spoilage of meat and meat products, as well as poultry, can be attributed to both chemical and biological causes.

7.7.1 Chemical factors contribute to the spoilage of Meat and Meat products and Poultry.

Oxidation: Exposure to oxygen in the air can cause oxidative reactions in meat and poultry, leading to changes in flavor, color, and texture. Oxidation can result in rancidity, off-putting odors, and a decrease in overall quality.

Lipid oxidation: The fats present in meat can undergo oxidation, particularly unsaturated fatty acids, resulting in the development of off flavors, rancidity, and a deterioration of sensory attributes.

Maillard reaction: The Maillard reaction is a chemical reaction between amino acids and reducing sugars that can occur during cooking or processing. In meat products, excessive Maillard reactions can lead to the formation of undesirable flavors, browning, and a decrease in quality.

Contamination with chemicals: Improper use of chemicals during processing, preservation, or storage can contaminate meat and poultry products. This includes excessive use of preservatives, additives, or pesticides, which can have detrimental effects on product quality and safety.

Interaction with packaging materials: Certain packaging materials, such as low-quality plastics or reactive metals, can interact with meat and poultry products, leading to chemical spoilage. This can result in off flavors, off odors, and a deterioration of product quality.

7.7.1 Biological factors contribute to the spoilage of Meat and Meat products and Poultry.

Natural enzymes present in meat can cause biochemical changes over time, leading to spoilage. These enzymes can break down proteins and fats, resulting in texture changes, off flavors, and the formation of off-putting compounds.

Microbial growth, The growth of bacteria, yeasts, and molds on meat and poultry can lead to spoilage. These microorganisms can multiply under favorable conditions, causing off odors, sliminess, discoloration, and an overall unpleasant appearance. Some bacteria can also produce toxins that make the food unsafe for consumption.

Insect infestation: Due to insect infestation such as flies or beetles can invade meat products, laying eggs or contaminating them with their presence, resulting in spoilage.

Contamination with trichinae and worms like *Trichinellaspiralis* (trichinae) and various types of worms can contaminate meat, especially pork, if it is not properly cooked, leading to health risks and making the meat unfit for consumption.

Some time, Physical/ Mechanical damage during processing, handling, or storage can result in tissue breakdown, bruising, or exposure to contaminants. This can accelerate spoilage and render the meat or poultry unacceptable.

7.3 Role of Micro-organisms

Meat and meat products can harbor various microorganisms, including bacteria, yeasts, and molds, some of which can be harmful pathogen. The primary sources of these microorganisms are the animal's intestinal tract and skin. The composition of the microbial flora in meat is influenced by several factors, such as pre-slaughter husbandry practices, the animal's age at slaughter, handling during slaughtering and processing, temperature controls, preservation methods, packaging, and consumer handling and storage. Common bacteria found in meat before spoilage include Staphylococcus, Bacillus, Campylobacter, Clostridium, Listeria, and Salmonella. Mold species such as Cladosporium, Geotrichum, Penicillium, and Mucor, as well as yeast species like Candida spp and Cryptococcus sp, can also be present.

The storage conditions influence the types of microbes that develop in meat and meat products. The pH range of 5.5-7.0 is favorable for the growth of spoilage bacteria in meat, leading to slime formation, degradation of structural components, off-odors, and changes in appearance.

7.3.1 Types of Microorganism: Types of Microorganism to spoil the meat and its products: There are two main types of meat spoilage: aerobic spoilage and anaerobic spoilage.

Under aerobic conditions, bacteria can cause the following conditions:

Surface slime: Caused by species of Pseudomonas, Moraxella, Streptococcus, Bacillus, and Micrococcus. It is an early indication of spoilage.

Changes in color of meat pigment: The red color of meat can change to shades of green, brown, or grey due to the production of oxidizing compounds like hydrogen peroxide and hydrogen sulfide. Lactobacillus is mainly responsible for this change.

Changes in fats: Oxidation of unsaturated fats, known as oxidative rancidity, can occur chemically in the presence of air and may be catalyzed by light and copper. Pseudomonas or yeast can be responsible for oxidative rancidity.

Various surface colors due to pigmented bacteria: Red spots may be caused by bacteria such as Serratiamarcescens. Pseudomonas syncyanea can impart a blue color, while Chromobacteriumlividum and other bacteria can cause greenish-blue or brownish-black spots.

Aerobic growth of molds: This can lead to stickiness, whiskers (limited mycelial growth without sporulation), green patches (due to species of Penicillium), and decomposition of fats (as many molds have lipase).

Spoilage under anaerobic conditions, facultative and anaerobic bacteria are involved, resulting in the following changes:

Souring: Imparts a sour taste to meat due to the production of acids such as formic, propionic, and acetic acids.

Putrefaction: Anaerobic decomposition of protein with the production of foul-smelling compounds like hydrogen sulfide, indole, ammonia, and amines, mainly caused by species of Clostridium

Taint: Undesirable odors and tastes.

Meat spoilage can be characterized by off odors/off flavors, discoloration, and gas production. Off odors such as sweet and fruity, putrid, sulfurous, and cheesy can occur due to Pseudomonas species and sulfur compounds. Discoloration happens when bacteria produce hydrogen sulfide (H_2S), which converts muscle pigment to green sulfmyoglobin. Gas production is associated with Clostridium species, particularly in vacuum-packaged beef, accompanied by foul odors.List of micro-organisms are responsible for changes, including

***Salmonella*:** This bacterium is commonly found in raw poultry, eggs, and meat products. Salmonella can cause foodborne illness, with symptoms ranging from diarrhea to severe dehydration.

***Campylobacter*:** This bacterium is commonly found in raw poultry, meat, and unpasteurized milk. Campylobacter can cause foodborne illness, with symptoms ranging from mild diarrhea to more severe symptoms such as fever and abdominal pain.

Escherichia.coli: The primary microbial to the beef industry is Escherichia coli O157: H7Certain strains of E. coli can cause severe foodborne illness, with symptoms ranging from diarrhea to kidney failure**.**

***Listeria*:** Listeria is a bacterium that can contaminate ready-to-eat meat products and poses a significant food safety concern. It is an adulterant with a zero tolerance policy, meaning that any detection of Listeria in these products is considered unacceptable.

Clostridium botulinum: This bacterium can produce a deadly toxin that causes botulism, a rare but serious foodborne illness. *Clostridium botulinum* can be found in improperly processed canned meats and other low-acid foods. *Clostridium* bacteria are commonly found in soil and are known for their ability to produce spores that can survive in harsh conditions. *Clostridium botulinum* is a type of bacteria that can produce a deadly toxin if meat is not processed and stored properly.

Pseudomonas*:Pseudomonas* bacteria are commonly found in soil, water, and on plant surfaces. They are also frequently found in raw meat and poultry. *Pseudomonas* can grow in refrigerated temperatures and can cause spoilage by producing slime, off-flavors, and odors.

***Brochothrix*:**Brochothrix bacteria are commonly found in raw meat and poultry. They grow well at refrigerated temperatures and can cause spoilage by producing a sour odor, slimy texture, and discoloration of the meat.

Enterobacter:*Enterobacter* bacteria are commonly found in soil, water, and on plant surfaces. They are also frequently found in raw meat and poultry. *Enterobacter* can cause spoilage by producing a rancid odor, off-flavors, and slime.

Lactobacillus: Lactobacillus bacteria are commonly found in raw meat and poultry. They are also used in the production of fermented meat products such as sausages. Lactobacillus can cause spoilage by producing a sour odor and flavor.

Some important Spoilage micro-organism

Gram-negative bacteria: *Neisseriaceae: Psychrobacterimmobilis, P. phenylpyruvica, Acinetobacter spp., A. twoffii, A. Johnsonii, Pseudomonadaceae: Pseudomonas fluorescens, P. lundensis, P. fragi, P. putida*

Gram-positive bacteria:*Brochothrixthermosphacta, Kurthiazophii, Staphylococcus spp., Clostridium estertheticum, Clostridium frigidicarnis, Clostridium casigenes, Clostridium algidixylanolyticum sp. nov.*

7.3.2 Deteriorative Effect of Micro-organisms on different Meat and Meat products and Poultry

Meat: Compared to fruits and vegetables, meat and its product primarily consists of protein and fats rather than carbohydrates. Water content in meat is typically around 71-76%, so moisture is not a concern except for cured meats susceptible to spoilage. After animals are slaughtered, meat is exposed to contaminants, making proper sanitation practices crucial for producing high-quality meat. The initial microbial load on meat after slaughter is a critical factor in determining its shelf life. The surface of beef carcasses can contain varying levels of bacteria (ranging from 10^1 to 10^7cfu/cm2), with psychrotrophic bacteria being the most prevalent. Chopping and grinding meat can increase microbial contamination due to increased surface area and nutrient availability. Various microbes are commonly found on meat, but the dominant spoilage organisms differ depending on factors such as pH, composition, texture, temperature, and packaging atmosphere. For example, *Pseudomonas spp.*are the primary spoilage bacteria in raw meat and poultry stored aerobically, producing

malodorous compounds as they metabolize gluconates and amino acids. Dark, firm, and dry meat with a higher pH spoils more rapidly, while *Shewanellaputrefaciens* can produce sulfides and ammonia, causing color changes even in the presence of glucose. *Brochothrixthermosphacta* is a significant spoilage organism on fresh meat stored aerobically at refrigeration temperatures. *Enterobacteriaceae*, particularly species like *Serratia, Enterobacter*, and *Hafnia*, are major causes of spoilage in vacuum-packed, high pH fresh meats, producing organic acids, hydrogen sulfide, and greening. Lactic acid bacteria (LAB) grow in vacuum and modified atmosphere-packaged meat, leading to aciduric off-odors, gas and slime formation, and greening. *Clostridium spp.,* psychrophilic anaerobes, are associated with spoilage of vacuum-packaged meats, causing "blown pack" spoilage characterized by excessive gas formation and off odors. Yeasts and molds have a limited role in meat spoilage due to slow growth and poor competition with bacteria.

Egg spoilage: Egg spoilage can occur when there are breaks or cracks in the eggshell due to transportation or mechanical damage, allowing microorganisms to enter the egg yolk and cause spoilage during storage called rotten. Stored eggs may experience moisture loss and weight reduction, while the egg white becomes thinner and more watery. The primary cause of egg spoilage is bacteria rather than molds. Common types of spoilage include green rot caused by Pseudomonas fluorescens, colorless rot caused by Pseudomonas, Acinetobacter, and other species, black rots caused by Proteus and Pseudomonas, red rots caused by Serratia spp., and custard rots caused by Proteus vulgaris and Pseudomonas intermedium. The growth of Aeromonas in the egg yolk can turn it black and produce a strong putrid odor due to the formation of hydrogen sulfide (H_2S). Storing eggs in a high-humidity environment can promote the growth of various molds on the eggshell surface, including species like Penicillium, Mucor, and Alternaria.

Poultry meat : Contamination in poultry meat can occur from various sources, similar to other types of meat. During different processing operations, the skin and lining of the body cavity can become contaminated. Salmonella spp. and Campylobacter jejuni are particularly significant bacteria associated with poultry. Additionally, Gram-negative psychrotrophic bacteria like Pseudomonas, Acinetobacter, and Flavobacterium have been detected in poultry carcasses. Ground turkey may also carry fecal streptococci. Rapid freezing of poultry is crucial to preserve its quality for extended periods. Freezing helps reduce the number of microorganisms in the meat, especially when stored at very low temperatures (-18 °C or below).

Spoilage of fish and sea food: Halophilic bacteria such as Serratia, Micrococcus, Bacillus, Alcaligenes, and Pseudomonas are responsible for spoilage of salt fish. Shellfish can be spoiled by Acinetobacter, Moraxella, and Vibrio. Crab meat is susceptible to spoilage by Pseudomonas, Acinetobacter, and Moraxella at low temperatures, and by Proteus at high temperatures. The microbial contamination in shrimps, oysters, and clams depends on the quality of the water they are harvested from. If water bodies are contaminated with sewage, the microbial quality of the seafood deteriorates. During handling, fecal coliforms, fecal streptococci, and Staphylococcus aureus can be introduced into the product. Oysters may also contain Salmonella due to water contamination. Seafood can be a source of various pathogens, including Pseudomonas spp., *Clostridium perfringens, Listeria monocytogenes, Vibrio parahaemolyticus, Salmonella entericaserovarEnteritidis* and *Typhimurium, Campylobacter jejuni, Yersinia enterocolitica, and enteroviruses*such as Hepatitis A. Pathogenic *Listeria monocytogenes* can also be found in smoked salmon and shrimps.

Meat Borne Diseases: Foodborne microbial hazards have a significant impact on human health. Some of the current concerns in fresh meat include pathogens like Salmonella, Campylobacter, enterohaemorrhagic Escherichia coli (including serotype O157:H7), and other enteric pathogens. Listeria monocytogenes infections from ready-to-eat meat and poultry products have also become a major problem. Other foodborne infections can be caused by Yersinia enterocolitica, Staphylococcus aureus, Clostridium botulinum, Clostridium perfringens, and Bacillus cereus. Pathogens like Vibrio cholerae, Vibrio vulnificus, Norovirus, Enterobactersakazakii, prions, and resistant bacteria have been recognized as foodborne pathogens since the 1970s. Animal health pandemics, such as Avian Influenza (AI) and Foot and Mouth Disease (FMD) viruses, have also been associated with foodborne pathogens. Avian influenza does not pose a risk to poultry meat safety when cooked properly at temperatures of 70°C or higher. Transmission through the oral route is less significant compared to non-foodborne routes. Pathogenic microorganisms are constantly adapting and developing resistance to antibiotics and traditional food preservation methods such as low pH, heat, cold temperatures, dryness, low water activity, and chemical additives. The development of antibiotic resistance in foodborne pathogens is a major concern for public health now and in the future.

Meat borne Disease	**Pathogens**	**Likely Contaminated Meat Products**
Salmonellosis	Salmonella spp.	Raw or undercooked poultry, beef, pork,

		and eggs
Campylobacteriosis	Campylobacter jejuni	Raw or undercooked poultry, unpasteurized milk
Escherichia coli (E. coli) Infections	Enterohaemorrhagic E. coli (EHEC), including serotype O157:H7	Undercooked ground beef, raw milk, contaminated water
Listeriosis	Listeria monocytogenes	Ready-to-eat deli meats, unpasteurized dairy products
Yersiniosis	Yersinia enterocolitica	Raw or undercooked pork, contaminated water
Staphylococcal Food Poisoning	Staphylococcus aureus	Processed meats, dairy products
Botulism	Clostridium botulinum	Improperly canned or preserved meats
Clostridial Food Poisoning	Clostridium perfringens	Cooked meats that are not reheated properly
Bacillus cereus Food Poisoning	Bacillus cereus	Cooked meat products
Vibrio Infections	Vibrio cholerae, Vibrio vulnificus	Raw or undercooked seafood, especially oysters
Norovirus Infections	Norovirus	Raw or undercooked seafood, contaminated water
Enterobactersakazakii Infections	Enterobactersakazakii	Powdered infant formula
Prion Diseases	Prions	Contaminated beef products
Antibiotic-Resistant Bacterial Infections	Resistant bacteria	Any meat product contaminated with resistant bacteria
Avian Influenza	Avian Influenza (AI) viruses	Raw or undercooked poultry
Foot and Mouth Disease	Foot and Mouth Disease (FMD) viruses	No specific meat product implicated

7.4 Role of different factors to control the Spoilage of meat products: To control the spoilage of meat and poultry food products, it is essential to manage both extrinsic and

intrinsic factors.these factor significantly reduce the spoilage of meat and poultry food products, extend their shelf life, and ensure their safety and quality for consumers.

Extrinsic Factors:

Temperature: Keep the temperature of meat and poultry products properly controlled throughout the supply chain, including during transportation, storage, and display. Refrigeration at temperatures below 4°C (40°F) slows down bacterial growth and extends shelf life.

Humidity: Maintain appropriate humidity levels to prevent excessive moisture or drying, as both can promote microbial growth or lead to quality deterioration. Packaging materials should provide a suitable moisture barrier.

Packaging: Use appropriate packaging materials and techniques to protect meat and poultry products from contamination, moisture loss, and exposure to oxygen. Vacuum packaging, modified atmosphere packaging (MAP), or a combination of both can help extend shelf life.

Light: Protect meat and poultry products from direct exposure to light, especially UV light, as it can lead to lipid oxidation and quality deterioration. Packaging should be opaque or provide adequate light-blocking properties.

Intrinsic Factors:

pH Level: Monitor and control the pH level of meat and poultry products, as it affects microbial growth. Lower pH (acidic conditions) inhibits the growth of spoilage and pathogenic bacteria. Marinades or ingredients with acidic properties can help control pH.

Water Activity (aw): Manage the water activity level in meat and poultry products by using appropriate formulations, drying techniques, and packaging. Controlling water activity limits microbial growth, as most bacteria require sufficient moisture for survival and multiplication.

Antimicrobial Ingredients: Use natural or approved antimicrobial ingredients such as salt, spices, herbs, and organic acids to inhibit microbial growth and extend the shelf life of meat and poultry products. These ingredients can be incorporated into marinades, brines, or coatings.

Quality of Raw Materials: Ensure the quality and freshness of raw meat and poultry products. Source them from reliable suppliers and maintain strict quality control measures to prevent initial contamination or spoilage.

Processing Conditions: Optimize processing conditions such as temperature, time, and hygiene practices during slaughter, butchering, and further processing to minimize bacterial contamination and maintain product integrity.

7.5 Different Prevention method of meat products

Refrigeration: Refrigeration is a commonly used preservation method for meat and poultry products. By storing the products at temperatures below 5°C (41°F), the growth of spoilage microorganisms is significantly slowed down. Cold temperatures inhibit the activity of bacteria, yeasts, and molds, extending the shelf life of the products.

Freezing: Freezing is another effective preservation method. By reducing the temperature of the meat or poultry products to below -18°C (0°F), microbial activity is virtually halted, preventing spoilage. Freezing also helps to maintain the quality, texture, and flavor of the products over a longer period.

Vacuum Packaging: Vacuum packaging involves removing air from the packaging material and sealing it tightly around the product. This method creates an oxygen-free (anaerobic) environment, which inhibits the growth of spoilage bacteria and extends the shelf life of the products. Vacuum packaging also helps to prevent freezer burn in frozen products.

Canning: Canning involves placing meat or poultry products in cans or jars and subjecting them to heat treatment. The heat destroys microorganisms and enzymes, making the products shelf-stable. Canned products can be stored at room temperature and have a long shelf life.

Drying: Drying or dehydration involves removing moisture from the meat or poultry products. This method inhibits the growth of spoilage microorganisms by eliminating the water necessary for their survival. Dried products, such as jerky or dried sausages, have an extended shelf life and can be stored at room temperature.

Smoking: Smoking is a preservation method that combines the effects of heat, drying, and the antimicrobial properties of smoke. The smoke contains compounds that inhibit microbial growth, while the heat and drying action further reduce moisture content. Smoking helps to preserve the flavor and extend the shelf life of the products.

Fermentation: Fermentation involves the use of beneficial microorganisms, such as lactic acid bacteria, to convert sugars in the meat or poultry products into lactic acid. The lowered pH created by fermentation inhibits the growth of spoilage and pathogenic bacteria. Fermented products, such as sausages or cured meats, have an extended shelf life and unique flavors.

Chemical Preservatives: Various chemical preservatives can be used to inhibit the growth of microorganisms in meat and poultry products. Common examples include sodium nitrite, sodium lactate, and citric acid. These preservatives can prevent the growth of bacteria and extend the shelf life of the products when used in appropriate concentrations.

Lets Sum Up

Table 1. Meat and poultry products undergoes different types of spoilage depending on its composition as shown in Table 7.1

Product	**Spoilage**	**Microorganism**
Fresh meat	Putrefaction	Alcaligenes Clostridium Proteus vulgaris Pseudomonas fluorescens
Cured meat	Moldy	AspergillusRhizopusPencillium
	Souring Pseudomonas Micrococcus	Pseudomonas Micrococcus
	Greening,	slime Lactobacillus Leuconostoc
Fish	Discoloration	Pseudomona
	Colourless rot	AlcaligenesFlavobacterium
Eggs	Green rot	Pseudomonas fluorescens
	Colourless rot	Pseudomonas alcaligenes
	Black rot	roteus
Poultry	Slime, odour	Pseudomonas Alcaligenes

Table2 : Sum up the different preservation methods

Preservation Method	Effect on Microorganisms
Refrigeration	Slows down microbial growth and extends shelf life.
Freezing	Inactivates microorganisms, preserving the product for an extended period.
Canning	High heat kills microorganisms, creating a sterile environment.
Drying	Removes moisture, inhibiting microbial growth.
Smoking	Exposure to smoke and heat inhibits microbial growth.
Salting	Draws out moisture, creating an inhospitable environment for microorganisms.
Pickling	Acidic brine inhibits microbial growth.
Vacuum Packaging	Creates an anaerobic environment, reducing microbial spoilage.
Modified Atmosphere	Altering the gas composition in packaging inhibits microbial growth.
Heat Treatment	High temperatures kill or inactivate microorganisms.
Chemical Preservatives	Use of approved antimicrobial substances inhibits microbial growth.
Fermentation	Encourages the growth of beneficial bacteria that inhibit spoilage microorganisms.

Check up your knowledge

Fill in the blanks:

a) Meat has a high-.: ..... activity which is ideal for, the growth of microorganisms.

b) An important microorganism that leads to fish spoilage under stored condition is .....

c) .....species is the primary spoilage organism in the meat at 10°C or below.

e) The oysters are spoiled at near .....temperatures by Pseudomonas.

2) Define the following terms:

I. **Ripening**
II. **Rotten**
III. **Thermophiles**
IV. **Meat Borne Diseases:**

List two major important factors that are involved in meat spoilage.

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Different Prevention method of meat products

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Halophilic bacteria

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Glossary:

Additives : Natural and man-made substances added to a food for an intended purpose (such as preservatives and colours) or unintentionally (such as pesticides and lubricants)

Adulteration : Deliberate contamination of foods with materials of low quality.

Aerobic : Requires oxygen

Antimicrobials : Preservatives that protect food by slowing the growth of bacteria, molds and yeasts.

Antioxidants : Preservatives that protect by preventing food molecules from combining with oxygen (air).

Biological Hazard : Danger posed to food safety by the contamination of food with pathogenic micro-organisms ornaturally occurring toxins.

Chemical Hazard : Danger posed to food safety by the contamination of food by chemical substances, such as pesticides, detergents, additives, and toxic metals.

BLOCK-III: CONTROL OF MICROORGANISM AND FOOD SPOILAGE OF MEAT,MILK AND INDICATORS OF FOOD SAFETY AND QUALITY

Unit VIII: Milk and Milk products, Canned foods.

1.0 Objective

2.0 Introduction

2.1 Composition of Milk and Contamination with microorganism

2.2 Spoilage of Raw milk and milk products:

2.3 Different Milk products Spoilage:

2.3.1 Pasteurized Milk Spoilage:

2.3.2 Spoilage of Concentrated Milk Products:

2.3.3 Ice Cream Spoilage

2.3.4 Fermented Milk Products Spoilage

2.4 Preservation of milk and milk products from microbial spoilage

2.5 Spoilage of Canned food

2.6 Spoilage and prevention of different Canned product:

2.6.1 Canned vegetables spoilage and Prevention:

2.6.2 Canned meat and poultry spoilage and prevention

2.6.3 Canned sauces and condiments spoilage and prevention

2.6.4 Spoilage of canned food can occur in various ways depending on the acidity of the food

2.7

Summary

1.0 Objective:

The objective of studying microbial spoilage in milk and milk products, as well as canned foods, is to identify and quantify the microorganisms responsible for spoilage. This chapter aims to understand the types of microorganisms present, their abundance, and their potential impact on the quality and safety of these food products. By studying microbial spoilage, we seek to contribute to the development of effective strategies for prevention, control, and preservation of milk, milk products, and canned foods, ultimately improving their shelf life and ensuring consumer safety.

2.0 Introduction

Milk undergoes various preservation methods due to its perishable nature and the presence of harmful pathogens. These treatments include pasteurization, commercial sterilization, fermentation, dehydration, refrigeration, and freezing. Milk products such as heated milk, yogurt, kefir, kumiss, cream, butter, cheese, condensed and dried milk, and others are marketed. Spoilage of milk and its products leads to off-flavors, odors, texture changes, and appearance alterations. This chapter provides an overview of milk and milk product contamination, spoilage, and the interaction of microorganisms with dairy foods.

2.1 Composition of Milkand Contamination of Raw milk with microorganism

Milk is a nutrient-rich source for microorganisms, containing carbohydrates, fats, casein, lactalbumin, and free amino acids. With a pH of 6.4, cow's milk has fats mainly present as globules in an oil-in-water emulsion, coated with proteins and polar lipids. Its fat composition consists of triglycerides (98%), diacetyl glycerol, phospholipids, and fatty acids.To be sold in fluid form, milk must undergo a minimum heat treatment of 72 °C for 15 seconds. However, most processors use higher temperatures and longer holding times. This pasteurization process eliminates pathogens and many spoilage microorganisms. However, it may promote the survival of heat-resistant spoilage microflora by destroying inhibitory chemicals and activating spores. Heat treatments impact microbial growth by increasing the availability of nitrogen through protein denaturation and sulfhydryl compounds.

Table 1: An approximate nutritional composition of raw cow's milk per 100 grams:

Nutrient	**Amount**
Calories	61 kcal
Protein	3.2 g
Fat	3.7 g
Carbohydrate	4.8 g
Sugar	4.8 g
Calcium	120 mg
Phosphorus	93 mg
Potassium	156 mg
Sodium	50 mg
Vitamin A	68 µg
Vitamin D	0.3 µg
Vitamin B12	0.4 µg
Riboflavin (B2)	0.18 mg

Initial microflora in raw milk can come from various sources such as the udder, udder surfaces, milking equipment, transport lines, storage tanks, environment (air and water), and workers. Animal manure, flies, soil, water, animals, and plant material can also contaminate raw milk. Undesirable bacteria found in raw milk include lactic Streptococcus, coliforms, psychrotrophic Gram-negative thermoduric bacteria (Micrococcus, Enterococcus, Bacillus, Brevibacterium), among others. Proper cleaning and sanitizing methods are crucial for controlling contamination. Raw milk may contain different levels of microorganisms depending on milking, cleaning, and handling practices. Psychrotrophic bacteria can grow in refrigerated raw milk and include genera such as Aerococcus, Bacillus, Lactobacillus, Leuconostoc, Microbacterium, Micrococcus, Propionibacterium, Proteus, Pseudomonas, Streptococcus, and coliforms. Pasteurization reduces psychrotrophic bacteria but some thermoduric and thermophilic bacteria can survive. Coliforms can be present on udder skin due to fecal contamination, and inadequate cleaning of this area or milk equipment can contribute to high coliform populations in raw milk. Water used for cleaning milking equipment can indirectly contaminate milk with psychrotrophic bacteria that produce enzymes. Spore-forming bacteria in raw milk, mainly Bacillus spp. (such as B. cereus, B. licheniformis, B. megaterium, B. subtilis), and low levels of Clostridium spp. can cause spoilage. The presence of spore-forming bacteria varies seasonally, with higher levels in winter due to contaminated bedding materials and spore-containing silage. Mastitis-causing

microorganisms commonly include Staphylococcus aureus, coagulase-negative Staphylococcus, Streptococcus agalactiae, and Streptococcus dysgalactiae.

Table:2 Types of microbial spoilage of milk.

Type of Spoilage	**Microorganism**	**Metabolic Product**
Lipolytic Spoilage	Pseudomonas spp.	Lipases - breakdown of milk fat into fatty acids
Proteolytic Spoilage	Pseudomonas spp., Bacillus spp.	Proteases - breakdown of milk proteins into peptides and amino acids
Acid Production Spoilage	Lactic acid bacteria (e.g., Lactobacillus spp.)	Lactic acid - leads to a decrease in pH and souring of milk
Yeast and Mold Spoilage	Yeasts and molds	Various enzymes (proteases, lipases, etc.) - degradation of proteins and lipids

2.3 Different Milk products Spoilage:

2.3.1 Pasteurized Milk Spoilage: When milk undergoes pasteurization, it effectively eliminates the majority of acid-forming bacteria. However, certain heat-resistant bacteria such as Bacillus and Clostridium can survive this process. Souring, a common form of spoilage in milk, is caused by thermoduric lactic acid bacteria, resulting in an unpleasant sour taste and odor. Bacillus spores have the ability to cause coagulation in milk without producing any off-flavors. During low-temperature storage, psychrotrophic*Bacillus* species can germinate, leading to a specific type of spoilage known as "bitty" and causing the aggregation of fat globules. Psychrotrophs also produce rennin-like enzymes that can induce sweet curdling in milk. Psychrotrophic Bacillus cereus and Bacillus polymyxa are known to spoil nonaseptic packaged milk, while *Bacillus* circulans dominates in aseptically packaged heat-treated milk, resulting in a sour flavor. Another psychrotrophic spore former in milk is *Bacillus*mycoides. Spoilage in milk can be visually observed as bacterial colonies, and the enzymatic breakdown of casein can contribute to a bitter taste.

Table 3: Types of microbial spoilage of milk.products

Milk Products	**Type of Spoilage**	**Bacteria Involved**
Pasteurized Milk	Bacterial Spoilage	Pseudomonas, Bacillus, coliforms

	Yeast and Mold Spoilage	Yeasts, molds
Concentrated Milk	Bacterial Spoilage	Pseudomonas, Bacillus, coliforms
	Yeast and Mold Spoilage	Yeasts, molds
Fluid Milk	Bacterial Spoilage	Pseudomonas, Bacillus, coliforms
	Yeast and Mold Spoilage	Yeasts, molds
Cream	Bacterial Spoilage	Pseudomonas, Bacillus, coliforms
	Yeast and Mold Spoilage	Yeasts, molds
Yogurt	Bacterial Spoilage	Lactic acid bacteria, Pseudomonas
	Yeast and Mold Spoilage	Yeasts, molds
Cheese	Bacterial Spoilage	Lactic acid bacteria, Pseudomonas, coliforms
	Yeast and Mold Spoilage	Yeasts, molds
Butter	Bacterial Spoilage	Pseudomonas, Bacillus, coliforms
	Yeast and Mold Spoilage	Yeasts, molds

2.3.2 Spoilage of Concentrated Milk Products: Concentrated milk products, such as evaporated milk, unsweetened condensed milk, and sweetened condensed milk, undergo heat treatments to kill microorganisms. Evaporated milk is sterilized in sealed cans, while condensed milk is concentrated and packed in cans. Heat-resistant bacteria like *Bacillus* and *Clostridium* can survive high-temperature treatments and cause coagulation, bitterness, and protein destruction. Gas-forming anaerobic spore formers and postprocess contaminant yeasts can lead to can swelling, off-flavors, and spoilage. Xerophilic fungi may cause blowing of cans. Poor hygiene can introduce spoilage microorganisms. Growth of spore-forming bacteria, such as *Bacillus coagulans* and *B. cereus*, can result in coagulation and protein destruction. *Clostridium sporogenes* can cause can swelling. "Flat sour" spoilage without gas production can occur due to the growth of certain *Bacillus* species. *S. aureus* growth is prevented by anaerobic conditions in condensed milk, but abuse of temperature can allow

growth. Listeria monocytogenes can grow faster in ultrafiltered milk compared to regular milk.

2.3.3 Ice Cream Spoilage: The production of ice cream involves several steps, including preheating, homogenization, and pasteurization of the mix. Flavors and ingredients are added after pasteurization, and the mix is aged to stabilize and crystallize the fat. Freezing, along with the introduction of air, is done to increase the volume of the ice cream. While heat treatments during production destroy microbes, contamination can still occur during the addition of ingredients and handling. Proper freezing methods are crucial to prevent microbial growth. Common contaminants in ice cream include *Streptococcus, Micrococcus, Pseudomonas,* spore formers, *coliforms,* and spoilage yeasts. Ice cream made with raw milk carries a higher risk of pathogenic microorganisms such as *Salmonella, Listeria, Staphylococcus,* and *Campylobacter*. Preventive measures for ice cream production include using high-quality ingredients, maintaining sanitation practices, preventing recontamination, and ensuring proper processing and storage conditions.

2.3.4 Fermented Milk Products Spoilage: Fermented milk products like buttermilk, yogurt, and cheese are produced by inoculating milk with specific starter cultures. Cheese is susceptible to spoilage by bacteria, yeasts, and molds. Factors influencing cheese spoilage include processing conditions, water activity, pH, salt, moisture ratio, temperature, starter culture characteristics, and microorganism types and viability. *Psychrotrophic* Gram-negative bacteria can contaminate and spoil cheese, causing visual and physical defects. LAB (lactic acid bacteria) can contribute unwanted flavors, gas production, and soft defects in cheese. Heterofermentative Lactobacillus and *Leuconostoc* spp. can cause gas and liquid accumulation. White crystalline deposits on the surface of ripened cheese are caused by *Lactobacillus* spp. producing insoluble calcium lactate crystals. *Lactobacillus casei* subsp. *alactosus* and *Lactobacillus casei subsp. rhamnosus* can lead to a phenolic flavor. LAB, particularly *Lactococcus* spp., can produce esterase, resulting in fruity off-flavors in cheese.

Butter is composed of water, fat, carbohydrates, proteins, and ash. Its quality depends on the cream and sanitary conditions used in processing. Butter can be spoiled by psychrotrophs, coliforms, yeasts, and LAB. Spoilage can manifest as surface taint, rancidity, flavor formations, discoloration, and excessive viscosity. Yeasts can cause off-flavors and metabolize diacetyl. Rancidity can result from lipolysis by microbial lipases or lipolytic bacteria. Uneven distribution of salt and moisture in butter can lead to the growth of

lipolyticpsychrotrophs. Desired flavor in butter depends on the growth of starter cultures and proper conditions. Pathogenic bacteria like *Staphylococcus aureus, Listeria monocytogenes,* and Campylobacter jejuni can survive and grow in butter due to contamination, poor hygiene, inadequate heat treatment, and temperature manipulation. The putrid, proteolytic fruity flavours in butter are caused by the psychrotrophic bacteria. Pseudomonas fluorescens and Pseudomonas fiagi are associated with the fruity odour in butter, They are both proteolytic and lipolytic i.e., they decompose proteins and fats.AFM1 mycotoxin can also contaminate butter, although most mycotoxins are removed during processing.

Sweetened condensed milk is a thick milk product with 8% milk fat and 23% total milk solids. It is sweetened with sucrose to prevent spoilage caused by reduced water activity. The milk is packed in sealed cans, which can be stored for long periods without refrigeration. Spoilage occurs when osmophillic yeasts or molds like *Tomlopsis* spp. grow in under-filled cans, causing gas formation and swelling. Spoilage organisms may enter through the canning equipment. Spray-dried milk powder, on the other hand, is prepared by concentrating the milk and then atomizing it into a hot air chamber. The drying air, heated to high temperatures, removes moisture from the milk particles. The dried powder is cooled, sifted to remove clumps, and then packed. The spoilage of dried milk depends on the initial presence of organisms in the raw milk and the processing sanitation conditions, with *Micrococcus* spp. and *Bacillus subtilis* being common heat-resistant organisms.

2.4 Preservation of milk and milk products from microbial spoilage

Milk and its products are rich in nutrients, making them an ideal medium for microorganism growth. To prevent spoilage and preserve the nutritional qualities of milk, various preservation methods are employed. Numerous techniques have been developed to control the growth of microorganisms in milk and its products.

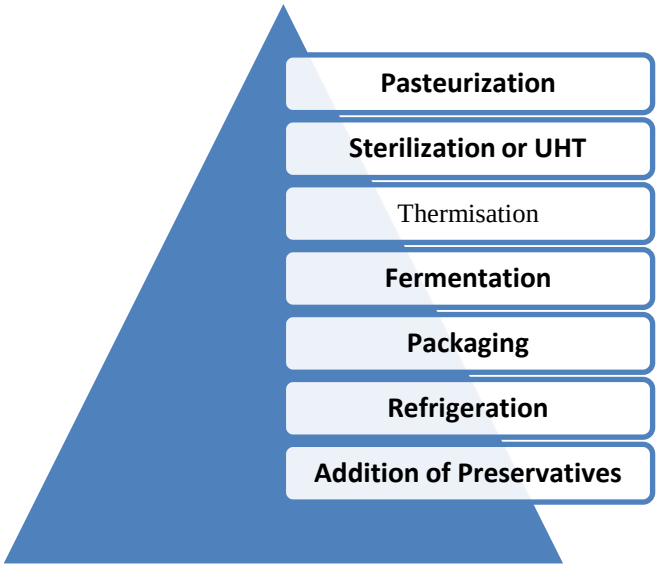

Fig 1: Different Types of Preservation Technique

Pasteurization: Pasteurization is a heat-based method used for food preservation, including milk. Heating milk to a specific temperature (usually around 72°C for 15-20 seconds) to kill pathogenic bacteria and reduce spoilage microorganisms. This process extends the shelf life of milk and ensures it is safe for consumption. It involves applying heat below 100°C for a specific duration to reduce the number of harmful pathogens and spoilage-causing microorganisms (such as *Coxiellaburnetii*, *Brucellaabortis*, and Mycobacterium tuberculosis) without compromising milk quality. The process kills thermoduric species found in milk, such as *Micrococcus spp., Enterococcus faecium*, *Enterococcus faecalis*, *Bacillus subtilis*, *Bacillus cereus*, and certain *lactobacilli*. There are four common types of milk pasteurization, which vary in temperature and duration. These include

- vat pasteurization (heated at 63°C for 30 minutes),
- high-temperature/short-time (HTST) pasteurization (heated at 72°C for 15 seconds),
- ultra-pasteurization (heated at 138° to 150°C for 1-2 seconds),
- ultra-high-temperature (UHT) pasteurization (heated at 280°F for 2 seconds).

Sterilization or UHT: InUltra-High Temperature (UHT) Treatment milk is heated to a very high temperature (above 135°C) for a short period (2-5 seconds) to achieve commercial sterility. UHT-treated milk can be stored without refrigeration for an extended period. Sterilized milk, for example, can be stored at room temperature for an extended period. There are two commonly used methods of sterilization.

- conventional or in-bottle sterilization method, where the product is packaged prior to heat treatment and heated at 105-110°C for 30-45 minutes.
- UHT or aseptic method, which involves heating the product at 135-150°C for 1-20 seconds, followed by immediate aseptic filling into sterile containers.

Thermisation: The most commonly employed method for milk preservation is known as thermisation. It involves heating the milk at a moderate temperature of 57-68 °C for 15-20 seconds, followed by rapid cooling to below 6 °C. While this method effectively controls spoilage-causing bacteria, it does not eliminate pathogens like L. *monocytogenes*. The primary goal of thermisation is to reduce the growth of psychrotrophic bacteria and prolong the shelf life of milk.

Refrigeration: Keeping milk and dairy products at temperatures below 5°C slows down microbial growth and helps to maintain freshness. This method is commonly used for liquid milk, yogurt, and other perishable dairy products.

Freezing: Freezing milk and dairy products significantly extends their shelf life. However, freezing can affect the texture and quality of certain dairy products, so it may not be suitable for all types.

Fermentation: The process of converting lactose (milk sugar) into lactic acid by adding beneficial bacteria or yeast cultures. Fermented milk products like yogurt, kefir, and cheese have a longer shelf life due to the acidic environment created by fermentation.

Packaging: Proper packaging plays a crucial role in preserving milk and dairy products. It should be airtight, light-resistant, and provide a barrier to moisture and external contaminants. Common packaging methods include bottles, cartons, pouches, and cans.

Addition of Preservatives: Preservatives are substances that are capable of inhibiting or retarding the growth of microorganisms. Such preservatives used in food can be divided into three types:

1. **Natural preservatives**
2. **Bio preservatives**
3. **Chemical preservatives**

Table 4: Preservatives that are used in milk and its products are:

	Product	Types of preservatives	Preservatives used
1.	Milk	Natural preservatives	Honey, lecithin
		Bio preservatives	LAB, bacteriocin, hydrogen peroxide
		Chemical preservatives	Benzoic acid, Sorbic acid, nisin, sodium diacetate, boric acid, formaldehyde,
2.	Cheese	Natural preservatives	Salt, essential oils (Thyme, Ginger, Cayenne, Clove, Cinnamon, Garlic, Lemongrass, Oregano, Basil), Lime juice
		Chemical preservatives	Sorbic acid, Potassium sorbate, propionic acid, Natamycin
		Bio preservatives	Lysozyme, Nisin, LAB,
3.	Ice cream	Chemical preservatives	Butyraldehyde, , Potassium sorbate, Diethyl glycol, Polysorbate 80
		Natural preservatives	Amyl acetate (banana oil), Piperonal (vanilla bean), corn starch, Soy lecithin,
4.	Butter	Natural preservatives	Salt, thymine, cumin
5.		Chemical preservatives	BHA(butylated hydroxyl anisole, BHT(butylated hydroxyl toluene), rosmaricin acid, gallic acid
6.	Yogurt	Chemical preservatives	Potassium sorbate& Sodium benzoate, , Antibiotic (Natamycin).

2.5 Spoilage of Canned food

Canned food has been a popular choice for preserving food for long periods due to its convenience and extended shelf life. However, despite the canning process designed to inhibit microbial growth, canned food is still susceptible to spoilage. Microbial spoilage in canned food can occur due to various factors, including improper processing, post-canning contamination, and storage conditions. Bacteria, yeasts, and molds are the main culprits behind canned food spoilage. Bacteria such as *Clostridium botulinum* and *Bacillus cereus* can survive the canning process and, under suitable conditions, grow and produce toxins that pose health risks. Yeasts and molds, known for their tolerance to low moisture levels, can also contaminate canned food, leading to texture changes, off-flavors, and visible signs of spoilage.

2.6 Spoilage and prevention of different canned product:

Canned food can be susceptible to microbial spoilage for three main reasons.

- First, spores of thermophilic bacteria can survive the canning process.
- Second, if there is inadequate cooling, insufficient heat treatment, or improper storage temperature, the surviving thermophilic bacteria can grow and cause spoilage.

- Finally, microorganisms can recontaminate the canned food if there is leakage in the can.

2.6.1 Canned vegetables spoilage and Prevention: are a popular and convenient food option, but they can be susceptible to bacterial spoilage if proper precautions are not taken during the canning process. One of the most concerning bacteria associated with canned vegetables is *Clostridium botulinum*. This bacterium thrives in low-acid, anaerobic conditions and can produce a dangerous toxin called botulinum toxin. If consumed, this toxin can cause botulism, a severe and potentially life-threatening illness.

In addition to *C. botulinum,* other bacteria such as Lactobacillus spp. and Bacillus spp. can also cause spoilage in canned vegetables. Lactobacillus bacteria, particularly certain species, can grow in canned vegetables that have high sugar or starch content. These bacteria produce lactic acid, leading to a sour taste, soft texture, and off-flavors in the vegetables.Bacillus bacteria are heat-resistant spore formers that can survive the canning process. They can be present in canned vegetables and may cause problems such as off-odors, gas production, and sliminess. These bacteria can be introduced during the canning process if proper hygiene practices are not followed or if the raw ingredients are contaminated.

It is important to note that commercially produced canned vegetables undergo rigorous quality control measures to ensure their safety. However, improper canning techniques, damaged cans, or contaminated ingredients can increase the risk of bacterial spoilage. Consumers should always inspect canned vegetables before consuming, looking for any signs of damage, bulging, or unusual odor. If any abnormalities are detected, it is best to discard the product to avoid the risk of foodborne illness.

2.6.2 Canned meat and poultry spoilage and prevention

Canned meat and poultry are popular food products that offer convenience and extended shelf life. However, they can be susceptible to bacterial spoilage if proper precautions are not taken during the canning process. Bacterial spoilage in canned meat and poultry is primarily caused by bacteria such as *Clostridium perfringens, Staphylococcus aureus,* and *Listeria monocytogenes*. *Clostridium perfringens* is a spore-forming bacterium that can cause foodborne illness when consumed in large numbers. It is commonly associated with improperly canned or undercooked meats. The spores of *C. perfringens* can survive the canning process, and if conditions are favorable (such as low oxygen levels and appropriate

temperature), they can germinate and grow, producing toxins that cause illness. Staphylococcus aureus is another bacterium that can cause spoilage in canned meat and poultry. It is commonly found on the skin and mucous membranes of humans and animals. If contaminated meat or poultry is not properly cooked before canning, *S. aureus* can survive and multiply. The toxins produced by this bacterium are heat-stable, so they can cause foodborne illness even if the canned product is reheated. *Listeria monocytogenes* is a pathogenic bacterium that can contaminate raw meat and poultry. It is a concern in canned products because it can survive and grow at refrigeration temperatures. Listeria contamination can occur during the processing of raw ingredients or due to cross-contamination during canning. Consumption of *L. monocytogenes*-contaminated canned meat and poultry can lead to severe illness, particularly in vulnerable populations such as pregnant women, the elderly, and individuals with weakened immune systems. To prevent bacterial spoilage and ensure the safety of canned meat and poultry, it is crucial to follow proper canning procedures. This includes thorough cooking of raw ingredients before canning, maintaining strict hygiene practices during processing, and utilizing adequate heat treatment during the canning process to destroy harmful bacteria. It is also important for consumers to carefully inspect canned meat and poultry for any signs of damage, bulging, or unusual odors before consumption. If any abnormalities are observed, it is best to discard the product to avoid the risk of foodborne illness.

2.6.3 Canned sauces and condiments spoilage and prevention: Canned sauces and condiments are susceptible to microbial spoilage, which can result in changes to flavor, texture, and safety of the product. Several types of microorganisms can cause spoilage in these canned products. Lactic acid bacteria, such as *Lactobacillus* and *Leuconostoc*species, are commonly found contaminants. They can ferment sugars and produce lactic acid, causing a decrease in pH and affecting the taste and consistency of the sauce or condiment. Yeasts, including Candida and Saccharomyces, are another group of microorganisms that can thrive in low-acid environments. They can ferment sugars, leading to the production of carbon dioxide and alcohol, which may cause fermentation off-flavors and gas formation in the product. Molds, such as *Aspergillus*and *Penicillium*, can grow on the surface of canned sauces and condiments. They can cause visible colonies, discoloration, and texture changes. In some cases, molds may produce mycotoxins, which are harmful compounds that can pose a health risk if consumed.

2.6.4 Spoilage of canned food can occur in various ways depending on the acidity of the food:Spoilage of canned food can occur in various ways depending on the acidity of the food and can result in specific defects in both the can and the food product it contains.

- **Acidic Spoilage**: In acidic canned foods, such as fruits and tomatoes, the spoilage can manifest in the following forms:
- **Souring:** The development of a sour or fermented taste due to the growth of acid-producing bacteria.
- **Can Corrosion**: The acidic nature of the food can cause corrosion of the can, leading to leaks and contamination.
- **Non-Acidic Spoilage**: Non-acidic canned foods, such as vegetables and meats, can experience spoilage in the following ways:
- **Flat Sour:** The absence of sourness in the spoiled food, but with an off-flavor and unpleasant odor caused by bacteria that do not produce gas.
- **Swell:** The growth of gas-producing bacteria can result in the bulging of the can, indicating spoilage.
- **Rancidity:** Fatty foods can undergo rancidity due to the oxidation of fats, leading to off-flavors and odors.

S. No	**Canned food based on pH**	**Examples of food**	**Cause of microbial spoilage**	**Defects**
1	Low-acid canned food (pH > 5.2)	Meat and meat products, milk, dairy products, and seafood.	Thermophilic flat-sour spoilage bacteria (*Geobacillusstearothermophilus* and *Bacillus coagulans*)	**Can:** flat, no gas, change in a vacuum **Product:** reducing pH, souring, off-odor, and flavor, sometimes cloudy juice.
		Corn and peas	Sulfide-producing anaerobic bacteria (*Clostridium nigrificans*, *Clostridium bifermentans*, and *Desulfotomaculumnigrificans)*	**Can:** flat, H2S produced are absorbed by food. **Product:** blackening," rotten egg" odor, iron sulfide precipitate
		Corn and spinach	Mesophilic putrefactive anaerobic bacteria (*Clostridium botulinum*)	**Can:** swells, may burst by gas production **Product:** partially

				digesting, increased pH, putrid odor, production of NH3, indoles, CO2, H2, and H2S
2		Corn and peas	Sulfide-producing anaerobic bacteria (*Clostridium nigrificans*, *Clostridium bifermentans*, and *Desulfotomaculumnigrificans)*	**Can:** flat, H2S produced are absorbed by food. **Product:** blackening," rotten egg" odor, iron sulfide precipitate
3		Corn and spinach	Mesophilic putrefactive anaerobic bacteria (*Clostridium botulinum*)	**Can:** swells, may burst by gas production **Product:** partially digesting, increased pH, putrid odor, production of NH3, indoles, CO2, H2, and H2S
4	Acid canned food (pH 4.5-3.7)	tomatoes, pears, figs, oranges, apricots, pineapples	Mesophilic spore-forming bacteria (*Bacillus polymyxa*, *Bacillus macerans*, and *Clostridium pasteurianum*),	**Can:** Flat or swelling, **Product:** coagulation, gas, and acid formation
			Butyric anaerobic bacteria(*Clostridium butyricu*m and *Clostridium tertium*)	**Can:** Swells, may burst **Product:** Fermentation, gas production (CO2, H2), butyric odor
			Aciduric bacteria *(G. stearothermophilus* and *B. coagulans*).	**Can:** flat, no gas, change in a vacuum **Product:** reducing pH, souring, off-odor, and flavor, sometimes cloudy juice.
5	High acid canned food (pH < 3.7)	pickled product, fermented product, ketchup, jams, jellies, marmalades, fruits, and butter	Yeasts *(Saccharomyces, Zygosaccharomyces)*	**Can:** Swells, may burst, and leakage by gas formation. **Product:** Fermentation, CO2 production, and yeasty odor.
			Molds *(B. fulva, Aspergillus, Penicillium, Citromyces)*	**Can:** Swells, may burst, and leakage by gas formation. **Product:** Fermentat

				ion, CO2 production, and yeasty odor.
			lactic acid bacteria (*L. brevis, L. mesentericus, L. dextranicum, L. mobilis)*	**Can:** swells, may burst by gas, or swelling may be arrested Pr**oduct:** Acid odor, Ropiness, and formation of acid and CO2

It is important to note that the defects caused by spoilage can impact the quality, taste, and safety of both the canned food and the integrity of the can itself. Regular inspection and proper storage conditions are essential to prevent and detect spoilage in canned products.

Preservation of canned food from microbial spoilage

Preventing microbial spoilage in canned sauces and condiments requires proper processing and packaging techniques. Thermal processing, including high-temperature sterilization, is crucial to destroy or inactivate microorganisms present in the product. It is important to follow recommended processing times and temperatures to ensure microbial safety. Additionally, maintaining strict sanitation practices throughout the production process is essential. This includes thorough cleaning and sanitization of equipment, facilities, and utensils to prevent cross-contamination and the introduction of spoilage microorganisms. Proper packaging is also vital to prevent microbial spoilage. The use of hermetically sealed containers, such as cans or jars, with appropriate closures helps create a barrier against microbial contamination. It is important to ensure that the containers are properly sealed to prevent the entry of air or microorganisms. Storage conditions play a significant role in preventing spoilage as well. Canned sauces and condiments should be stored in a cool, dry place away from direct sunlight and extreme temperature fluctuations. Proper storage helps maintain product quality and extends shelf life by minimizing the growth of spoilage microorganisms.

To prevent microbial spoilage of canned food and ensure its long-term storage, various preservation methods can be employed in combination with canning while maintaining the nutritional quality of the food.

Radiation: Radiation sterilization, known as radappertization, involves exposing sealed containers of food to ionizing radiation to eliminate spoilage-causing microorganisms. This method effectively targets the surfaces of canned jars and lids.

Preservatives: Preservative agents are substances that inhibit or slow down the growth of microorganisms. These substances, categorized as "Generally Regarded As Safe (GRAS)," include organic acids, sulfite, ethylene oxide, sodium nitrite, ethyl formate, LAB (lactic acid bacteria), and bacteriocins.

Chilling Storage: Canned foods can be stored at temperatures between 0°C and 5°C. Chilling storage aims to reduce microbial growth and enzymatic activities, thereby extending the shelf life of canned food.

Regular monitoring and quality control testing should be conducted to ensure the safety and quality of canned product. This includes microbial testing to detect any potential contamination or spoilage issues. By implementing these preventive measures, producers can minimize the risk of microbial spoilage in canned sauces and condiments, ensuring that consumers receive safe and high-quality products.

Summary:

Milk and milk products, as well as canned foods, are susceptible to spoilage caused by microbial growth. In the case of milk and its derivatives, such as dairy products, various measures are taken to prevent spoilage. One of the most common methods is pasteurization, which involves heating the milk to a specific temperature for a certain duration to eliminate or reduce harmful microorganisms. Refrigeration is another crucial aspect of milk preservation, as storing milk and dairy products at temperatures below 4°C slows down microbial growth and extends their freshness. Additionally, aseptic packaging techniques are employed to ensure the sterility of milk products. This involves using sterile packaging materials and maintaining aseptic conditions during the packaging process to prevent recontamination. Adhering to proper handling and hygiene practices throughout the milking, processing, and packaging stages is also essential in minimizing microbial contamination.When it comes to canned foods, specific strategies are implemented to prevent spoilage. Thermal processing is a key step in canning, where the food is subjected to heat treatment to achieve commercial sterility. This process involves heating the food to a

specific temperature and duration to eliminate or inactivate spoilage-causing microorganisms.Proper sealing of cans is crucial to prevent recontamination from microorganisms present in the surrounding environment. Regular inspections and quality control measures are employed during the canning process to identify and eliminate defective or compromised cans. It is also important to store canned foods in appropriate conditions, such as cool and dry environments, away from direct sunlight, extreme temperatures, and humidity.By implementing these preventive measures, including pasteurization, refrigeration, aseptic packaging, thermal processing, proper sealing, quality control, and appropriate storage, the growth of spoilage-causing microorganisms can be controlled, ensuring the safety and quality of milk, milk products, and canned foods.

Fill in the blanks:

I. Growth of spore-forming bacteria, such as …… and …….. can result in coagulation and protein destruction.

II. Bacteria associated with canned vegetables is…….which can grow at low-acid, anaerobic conditions and can produce a dangerous toxin called botulinum toxin.

III. ..... is 'the organism responsible for souring of milk while ..... causes ropiness.

IV. Enzyme lipases cause ..... off flavour and proteases lead to hydrolysis of milk .....

V. The putrid, proteolyticfruity flavours in butter are caused due to ..... and .....

VI. Bacteria such as ……. and …….. can survive the canning process and, under suitable conditions, grow and produce toxins that pose health risks

2) List the sources of spoilage of raw milk and ice cream

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3) Name a few psychrophilic organisms.

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4) 'What causes the spoilage of canned condensed milk?

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Mastitis-causing microorganisms

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Canned sauces and condiments spoilagae and prevention

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Answer :

- *Bacillus coagulans* and *B. cereus*
- *Clostridium botulinum*
- Breakdown of milk fat into fatty acids and Breakdown of milk proteins into peptides and amino acids
- Pseudomonas fluorescens and Pseudomonas fiagi
- *Lactococcus*
- *Clostridium botulinum* and *Bacillus cereus*

Unit IX: Indicators of Food Safety and Quality: Microbiological criteria of foods and their significance.

Content

9.0 Objective:

- The objective of studying indicators of food safety and quality, specifically microbiological criteria of foods, can be summarized in the following points:
- Identification: Identify specific microorganisms or their metabolic products that can serve as indicators of food safety and quality.

- Assessment: Establish guidelines and standards to assess the microbiological quality of foods based on these indicators.

9.1 Introduction

Ensuring the safety and quality of food is of paramount importance to protect public health and meet consumer expectations. One crucial aspect of assessing food safety and quality is the examination of microbiological criteria. These criteria serve as indicators, providing valuable insights into the presence and levels of microorganisms in food products. By analyzing microbiological parameters, such as the presence of pathogens or spoilage microorganisms, food manufacturers, regulatory bodies, and consumers can make informed decisions regarding the safety and suitability of food for consumption. Microbiological criteria encompass a set of standards and guidelines that outline acceptable microbial levels in various food categories. These criteria are established based on scientific knowledge, risk assessment, and the analysis of potential hazards associated with microorganisms. They are designed to assess the hygienic practices employed during food production, processing, storage, and distribution, and ensure that these practices effectively prevent or reduce the risk of microbial contamination.

The significance of microbiological criteria lies in their ability to identify potential hazards and ensure that food is safe for consumption. Pathogenic microorganisms, such as *Salmonella, Escherichia coli (E. coli), Campylobacter, and Listeria monocytogenes*, can cause foodborne illnesses with severe consequences for human health. Monitoring these microorganisms and setting appropriate limits for their presence in food products helps prevent outbreaks of foodborne diseases and protects consumers from harm. Furthermore, microbiological criteria also play a crucial role in determining the quality and shelf life of food products. Certain microorganisms, such as spoilage bacteria and yeasts, can cause undesirable changes in taste, texture, appearance, and odor, rendering the food unpalatable or unfit for consumption. By establishing microbiological criteria for these spoilage microorganisms, food manufacturers can assess the freshness and quality of their products and ensure that they meet consumer expectations.

Microbiological criteria serve as important indicators of food safety and quality. By establishing standards and guidelines for acceptable microbial levels, they enable the identification and control of potential hazards, thereby protecting public health and ensuring consumer confidence. Through their significance in assessing both the safety and suitability

of food for consumption, microbiological criteria play a crucial role in the overall management of food safety and quality systems. Microbiological criteria for foods refer to established guidelines and limits for the presence and levels of microorganisms in food products. These criteria are based on scientific knowledge and aim to ensure food safety, quality, and suitability for consumption. They play a crucial role in assessing and managing the microbiological risks associated with foodborne illnesses.

9.2 Current Role of criteria in Microbiological Control in food

Food Safety: The primary objective of the food safety system is to effectively recognize and mitigate potential dangers that could cause the degradation of food products. A crucial aspect of this system is the implementation of good manufacturing practices, which guarantee that products adhere to food safety, quality, and legal standards. To achieve this, the hazard analysis and critical control point system (HACCP) is employed in food safety management, focusing on the control of critical points throughout the food handling process to proactively avert food safety issues. Microbiological criteria help protect consumers from the risks of foodborne pathogens and toxins such as *Salmonella, Escherichia coli, Listeria monocytogenes*, and *Campylobacter*. These pathogens can cause severe foodborne illnesses, and microbiological criteria help identify and control their presence in food, ensuring consumer safety.

Quality Assurance: Microbiological criteria also contribute to maintaining food quality and freshness. They help control spoilage microorganisms that can cause off-flavors, odors, and texture changes in food products. By adhering to microbiological standards, manufacturers can ensure that their products meet specific quality requirements.

Process Control: Microbiological criteria serve as a tool for monitoring and controlling food production processes. They help assess the effectiveness of preventive measures, such as sanitation practices, temperature control, and hygiene protocols. Regular testing and analysis of microbiological parameters allow for early detection of deviations and enable corrective actions to be taken promptly.

Compliance and Regulation: Microbiological criteria provide a basis for regulatory compliance in the food industry. Food safety authorities and regulatory agencies establish and enforce these criteria to ensure that food producers meet the required standards. Compliance

with microbiological criteria is often a legal requirement and may involve routine testing and inspection of food products.

Verification of Good Manufacturing Practices (GMPs): Microbiological criteria provide a means to assess the effectiveness of GMPs and hygiene practices implemented in food processing facilities. They help verify the adequacy of sanitation procedures, personnel hygiene, facility design, and equipment sanitation to ensure microbial control.

Public Health Protection: Microbiological criteria are designed to protect public health by setting limits for harmful microorganisms. Compliance with these criteria ensures that food products are within acceptable microbial limits, reducing the risk of foodborne infections and outbreaks.

Regulatory Compliance: Microbiological criteria are often established by regulatory agencies and food safety authorities. Compliance with these criteria is essential for food producers to meet regulatory requirements and demonstrate their commitment to food safety and quality standards.

International Trade: Microbiological criteria facilitate international trade by providing a common basis for assessing the safety and quality of food products. Harmonized standards and guidelines, such as those developed by international organizations like Codex Alimentarius, promote consistency in microbiological criteria worldwide. This helps ensure fair trade practices and consumer protection across different countries.

Therefore microbiological criteria of foods are significant indicators of food safety and quality. They play a crucial role in protecting public health, ensuring process control, maintaining product quality, complying with regulations, verifying GMPs, and facilitating international trade. Compliance with these criteria helps ensure that food products are safe for consumption and meet the desired quality standards.

9. 3 Food Standards Program

A food standards program refers to a set of regulations, guidelines, and processes established by regulatory authorities to ensure the safety, quality, and integrity of food products. These programs are typically implemented by government agencies or regulatory bodies with the aim of protecting public health, promoting fair trade practices, and providing consumers with reliable information about the food they consume.

The different food standards program implemented by different countries and organizations:

United States: The food standards program in the United States is primarily regulated by the U.S. Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA). The FDA oversees food safety regulations through programs like the Food Safety Modernization Act (FSMA), while the USDA is responsible for standards related to meat, poultry, and processed egg products through its Food Safety and Inspection Service (FSIS).

European Union: In the European Union (EU), the food standards program is governed by the European Food Safety Authority (EFSA) and the European Commission. The EU establishes food safety and quality standards through regulations such as the General Food Law, the Hygiene Package, and the Novel Food Regulation.

Australia and New Zealand: The food standards program in Australia and New Zealand is managed by Food Standards Australia New Zealand (FSANZ). FSANZ develops and administers the Food Standards Code, which sets out requirements for food safety, labeling, additives, and contaminants.

Canada: In Canada, the food standards program is overseen by the Canadian Food Inspection Agency (CFIA). CFIA sets standards and regulations under the Food and Drugs Act and the Safe Food for Canadians Act to ensure food safety, quality, and consumer protection.

Codex Alimentarius: The Codex Alimentarius Commission is an international body jointly established by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). It develops international food standards, guidelines, and codes of practice to promote fair trade and protect consumer health. These standards and guidelines serve as a reference for many countries' food standards programs.

In India, the food standards program is primarily governed by the Food Safety and Standards Authority of India (FSSAI). FSSAI is the regulatory body responsible for ensuring the safety and quality of food products throughout the country. The food standards program in India is implemented through various regulations and guidelines issued by FSSAI

9.4 Codex Alimentarius Food Standard

Microbiological criteria are applicable at the international, federal, state (both health and agriculture departments), and local (city-county) levels, as well as by the food industry. The types of criteria at each of these levels are influenced by the mission and responsibilities of the agency or organization involved.Microbiological criteria for foods at the international level are applied primarily within the Joint FAO/WHO Food Standards Program as implemented by the Codex Alimentarius Commission.

The Codex Alimentarius Commission was created to implement the program through which international food standards for processed, semi-processed, and raw foods are established. Membership in the commission is voluntary and is made up of member and associate member nations of FAO and WHO. At present, 121 nations are members (Kimbrell, 1982). Each member government is free to adopt each standard at any one of four levels of participation:

1. full acceptance of the standard and food(s) affected;
2. acceptance with specified deviations from the standard and food(s) affected;
3. target acceptance of food products in anticipation of later approval of Codex Standard;
4. nonacceptance with free distribution of food(s) conforming to Codex Standard.

The specific objectives of the Codex program are:

1. to develop international food standards on a worldwide or regional basis;
2. to publish these standards in a food code (Codex Alimentarius);
3. to record acceptance and implementation of these standards by governments.

To date, 148 international standards have been adopted and 19 more are under development (Kimbrell, 1982). These Codex standards are very similar to standards of identity; microbiological criteria have seldom been included as part of a standard.

9.5 The Major component of microbiological criteria for food safety and quality include:

9.5.1 Aerobic Colony Count (ACC): The ACC measures the total number of viable aerobic microorganisms present in a food sample. It provides an indication of the overall microbial load and hygiene level of the product. Elevated ACC levels may suggest poor hygiene practices or potential for spoilage.

9.5.2 Hygiene Indicator Organisms - *E. coli and Enterobacteriaceae*: These indicator organisms serve as markers for fecal contamination and poor hygiene practices. The presence of Escherichia coli (E. coli) in substantial number, food suggests a general lack of cleanliness in handling and improper storage. Enterobacteriaceae in food samples indicates a potential risk of pathogenic contamination and inadequate sanitation.Enterobacteriaceae can contribute to the formation of histamine (scombrotoxin) in foods such as scombroid fish and occasionally some cheeses if these are not processed properly and/or stored at an adequate refrigeration temperature.

9.5.3 Specific Foodborne Pathogens: Microbiological criteria also focus on specific bacterial pathogens known to cause foodborne illnesses. These criteria set limits for the presence or absence of ten specific bacterial pathogens, including *Salmonella spp., Listeria monocytogenes, Campylobacter spp., Escherichia coli O157:H7,* and others. Compliance with these limits ensures the absence of these pathogens in food products, reducing the risk of foodborne infections.

9.6 Application of Microbiological Criteria to Foods and Food Ingredients in India

The foods or groups of foods, the subcommittee has attempted to address the following basic issues in each section: (1) the sensitivity of the food product(s) relative to safety and quality, (2) the needs for a microbiological standard(s) and/or guideline(s), (3) assessment of information necessary for establishment of a criterion if one seems to be indicated, and (4) where the criterion should be applied.

Microbial food safety in India is regulated by several authorities and laws to ensure the safety and quality of food products. The primary regulatory body responsible for food safety in India is the Food Safety and Standards Authority of India (FSSAI). FSSAI has established regulations and guidelines that specifically address microbial food safety. Prevention of Food Adulteration Rules, 1956 is a set of regulations in India that aim to prevent the adulteration of food and ensure its safety and quality. These rules were enacted under the Prevention of Food Adulteration Act, 1954, which provides a legal framework for regulating food standards and preventing the sale of adulterated or substandard food products.

9.6.1 Microbial Standards of quality of different food product:

9.6.1.1 Infant milk food infant formula appendix b definitions and standards of qualit y infant. formula milk –cereal based complementary food , processed cereal based complementary food ,Follow -up formula up formula -complementary food complementary

Microbial counts

1. Bacterial Count per gram (not more than) 10,000 \
2. Coliform Count absent in 0.1 gram
3. Yeast and mould count absent in 0.1 gram
4. Salmonella and Shigella absent in 25 gram
5. E.coli absent in 0.1 gram
6. Staphylococcus aureas absent in 0.1 gram

9.6.1.2 Spices and Condiments:List of Spices and Condiments Produc

Caraway Whole, Caraway Black Whole, Caraway Powder, Cardamom (Whole, Seeds and Powder), Large Cardamom (Whole, Seeds and Powder), Chillies and Capsicum, Whole and Powder), Cinnamon (Whole and Powder), Cassia (Whole and Powder), Cloves (Whole and Powder), Coriander (Whole and Powder), Cumin (Whole and Powder), Cumin Black (Whole and Powder), Fennel (Whole and Powder), Fenugreek (Whole and Powder), Ginger (Whole and Powder), Mace (Whole and Powder), Mustard ( ), g ( ), pp (Whole and Powder), Nutme g (Whole and Powder), Pepper Black (Whole and Powder), Saffron (Whole and Powder), Turmeric (Whole and Powder), Aniseed Whole, Ajowan, Pepper White (Whole and Powder) and Dried Garlic Whole.

In all the Spices and condiment, Salmonella is absent in 25g

9.6.1.3 Malted Milk Food

a) Without cocoa powder

- Bacterial count Not more than 50,000 per gram
- Coliform count Not more than 10 per gram

b) With cocoa powder

- Bacterial count Not more than 50,000 per gram
- Coliform count Not more than 10 per gram

- Yeast and Mould count Yeast and Mould count absent in 0 1 gram absent in 0.1 gram
- Salmonella and Shigella absent in 0.1 gram
- E.coli absent in 0.1 gram
- Vibrio cholera and V Paraheamolyticus Vibrio cholera and V. Paraheamolyticus absent in 0 1 gram
- Faecal streptococci and Staphylococcus aureas sent in 0.1 gram

9.6.1.4Malt Based Foods (Malt food)

- Total Plate Count Total Plate Count Not more than 50 000 per gram
- Coliform count Not more than 10 per gram
- Yeast and Mould count Not more than 10 per gram
- Salmonella and Shigella absent in 25 gram
- Vibrio cholera and V. Paraheamolyticus absent in 0.1 gram
- Faecalstre p pytococci and Sta p h ylococcusaureas absent in 0.1 gram

9.6.1.5 Solvent extracted Soya flour, Quality, Solvent Extracted Groundnut flour , Solvent extracted Sesame flour, Solvent extracted Coconut flour , Solvent extracted cotton seed flour

- Total bacterial count not more than 50,000 per gram
- Coliform bacteria Coliform bacteria not more than 10 per gram
- Salmonella bacteria Nil in 25 gram

9.6.1.6 Mineral Water

- Yeast and mould counts Absent
- Salmonella and Shigella
- E. coli or thermotolerant Coliforms 1 x 25 ml Absent
- Total coliform bacteria 1 x 250 ml Absent
- Faecalstre p pytococci and sta p h ylococcusaureus 1 x 250 ml Absent
- Pseudomonas aeruginosa 1 x 250 ml Absent
- Sulphite-reducing anaerobes 1 x 50 ml Absent
- Vib h l Vibroc h olera 1 250 l Ab t 1 x 250 ml Absen t
- V. paraheamolyticus 1 x 250 ml Absent

9.6.1.7Packaged Drinking Water (Other than mineral water)

- Yeast and mould counts 1 x 250 ml Absent
- S l ll d Shi ll S almonella an d Shigell a 1 250 l Ab t 1 x 250 ml Absen t
- E. coli or thermotolerant Coliforms 1 x 250 ml Absent
- Coliform bacteria 1 x 250 ml Absent
- Fecasteptococc a d stap y ococcus au eus l streptococci a n d stap h ylococcus au reus 1 x 250 bse t ml Abse n t
- Pseudomonas aeruginosa 1 x 250 ml Absent
- Sulphite-reducing anaerobes 1 x 50 ml Absent
- Vibrocholera 1 x 250 ml Absent
- V paraheamolyticus V. paraheamolyticus 1 x 250 ml Absent 1 x 250 ml Absent
- Aerobic Microbial Count The total viable colony count shall not exceed 100 per ml at 20 C to 22 C in 72 h on agar-agar or on agar-gelatin mixture, and 20 per ml at 37 C in 24 h on agar-agar.

9.6.1.8 **MEAT AND MEAT PRODUCTS CORNED BEEF, LUNCHEON MEAT, COOKED HAM, CHOPPED MEAT, CANNED CHICKEN, CANNED MUTTON AND GOAT MEAT**

- Total plate count 1000/gram maximum
- E. coli Absent in 25 gram
- Samonella Absent in 25 gram
- Staphylococcus aureus Absent in 25 gram
- Clostridium perfringens and Clostirdiumbotulinum Absent in 25 gm

9.6.1.9 FROZEN MUTTON GOAT BEEF AND BUFFALO MEAT

- Total plate count 10000/gram maximum
- E. coli 100/gram maximum
- Samonella Absent in 25 gram Absent in 25 gram
- Staphylococcus aureus 100/gram maximum
- Clostridium perfringens and ClostirdiumBotulinum 30/gm max
- Listeria monocytogenes Listeria monocytogenes Absent in 25 gram Absent in 25 gram
- Yeast and mould count 1000/gram maximum

9.6.1.10 MICROBIOLOGICAL REQUIREMENTS FOR SEA FOOD

Sl.No.	**Name of product**	**Total Plate Count**	**E. coli**	**Staphylococcusaureus**	**Salmonella and Shigella**	**Vibrio cholerae**	**Vobrioparahaemolyticus**	**Clostridium prefringens**
1	Frozen shrimps or							

	Prawns							
	Raw	Not more than five lakhs/gm	Not more than 20/gm	Not more than 100/gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	
	Cooked	Not more than one lakhs/gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	
2	Frozen Lobsters							
	Raw	Not more than five lakhs/gm	Not more than 20/gm	Not more than 100/gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	
	Cooked	Not more than one lakhs/gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	
3	Frozen Squid	Not more than five lakhs/gm	Not more than 20/gm	Not more than 100/gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	
4	Frozen Fin Fish	Not more than five lakhs/gm	Not more than 20/gm	Not more than 100/gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	
5	Frozen fish fillets or minced fish flesh	Not more than five lakhs/gm	Not more than 20/gm	Not more than 100/gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	
6	Dried shark fins	Not more than five lakhs/gm	Not more than 20/gm	Not more than 100/gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	
7	Salted fish/dried salted fish	Not more than five lakhs/gm	Absent in 25 gm.	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm.	

8	Canned finfish	Nil	Absent in 25 gm.	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm.	Absent in 25 gm.
9	Canned Sardines	Nil	Absent in 25 gm.	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm.	
10	Canned shrimp sardine type products	Nil	Absent in 25 gm.	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm.	
11	Canned salmon	Nil	Absent in 25 gm.	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm.	
12	Canned crab meat	Nil	Absent in 25 gm.	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm.	
13	Canned Tuna and Bonito	Nil	Absent in 25 gm.	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm.	

9.6.1.11 MICROBIOLOGICAL REQUIREMENTS FOR FOOD PRODUCTS

S. No	Product	Parameter	Limits
1.	Thermally processed fruits and vegetable products	Total plate count b) Incubation at 37^{o}C for 10 days and 55 C for 7 days	Not more than 50/ml No change in pH
2.	Dehydrated fruits and vegetable products ii) Soup powders iii) Desiccated coconut powder iv) Table olives v) raisins vi) Pistachio nuts vii) dates viii) Dry fruits and nuts	Total plate count	Not more than 40000/gm

3.	Carbonated beverages, ready-to-serve beverages including fruit beverages	Total Plate Count b) Yeast and mould count c) Coliform count	Not more than 50cfu/ml Not more than 2.0cfu/ml Absent in 100 ml Absent in 100 ml
4.	Tomato Products a)Tomato juices and soups b)Tomato Puree and Paste c)Tpmato ketchup and Tomato Sauces	Mould Count b) Yeast and Spores a) Mould Count a) Mould Count b) Yeast and Spores c) Total Plate count	Positive in more than 40.0 percent of the field examined Not more than 125 per 1/60 c.m.m. Positive in more than 60.0 percent of the field examined of the field examined Positive in more than 40.0 percent of the field examined Not more than 125 per 1/60 c.m.m. Not more than 10000/ml
5.	Jam/Marmalade/Fruit Jelly/Fruit Chutney and Sauce	a) Mould Count b) Yeast and Spores	Positive in more than 40.0 percent of the field examined
6.	Other fruits and Vegetables products Covered under item A.16 to Appendix B.	Yeast and Mould Count	Not more than 125 per 1/60 c.m.m.
7.	Frozen Fruits and Vegetable products	Total Plate Count	Not more than 40000/gm
8.	Preserves	Mould Count	Absent in 25 gm/ml
9.	Pickles	Mould Count	Absent in 25 gm/ml
10.	Fruit Cereal Flakes	Mould Count	Absent in 25 gm/ml
11.	Candied and Crystalised or Glazed Fruit and Peel	Mould Count	Absent in 25 gm/ml
12.	i) All Fruits Vegetable Products and Ready to Serve Beverages including Fruit Beverages and Synthetic Products covered under A. 16 of Appendix 'B' ii)Table olives iii)Raisins iv) Pistachio nuts v)Dates vi)Dryfruits and nuts vii)vinegars	a)Flat Sour Organisms b) Staphyloccusaureus c) Salmonellad) Shigella e) Clostridium botulinum f) E.co;i. g) Vibrio Cholera	Not more than 1000 cfu/gm for those products which have pH less than 5.2 Absent in 25 gm/ml Absent in 25 gm/ml Absent in 25 gm/ml Absent in 25 gm/ml Absent in1 gm/ml Absent in 25 gm/ml Absent in 25 gm/ml

9.6.1.11 MICROBIOLOGICAL REQUIREMENTS FOR FOOD PRODUCTSCont…..

Total Plate Count	Ice Cream/frozen Dessert/Milk Lolly/ Ice Candy/Dry ice cream mix	Cheese/ Processed	Evaporated Milk	Sweetened Condensed Milk	Butter	Butter Oil/Butter Fat and Ghee	Yoghurt/ Dahi
Total Plate Count	Not more than 2,50,000/gm	Not more than 50,000/ gm	Not more than 500/gm	Not more than 500/gm	Not more than 500/gm	Not more than 500/gm	Not more than 10,00,000 /g m
Coliform Count	Not more than 10/gm	Absent in 0.1 gm	Absent in 0.1 gm	Absent in 0.1 gm	Not more than 5/gm	Absent in 0.1 gm	Not more than 10/gm
E.Coli	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm
Salmonella	Absent in 25 gm.	Absent in 25 gm.	Absent in 25 gm.	Absent in 25 gm.	Absent in 25 gm.	Absent in 25 gm.	Absent in 25 gm.
Shigella	Absent in 25 gm.	Absent in 25 gm.	Absent in 25 gm.	Absent in 25 gm.	Absent in 25 gm.	Absent in 25 gm.	Absent in 25 gm.
Staphylococusaureus	Absent in 1 gm	Absent in 1 gm	Not more than 100/gm	Not more than 100/gm	Absent in 1 gm	Absent in 1 gm	Not more than 100/gm
Yeast and Mould Count	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	not more than 10/gm	not more than 20/gm	Absent in 1 gm	Not more than 100/gm
Anaerobic Spore Count	Absent in 1 gm	Absent in 1 gm	Not more than 5/gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm
Listeria monocytogene s	Absent in 1 gm	Cheese aother than hard cheese: Absent in 25 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm

9.6.1.11 MICROBIOLOGICAL REQUIREMENTS FOR FOOD PRODUCTS Conti…..

Requirement	**Milk Powder/Cream Powder**	**Edible Casein Products**	**UHT Milk/UHT Flovoured Milk**	**Pasteurise**	**Sterilised Muilk/Sterilised floured milk**	**Khoya/Chhenna/paneer**	**Chakka/Srikhand**
total plate count	Not more then 50,000/gm	Not more then 50,000/gm		30,000/gm		Not more then 50,000/gm	Not more then 50,000/gm
Coli form count	Not more than 0.1/gm	Absent in 0.1 gm	Absent in 0.1 gm	Absent in 0.1 gm	Absent in 0.1 gm	Not more than 90/gm	Not more than 10/gm
E. coli	Absent in 0.1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm
Salmonella	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm
Shigella	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm
Stapylloccusaureus	Absent in 0.1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Not more than 100/gm	Not more than 100/gm
Yeast and mold	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Not more then 250/gm than 10/gm	Chakka: Not more than 10/gm

Anarobic count	Absent in 1 gm	Absent in 1 gm	not more then 5 g	Absent in 1 gm	not more then 5 g	Absent in 1 gm	Absent in 1 gm
Listeria monocytogenes	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm

9.7 Some Indicators of Product Quality: Indicators of product quality are organisms or their metabolic products used to assess or predict shelf life. Criteria for indicator organisms include:

- Presence and detectability in all relevant foods.
- Negative correlation between their growth/numbers and product quality.
- Easy detection, enumeration, and distinction from other organisms.
- Quick enumeration, ideally within a working day.
- Unaffected growth by other components of the food microbiota.

Reliable indicators are often product-specific, with restricted biota and spoilage caused by a single organism. Monitoring can be done through selective culturing or impedance with appropriate selective media. Microbial quality indicators are spoilage organisms, and their increasing numbers lead to a loss of product quality.

Table:1Some Organisms that are Negatively Correlated with Product Quality

Organisms	**Products**
Acetobacterspp	Fresh cider
Bacillus spp	Bread dough
Byssochlamysspp	Canned fruits
Clostridium spp	Hard cheeses
Flat-sour spores	Canned vegetables
Geotrichumspp	Fruit cannery sanitation
Lactic acid bacteria	Beers, wines
Lactococcuslactis	Raw milk (refrigerated)

Leuconostocmesenteroides	Sugar (during refinery)
Pectinatuscerevisiiphilus	Beers
“Pseudomonas putrefaciens”	Butter
Yeasts	Fruit juice concentrates
Zygosaccharomycesbailii Mayonnaise,	 salad dressing

Metabolic products are useful for assessing and predicting microbial quality in certain products. Examples include diamines, histamine, polyamines, diacetyl, ethanol, lactic acid, and trimethylamine (TMA). Diacetyl is a negative predictor of quality in frozen orange juice concentrates, while ethanol is suggested as a quality index for canned salmon. Lactic acid is commonly found in spoiled canned vegetables, and TMA production is used as a quality or spoilage index in fish. Total volatile substances are also measured as indicators in some cases.

9.8 Assessment of food safety and quality:

The assessment aspect of studying indicators of food safety and quality involves establishing guidelines and standards to evaluate the microbiological quality of foods using these indicators. This includes:

Defining acceptable levels: Determine the acceptable limits or thresholds for indicator microorganisms or their metabolic products in different food products.

Setting sampling plans: Develop protocols for sampling food products to obtain representative samples for microbiological analysis.

Testing methods: Identify appropriate testing methods and techniques to detect and quantify indicator microorganisms or their metabolic products.

Interpretation of results: Define criteria for interpreting the test results, such as determining if the microbial levels are within acceptable limits or if the product meets specific quality requirements.

Compliance assessment: Use these guidelines and standards to assess the compliance of food products with microbiological quality requirements.

Risk categorization: Classify food products based on the level of microbiological risk they pose to consumers, considering the indicator results.

Continuous improvement: Continuously review and update the guidelines and standards based on scientific advancements and emerging knowledge in the field of food microbiology.

By establishing clear guidelines and standards for microbiological quality assessment, stakeholders in the food industry can effectively monitor and ensure the safety and quality of food products, thereby safeguarding public health and consumer confidence.

Lets Sum UP: The study of indicators of food safety and quality, particularly microbiological criteria of foods, plays a crucial role in ensuring the safety and quality of food products. It involves identifying specific microorganisms or their metabolic products that serve as indicators, establishing guidelines and standards for assessment, and implementing control measures to minimize microbial contamination. By focusing on these indicators, food safety professionals can improve processes, comply with regulations, extend product shelf life, and enhance consumer confidence in the safety and quality of food. Ultimately, the goal is to protect public health by preventing foodborne illnesses and maintaining the overall integrity of food products.

Check your Knowledge

Fill in the Blank

1. ACC measures the…… present in a food sample
2. These ……indicator organisms serve as markers for fecal contamination and poor hygiene practices
3. Enterobacteriaceae can contribute to the formation of …… in foods such as scombroid fish and cheeses
4. E.coli stain …… absence of these pathogens in food products, reducing the risk of foodborne infections.
5. Codex Alimentarius Commission is an international body jointly established by the ……

Write the Major component of microbiological criteria for food safety and quality.

……
……
……
……..

Some Organisms that are Negatively Correlated with Product Quality

……
……
……
……
……

Major component of microbiological criteria for food safety and quality include

……
……
……
……
……
…….

Answer Key

Atotal number of viable aerobic microorganisms

E. coli and*Enterobacteriaceae*

histamine (scombrotoxin)

Escherichia coli O157:H7

FAO/WHO

Unit X: Public Health Hazards Due to Contaminated Foods: Food borne infections and intoxications- symptoms, mode and sources of transmission and methods of preservation.

Investigation and detection of food borne diseases.

Content

10 **Objective:**

After studying this unit, you will be able to:

1. Discuss different types food contamination
2. Investigate and understand the various types of diseases caused by the ingestion of contaminated food.
3. Identify the specific borne infections, intoxications and toxic infections,
4. Discuss about the causative agents and methods to control them, and
5. Examine the symptoms and severity of these diseases, analyze the factors contributing to disease outbreaks,

10.1 **Introduction**

Foodborne infections and intoxications pose significant risks to public health worldwide. These conditions result from consuming foods contaminated with pathogens or toxic substances, leading to various symptoms and health complications. Understanding the mode and sources of transmission, as well as methods of preservation, is crucial for mitigating these hazards. Additionally, effective investigation and detection

of foodborne diseases are essential for preventing outbreaks and ensuring the safety of the food supply chain.

Foodborne infections are caused by ingesting foods contaminated with harmful bacteria, viruses, parasites, or fungi. These pathogens can multiply in food, causing illness when consumed. Common symptoms of foodborne infections include nausea, vomiting, diarrhea, abdominal pain, fever, and sometimes even severe complications such as dehydration or organ failure. On the other hand, foodborne intoxications occur when toxins produced by certain bacteria or other microorganisms contaminate the food and cause illness upon ingestion. Symptoms of foodborne intoxications can range from mild gastrointestinal discomfort to more severe conditions affecting the nervous system or other organs.The transmission of foodborne diseases can occur through various routes. One of the primary modes is through the consumption of raw or undercooked contaminated food, including meats, eggs, seafood, and unpasteurized dairy products. Contamination can also occur during food preparation or processing, leading to cross-contamination between raw and cooked foods. Additionally, contaminated water, contaminated utensils, poor hygiene practices, and inadequate sanitation can contribute to the spread of foodborne pathogens. Understanding these sources of transmission is crucial for implementing preventive measures to minimize the risks associated with contaminated foods.

Preservation methods play a crucial role in ensuring food safety and preventing foodborne diseases. Techniques such as proper storage, refrigeration, and freezing can help inhibit the growth of pathogens in perishable foods. Canning, pickling, and fermentation are effective methods for preserving food by creating an environment that inhibits the growth of bacteria or other microorganisms. Food processing techniques, such as pasteurization, can also eliminate or reduce pathogens in food products. Additionally, adopting good hygiene practices, both during food production and in the home, is essential for preventing contamination and maintaining food safety.

Investigation and detection of foodborne diseases are paramount for effective public health response and prevention of outbreaks. When outbreaks occur, it is crucial to identify the specific pathogen responsible for the illnesses, trace its source, and implement appropriate control measures. This process involves surveillance systems,

laboratory testing, epidemiological investigations, and collaboration between public health agencies, healthcare providers, and food regulatory authorities. Timely detection and response can help prevent further cases, identify and address potential gaps in the food supply chain, and improve overall food safety standards.

In conclusion, foodborne infections and intoxications pose significant public health hazards. Understanding the symptoms, modes, and sources of transmission, as well as implementing effective preservation methods, are essential for mitigating these risks. Furthermore, the investigation and detection of foodborne diseases are crucial for preventing outbreaks and ensuring the safety of the food supply chain. By addressing these aspects comprehensively, we can work towards a safer and healthier food environment for everyone.

10.2 Food Contamination:

Food contamination refers to the presence of harmful substances or pathogens in food that can cause illness or injury when consumed. Food contamination can occur through various sources and can be classified into several categories:

Biological Contamination: This type of contamination involves the presence of microorganisms, such as bacteria, viruses, parasites, or fungi, in food. Examples include Salmonella, E. coli, Listeria, and Norovirus. Biological contamination can occur due to poor hygiene practices, improper handling of food, contaminated water, or contact with animals or their waste.

Chemical Contamination: Chemical contamination refers to the presence of harmful chemicals or toxic substances in food. This can include naturally occurring toxins in certain plants or seafood, as well as chemical contaminants such as pesticides, heavy metals, cleaning agents, or food additives. Chemical contamination can result from improper use of chemicals, environmental pollution, or contamination during food processing or packaging.

Physical Contamination: Physical contamination involves the presence of foreign objects in food that can cause harm if ingested. This can include objects like glass, metal fragments, plastic, hair, or other foreign materials. Physical contamination can occur due to mishandling of food, inadequate quality control, or improper storage and packaging practices.

Cross-Contamination: Cross-contamination refers to the transfer of harmful microorganisms or substances from one food item to another. It can occur when contaminated raw foods come into contact with ready-to-eat foods, food preparation surfaces, utensils, or hands. Cross-contamination can lead to the spread of pathogens and cause foodborne illnesses.

10.3 Effect of contaminated foods on public health hazards :

Contaminated foods can pose significant public health hazards, leading to various illnesses and outbreaks. Contaminated foods can harbor harmful microorganisms, such as bacteria, viruses, parasites, or fungi, which can cause foodborne illnesses. These illnesses can range from mild gastrointestinal discomfort to severe infections and even life-threatening conditions. Common foodborne illnesses include Salmonellosis, Campylobacteriosis, E. coli infections, Listeriosis, and Norovirus infections.

- **Outbreaks and Epidemics:** Contaminated foods can result in outbreaks and epidemics, where a large number of people become ill after consuming the same contaminated food or from a common source of contamination. These outbreaks can occur in various settings, such as restaurants, catering events, schools, or community gatherings. They often require investigation, public health interventions, and measures to prevent further spread.
- **Long-term Health Effects:** Some contaminants in food, such as certain chemicals or toxins, may not cause immediate illness but can lead to long-term health effects. For example, exposure to certain heavy metals, pesticides, or food additives over time can contribute to chronic health conditions, including cancer, neurological disorders, or hormonal imbalances.
- **Vulnerable Populations**: Certain groups of people are more susceptible to foodborne illnesses and their complications. This includes young children, older adults, pregnant women, and individuals with weakened immune systems or underlying health conditions. Contaminated foods can have more severe health consequences for these vulnerable populations.
- **Economic Burden:** Contaminated foods not only affect public health but also result in economic burdens. Foodborne illnesses can lead to hospitalizations, medical expenses, lost productivity, and decreased consumer confidence in food products and establishments. The costs associated with investigating outbreaks, implementing recalls, and implementing preventive measures can also be substantial.

  To mitigate the public health hazards associated with contaminated foods, it is crucial to implement comprehensive food safety measures. This includes ensuring proper hygiene practices during food handling, implementing robust quality control systems, enforcing regulatory standards, conducting regular inspections, and promoting public

awareness and education on safe food handling and consumption. Collaboration between government agencies, food producers, retailers, and consumers is essential to ensure the safety and integrity of the food supply chain and protect public health.

10.4 Food borne diseases:

Foodborne diseases, commonly known as food poisoning, occur when individuals consume food that is contaminated with toxic or infectious agents. In India, these diseases are referred to as food poisoning and are characterized by the harmful effects resulting from the ingestion of food contaminated by microorganisms.

Foodborne disease outbreaks are frequently observed in both developed and developing countries. An outbreak is defined as an incident in which two or more people experience similar illnesses, typically gastrointestinal symptoms, after consuming a common food item identified as the source of the illness. More than 250 different foodborne diseases have been described, primarily caused by a variety of bacteria, viruses, parasites, and harmful toxins or chemicals present in contaminated food. Certain types of mushrooms and molds produce toxins, known as mycotoxins, that can contaminate food and lead to illness. Foodborne diseases are considered a significant global health problem, adversely affecting economic prosperity. In fact, the occurrence of foodborne diseases is second only to the common cold. In developing countries, the true magnitude of the problem is often underestimated. The World Health Organization (WHO) estimates that the ratio of actual cases to reported cases of foodborne diseases ranges from 25:1 to 100:1.

In India, foodborne diseases are rarely recorded, and when they are, they are often categorized under gastroenteritis, which involves inflammation of the stomach and the small and large intestines. Due to the mild and relatively short duration of the effects, affected individuals may overlook the connection between their illness and the consumed food, and may not seek medical help. Even if they do seek medical aid, it is usually on an individual basis, except in rare cases where a large number of people are affected simultaneously. Foodborne diseases typically manifest as disturbances of the gastrointestinal tract, with symptoms including abdominal pain, diarrhea, and sometimes vomiting. The severity of foodborne illnesses can range from mild gastroenteritis to life-threatening neurologic, hepatic, and renal complications.

10.5 Type of food borne Diseases

There are various types of foodborne diseases caused by different pathogens, toxins, and chemicals. Classifications and subtypes of foodborne diseases may exist based on specific pathogens, toxins, or modes of transmission. Understanding the different types of foodborne diseases helps in their prevention, identification, and treatment.

Food Intoxication: Food intoxication occurs when individuals consume food containing pre-formed toxins produced by certain bacteria or fungi. Examples include:

- Staphylococcal food poisoning (caused by Staphylococcus aureus toxins)
- Bacillus cereus food poisoning (caused by toxins produced by Bacillus cereus)
- Clostridium botulinum food poisoning (causing botulism due to botulinum toxin)

Food Infection: Food infection refers to illnesses caused by ingesting food contaminated with live pathogens, which then multiply and cause infection in the body. Examples include:

- Salmonellosis (caused by Salmonella bacteria)
- Campylobacteriosis (caused by Campylobacter bacteria)
- Shigellosis (caused by Shigella bacteria)
- Escherichia coli (E. coli) infection, including enterohemorrhagic E. coli (EHEC)
- Listeriosis (caused by Listeria monocytogenes)
- VibroParahaemolyticus
- Hepatitis A

Foodborne Toxic Infection: Foodborne toxic infections occur when individuals ingest food contaminated with pathogens that can produce toxins within the body. The infection itself, as well as the toxins produced, contribute to the illness. Examples include:

- Enterohemorrhagic E. coli (EHEC) infections leading to hemolytic uremic syndrome (HUS)
- Shiga toxin-producing E. coli (STEC) infections
- *Clostridium Perfringens* Gastroenteritis
- Cholera
- *Yersinia Enterocolitica* Gastroenteritis
- *Enterotoxigenic*Escherichia Coli Gastroenteritis

In addition to the three main types of foodborne diseases (food intoxication, food infection, and foodborne toxic infection), certain molds and fungi can also contribute to foodborne illnesses by producing mycotoxins. Mycotoxins are toxic compounds that can contaminate various food products. Aflatoxin poisoning is caused by molds like Aspergillusflavus and Aspergillusparasiticus, which commonly contaminate crops such as peanuts and maize. Ochratoxin poisoning is associated with molds like Aspergillus and Penicillium, found in cereals and spices. Fusariummolds can produce mycotoxins like deoxynivalenol and zearalenone, contaminating grains. Ergotism, caused by Clavicepspurpurea, can occur from consuming grains like rye. Proper food storage, handling, and adherence to food safety regulations are crucial in preventing mycotoxin-related foodborne diseases.

10.5.1Food Borne Intoxication

Foodborne intoxication refers to a type of foodborne disease where individuals become ill after consuming food that contains toxins produced by certain bacteria or fungi. In this case, the toxins are already present in the food and can cause illness when ingested. The toxins may be produced by the microorganisms themselves or may be produced prior to ingestion, such as during food processing or storage. Unlike foodborne infections where live pathogens cause illness, in foodborne intoxication, the toxins themselves are responsible for the symptoms and can lead to various gastrointestinal issues, such as nausea, vomiting, abdominal cramps, and diarrhea. It is important to practice proper food handling, storage, and hygiene to prevent the growth and production of toxins in food and minimize the risk of foodborne intoxication.

The most important food bornefood intoxication are i) Staphylococcal food poisoning .ii) Bacillus cereus food poisoning iii) Clostridium botulinum food poisoning

Staphylococcal food poisoning (caused by *Staphylococcus aureus* toxins): Staphylococcus aureus is capable of growing in a wide range of food types, especially those that provide favorable conditions for bacterial growth**Fig:1**. However, as a general guideline, *Staphylococcus aureus* cells typically have a diameter ranging from 0.5 to 1.0 micrometers (µm).*Staphylococcus aureus* cells, often form grape-like clusters or irregular aggregates due to their characteristic pattern of division and growth. *Staphylococcus aureus* isfacultative anaerobe bacteria produces various virulence factors that contribute to its pathogenicity. These include toxins (such as enterotoxins and

exotoxins), enzymes (such as coagulase, catalase, and hyaluronidase), adhesion factors (such as protein A), and biofilm formation ability.*Staphylococcus aureus* is capable of growing in a wide range of food types, especially those that provide favorable conditions for bacterial growth. It is a resilient bacterium that can tolerate various environmental conditions, including high salt concentrations and low water activity.Staphylococcusaureus grows best in the temperature range of 10°C to 46°C (50°F to 115°F). The optimal temperature for growth is around 37°C (98.6°F), which is body temperature.

Fig:1*Staphylococcus aureus*

Staphylococcus aureus can produce toxins that cause illness when present in contaminated food. These toxins are typically heat-stable and resistant to digestion, allowing them to remain active even after cooking or ingestion. When ingested, the toxins can cause symptoms of staphylococcal food poisoning.The primary toxins associated with Staphylococcus aureus food poisoning are known as *staphylococcal* enterotoxins (SEs). These toxins are produced by certain strains of Staphylococcus aureus and can cause rapid onset of symptoms, typically within a few hours after consuming contaminated food.Staphylococcal enterotoxins act as superantigens, stimulating a strong immune response in the body. This immune response leads to symptoms such as nausea, vomiting, abdominal cramps, and diarrhea. Other symptoms may include headache, dizziness, and occasionally fever.

To prevent Staphylococcus aureus toxin formation in food, it is essential to follow proper food safety practices, including:

- Practicing good personal hygiene, such as regular handwashing, especially before handling food.Maintaining clean food preparation surfaces and utensils.
- Properly storing food at the correct temperature, such as refrigerating perishable foods below 5°C (41°F).

- Avoiding leaving cooked foods at room temperature for extended periods.
- Ensuring that food handlers follow strict hygiene practices and are trained in safe food handling.

By adhering to these preventive measures, the risk of Staphylococcus aureus contamination and subsequent toxin production in food can be significantly reduced.

10.5.1.2 Bacillus cereus food poisoning (caused by toxins produced by Bacillus cereus):

Bacillus cereus is a Gram-positive, rod-shaped bacterium that can be found in raw or processed food**Fig:2**. The size of Bacillus cereus cells can vary, but as a general guideline, they are typically around 1 to 1.5 micrometers (µm) in width and 3 to 5 µm in length. These measurements represent the dimensions of individual bacterial cells.Bacillus cereus is a spore-forming bacterium, meaning it can form dormant, tough-walled structures called endospores. These endospores allow the bacterium to survive harsh conditions, such as high temperatures and low moisture, and can persist in the environment for extended periods.Spores are typically oval or ellipsoidal in shape, and their size can vary depending on the growth conditions and strain of the bacterium. Bacillus cereus spores are usually around 1 to 1.5 µm in width and 3 to 8 µm in length. The optimal temperature range for the growth of Bacillus cereus is between 20°C (68°F) and 45°C (113°F). Within this range, the bacterium can multiply and proliferate rapidly, potentially leading to higher levels of contamination in food. Some strains of Bacillus cereus are psychrotrophic, meaning they can grow and survive at lower temperatures, including refrigeration temperatures. Psychrotrophic strains of Bacillus cereus can pose a risk because their spores can survive cooking and subsequently germinate and grow at refrigeration temperatures (4°C to 8°C or 39°F to 46°F). This ability to grow at lower temperatures increases the importance of proper food storage and handling practices. Bacillus cereus spores are heat-resistant and can survive cooking temperatures. While the bacterial cells are more susceptible to heat, the spores are more resilient. Therefore, even if Bacillus cereus cells are killed during cooking, any surviving spores can germinate and grow if food is not properly handled and stored.

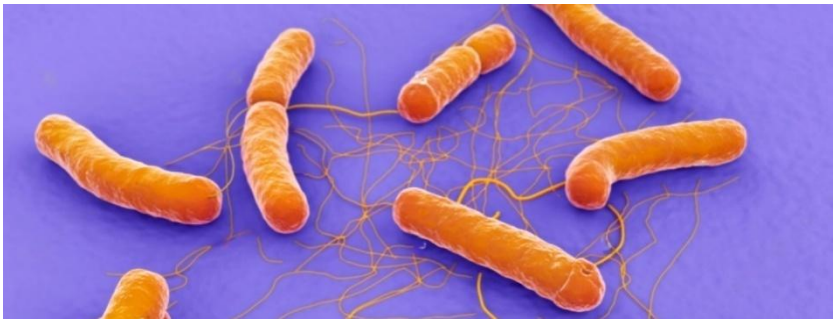

Fig:2*Bacillus cereu*

Bacillus cereus is a bacterium commonly associated with foodborne illness. It produces toxins that can cause two types of food poisoning: emetic syndrome and diarrheal syndrome. These toxins are heat-stable and can survive cooking and improper food storage conditions.

Emetic Syndrome: This type of food poisoning is characterized by the sudden onset of nausea and vomiting, typically occurring within 1 to 6 hours after consuming contaminated food. The toxin responsible for emetic syndrome is called cereulide. It is preformed in food and resistant to heat, making it resistant to destruction during cooking. Foods commonly implicated in emetic syndrome include cooked rice dishes, pasta, and other starchy foods.

Diarrheal Syndrome: This form of food poisoning is characterized by abdominal cramps and watery diarrhea. The toxins responsible for diarrheal syndrome are produced in the intestines after consuming food contaminated with certain strains of Bacillus cereus. These strains produce enterotoxins, such as hemolysin BL and non-hemolytic enterotoxin. Foods commonly associated with diarrheal syndrome include meat, vegetables, dairy products, and other cooked foods that have been improperly handled or stored.

10.5.1.3 *Clostridium botulinum* food poisoning (causing botulism due to botulinum toxin)

*Clostridium botulinum*is a Gram-positive, rod-shaped spore-forming bacterium. The size of individual cells can vary depending on the growth conditions and the specific strain. Generally, *Clostridium botulinum* cells have a length ranging from 2 to 10 micrometers (µm) and a width of about 0.5 to 2 µm**Fig:3**. *Clostridium botulinum* is a thermophilicbacterium, meaning it prefers to grow in warm

environments. The optimal growth temperature for most strains of *Clostridium botulinum* is between 37°C (98.6°F) and 45°C (113°F). However, certain strains, such as Group II (non-proteolytic) and Group III, are psychrotrophic and can grow at lower temperatures, including refrigeration temperatures (4°C to 8°C or 39°F to 46°F). One notable characteristic of *Clostridium botulinum*is its ability to form highly resistant spores. These spores are heat-resistant and can survive harsh conditions, including cooking and pasteurization. Spores are typically oval or ellipsoidal in shape and larger than the vegetative cells. The size of *Clostridium botulinum*spores can range from 1 to 2 µm in width and 4 to 10 µm in length.

Fig:3*Clostridium botulinum*

Clostridium botulinum is a bacterium that can produce a potent neurotoxin called botulinum toxin. Botulism is a severe form of food poisoning caused by the ingestion of food contaminated with this toxin. There are several different strains of Clostridium botulinum, classified into groups A to G, based on the specific toxin they produce.

Botulinum Toxin: The botulinum toxin is one of the most potent toxins known to humans. It acts by blocking nerve signals, leading to muscle paralysis. There are seven known serotypes of botulinum toxin, labeled from A to G. Types A, B, E, and, rarely, F are associated with human botulism.

Symptoms: Botulism symptoms typically manifest within 12 to 72 hours after consuming contaminated food. Common symptoms include blurred or double vision, drooping eyelids, difficulty swallowing, slurred speech, muscle weakness, and paralysis. Botulism can be life-threatening, as the paralysis can affect respiratory muscles, leading to respiratory failure.

Treatment and Prevention: Botulism requires immediate medical attention. Hospitalization and supportive care, such as respiratory support and administration of botulinum antitoxin, are critical. Prevention involves proper food handling, preparation, and storage practices, including:

- Using proper canning techniques for home-canned foods.
- Ensuring that commercial canned foods are not bulging, leaking, or damaged.
- Heating foods at a temperature and duration sufficient to destroy botulinum spores (e.g., 121°C/250°F for 3 minutes).
- Discarding any canned food with an unusual odor, appearance, or spoiled content.
- Not feeding honey to infants under one year old, as it can contain spores that infants' digestive systems cannot handle.
- It is essential to adhere to proper food safety practices to minimize the risk of botulism and ensure the safety of the food we consume. If you suspect botulism or have consumed potentially contaminated food, seek immediate medical attention.

10.5. 2Food borne Infection:

Foodborne infections occur when individuals consume food contaminated with pathogenic microorganisms that can invade the intestinal mucosa and multiply in the body or migrate to other tissues. Some common examples of foodborne infections include Salmonellosis, Shigellosis, Vibrio parahaemolyticus gastroenteritis, Escherichia coli (E. coli) diarrhea, and infectious hepatitis. In these infections, the ingested pathogens can cause various symptoms and affect different parts of the body.

10.5.2.1 Salmonellosis (caused by Salmonella bacteria):

Salmonellosis is a widespread foodborne illness affecting people worldwide. There are approximately 1,600 different strains, or serotypes, of Salmonella. The most common serotype is *Salmonella typhimurium*, which can cause typhoid fever**Fig 4**. Salmonella organisms are short, motile, gram-negative rods that do not form spores. They can survive in aerobic and facultative anaerobic conditions.

Fig:4*Salmonella*

Salmonella grows best at around 38°C (100.4°F), but its growth is slow at temperatures below 10°C (50°F). Heat treatments, such as pasteurization or exposure to temperatures of 60°C (140°F) for 15-20 minutes, can destroy the bacteria. The primary source of Salmonella is animals, but human carriers can indirectly transmit the bacteria after becoming infected.

Domestic animals like chickens, pigs, and turkeys are the main reservoirs of Salmonella. The bacteria can spread from one carcass to another in slaughterhouses. Various types of foods have been implicated in foodborne salmonellosis, including cereal and grain products, desiccated coconut, chocolate, dairy products, eggs, meat, and meat products. Milk and milk products, such as fermented milks, ice cream, and cheese, can also be sources of contamination. Cross-contamination can occur in the kitchen through contaminated equipment and utensils.

It is important to practice proper food handling, cooking, and storage techniques to prevent *Salmonella* contamination. Thoroughly cooking food, pasteurizing certain products, preventing cross-contamination, and maintaining proper hygiene can help reduce the risk of Salmonella-related foodborne illnesses.

10.5.2.2Campylobacteriosis (caused by *Campylobacter* bacteria):

Campylobacteriosis is a common foodborne infection caused by *Campylobacter* bacteria. It is one of the leading causes of bacterial gastroenteritis worldwide. The two most common species responsible for human infections are *Campylobacter jejuni* and *Campylobacter coli.*

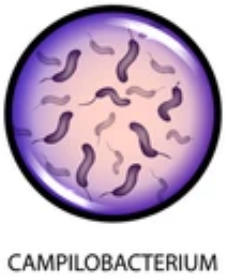

Fig 5.*Compilobacter*

Transmission of Campylobacteriosis primarily occurs through the consumption of contaminated food, particularly undercooked poultry, raw or unpasteurized milk, and contaminated water. The bacteria can also be present in the feces of infected animals, and direct contact with infected animals or their environment can lead to infection. Once ingested, the Campylobacter bacteria can invade the lining of the small intestine, leading to inflammation and symptoms of gastroenteritis. The incubation period for Campylobacteriosis is typically 2 to 5 days after exposure, although it can range from 1 to 10 days.

Common symptoms of Campylobacteriosis include:

- Diarrhea (often watery and sometimes bloody)
- Abdominal pain and cramping
- Fever
- Nausea and vomiting
- Fatigue

In most cases, the illness is self-limiting and resolves within a week without specific treatment. However, in some cases, complications can arise, especially in individuals with weakened immune systems. These complications may include bloodstream infections, Guillain-Barré syndrome (a rare neurological disorder), and reactive arthritis.

Prevention of Campylobacteriosis: primarily involves practicing good hygiene and safe food handling practices. Here are some measures to reduce the risk of infection:

- Thoroughly cook poultry products, including chicken and turkey, until the juices run clear, and there is no pink meat.
- Avoid consuming raw or undercooked meat, especially poultry.

- Practice proper hand hygiene by washing hands with soap and water before handling food, after using the restroom, and after contact with animals.
- Use separate cutting boards and utensils for raw and cooked foods to prevent cross-contamination.
- Drink pasteurized milk and use safe water sources.
- Store and handle food properly, refrigerating perishable items promptly.

10.5.2.3 Shigellosis (caused by *Shigella* bacteria):

Shigellosis is a common intestinal infection caused by the genus Shigella. Shigella has four serological groups, with *S. sonnei* (Subgroup D) and *S. flexneri* (Subgroup B) being the most significant. *Shigella* organisms are non-motile, gram-negative rods that are aerobic and facultative anaerobic. They do not form spores and belong to the Enterobacteriaceae family.

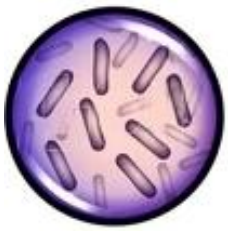

Fig: 6 Shigella

The optimal temperature for *Shigella* growth is 37°C (98.6°F), with a temperature range of 10 to 40°C (50 to 104°F). *Shigella* can tolerate salt concentrations up to 6% but is sensitive to heat. It utilizes glucose and other carbohydrates for growth and is inhibited by potassium cyanide.*Shigella* is mainly transmitted from human to human, and the number of organisms required to cause illness is low. The incubation period varies depending on the specific *Shigella* strain, the age and health of the affected person. Typically, the incubation period is short, ranging from 1 to 7 days, with an average of 1 to 3 days. Symptoms of shigellosis include diarrhea, fever, nausea, vomiting, and abdominal cramps. Stools may contain blood, mucus, and pus.

Although shigellosis is often transmitted through contaminated water, food can also be a vehicle for infection. Milk, shellfish, raw vegetables, Mexican dishes, and various salad preparations have been associated with shigellosis outbreaks. Poor

personal hygiene of food handlers who act as carriers can lead to contamination of food and food preparation surfaces.

To minimize the problem of shigellosis, the following measures are important:

- Educating food handlers about safe food handling practices and strict personal hygiene, including handwashing after using the toilet.
- Rapid chilling or holding leftover foods at temperatures above 40°C (104°F) to prevent Shigella growth.
- Ensuring proper cooking and reheating of foods to safe temperatures to eliminate Shigella bacteria.

10.5.2.4*Escherichia coli* (E. coli) infection, including enterohemorrhagic*E. coli* (EHEC):*Escherichia coli* (E. coli) is a type of bacteria commonly found in the intestines of humans and animals. While most strains of *E. coli* are harmless and even beneficial, some can cause infections and illness. One specific strain of *E. coli* known as enterohemorrhagic E. coli (EHEC) is of particular concern. EHEC can produce toxins that can cause severe gastrointestinal symptoms. The most well-known strain of EHEC is *E. coli* O157:H7, but there are other serotypes as well. EHEC is primarily transmitted through contaminated food or water, often associated with undercooked ground beef, unpasteurized milk or juice, raw fruits and vegetables, and contaminated water sources.

Fig. 6 *E. Coli*

Symptoms of EHEC infection can vary but often include severe abdominal cramps, bloody diarrhea, and sometimes fever. In more severe cases, it can lead to a complication called hemolytic uremic syndrome (HUS), which can cause kidney damage.

Prevention of *E. coli* infections, including EHEC, involves practicing good hygiene and safe food handling techniques. This includes thoroughly cooking meats, especially ground beef, pasteurizing milk and juice, washing fruits and vegetables, avoiding cross-contamination of raw and cooked foods, and ensuring proper handwashing.

10.5.2.5Listeriosis (caused by Listeria monocytogenes)

Listeria monocytogenes is a gram-positive bacterium that can be found in various environments, including soil, water, and animal feces. It can contaminate a wide range of foods, particularly ready-to-eat foods such as deli meats, soft cheeses, and refrigerated smoked seafood.

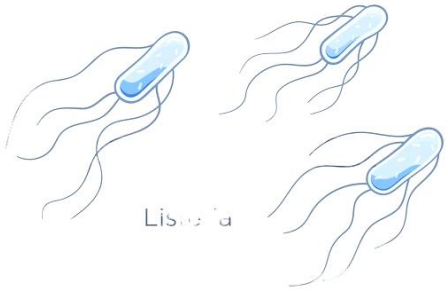

Fig. 7*Listeria monocytogenes*

Transmission: *Listeria monocytogenes* can be transmitted through the consumption of contaminated food, especially if it is not properly cooked or stored. It can also be transmitted from mother to fetus during pregnancy, leading to severe complications for the newborn.

Risk groups: Certain individuals are at a higher risk of developing severe listeriosis, including pregnant women, newborns, older adults, and people with weakened immune systems. For pregnant women, listeriosis can result in miscarriage, stillbirth, or serious illness in the newborn.

Symptoms: The symptoms of listeriosis can vary but commonly include fever, muscle aches, nausea, diarrhea, and headache. In severe cases, it can progress to more serious complications, such as meningitis or septicemia.

Prevention: To prevent listeriosis, it is important to practice proper food safety measures. This includes thoroughly cooking raw foods, particularly meat and poultry,

avoiding unpasteurized dairy products and soft cheeses, practicing good hygiene in the kitchen, and promptly refrigerating perishable foods.

Treatment: Listeriosis is typically treated with antibiotics. Listeriosis can be a severe and potentially life-threatening infection, especially for vulnerable populations. So, if any one suspect to have listeriosis or are at a higher risk due to pregnancy or a compromised immune system, it is important to seek medical attention for proper diagnosis and treatment.

10.5.2.6*VibroParahaemolyticus* Gastroenteritis

Vibrio parahaemolyticus is a gram-negative bacterium commonly found in marine environments, such as coastal waters and seafood. It can multiply rapidly in warm temperatures, particularly during the summer months.

Transmission: Vibrio parahaemolyticus gastroenteritis is primarily transmitted through the consumption of raw or undercooked seafood, particularly shellfish like oysters, clams, and mussels. Contaminated water and cross-contamination in the kitchen can also contribute to the spread of the bacteria.

Symptoms: The symptoms of *Vibrio parahaemolyticus* gastroenteritis typically appear within 24 to 48 hours after consuming contaminated food or water. They may include watery diarrhea, abdominal cramps, nausea, vomiting, and sometimes fever. The illness is usually self-limiting and resolves within a few days to a week.

Risk factors: Individuals with weakened immune systems, underlying medical conditions, or those who consume large quantities of raw or undercooked seafood are at a higher risk of developing severe symptoms from Vibrio parahaemolyticus infection.

Prevention: To prevent Vibrio parahaemolyticus gastroenteritis, it is important to cook seafood thoroughly, especially shellfish, to kill the bacteria. Avoid consuming raw or undercooked seafood, particularly if you have underlying health conditions. Practice good hygiene, such as proper handwashing, and avoid cross-contamination in the kitchen.

Treatment: Most cases of Vibrio parahaemolyticus gastroenteritis do not require specific treatment and resolve on their own. It is important to stay hydrated by

drinking plenty of fluids. In severe cases or for individuals with compromised immune systems, antibiotics may be prescribed.

By practicing proper food safety measures and being cautious when consuming seafood, the risk of *Vibrio parahaemolyticus*gastroenteritis can be reduced.

10.5.2.7 Hepatitis A: Hepatitis A is a viral infection that can be transmitted through contaminated food and water. Important points about Hepatitis A as a foodborne infection:

Hepatitis A virus (HAV): Hepatitis A is caused by the Hepatitis A virus, which primarily affects the liver. The virus is found in the feces of infected individuals and can contaminate food and water.

Transmission: Hepatitis A is typically spread through the ingestion of fecal matter, either by consuming contaminated food or water or through close contact with an infected person. Foodborne transmission can occur when food handlers with poor hygiene practices contaminate food during preparation.

Symptoms: Symptoms of Hepatitis A can include fatigue, loss of appetite, nausea, abdominal discomfort, dark urine, clay-colored stools, and yellowing of the skin and eyes (jaundice). Not everyone infected with Hepatitis A experiences symptoms, especially young children who may have mild or asymptomatic cases.

Prevention: Hepatitis A can be prevented by practicing good hygiene, including thorough handwashing with soap and water before handling food or eating. It is also important to consume properly cooked food, particularly shellfish, fruits, and vegetables that may be at risk of contamination. Vaccination against Hepatitis A is available and recommended, especially for individuals at higher risk.

Treatment: There is no specific treatment for Hepatitis A. Most individuals recover on their own with supportive care, such as rest, adequate hydration, and a healthy diet. In severe cases, hospitalization may be required.

Outbreaks and Public Health Measures: Hepatitis A outbreaks can occur, particularly in settings where hygiene practices are compromised or in areas with inadequate sanitation. Public health measures may include investigation of the source of contamination, implementation of preventive measures, and vaccination

campaigns.It is important to note that Hepatitis A is a preventable infection, and practicing good hygiene and safe food handling practices can significantly reduce the risk of transmission.

10.5.3 Foodborne Toxic Infection

Foodborne toxic infection is a type of foodborne disease where individuals become ill after consuming food contaminated with pathogens that can produce toxins within the body. In this case, the pathogens themselves cause an infection, and the toxins they produce contribute to the illness. A well-known example of foodborne toxic infection is enterohemorrhagic Escherichia coli (EHEC) infection, which can lead to a condition called hemolytic uremic syndrome (HUS). EHEC produces toxins that damage the lining of blood vessels and can cause severe complications. Other pathogens, such as certain strains of Salmonella and Clostridium difficile, can also cause foodborne toxic infections. These infections can result in a range of symptoms, including diarrhea, abdominal cramps, and in severe cases, organ damage. Proper food handling, thorough cooking, and adherence to food safety practices are crucial in preventing the transmission of pathogens and reducing the risk of foodborne toxic infections.

10.5.3.1*Clostridium perfringens*-associated gastroenteritis is a food borne disease which is frequently reported and is a common cause of illness. The organism Clostridium perfringens is a large (2-8 pm long; 1 pm wide), non-motile, gram positive, obligate anaerobe and spore-forming rod as can be seen in Figure 5.10. The organism does not produce spores while growing in the food but it forms spores when it reaches the intestinal tract.

Fig: 8 *Clostridium perfringens*

The spores of *C. perfringens* can withstand boiling temperatures for up to 6 hours. The optimum temperature for growth is between 43°C to 45"C, whereas, the maximal temperature at which it can grow is 55°C. Its growth gets restricted at the temperatures of about 15-20°C. The vegetative cells of the organism are usually destroyed at the temperatures of 60°C and above. The strains of *C. perfringens* are classified into 5 types-A to E, depending on the type of toxin produced. The strains of *C. perfringens* type A, the spores of which are heat resistant survive at temperatures from 95-100°C for periods of upto one hour. These are responsible for several outbreaks in the United Kingdom. The incubation period for the disease ranges between 8 to 22 hours, but normally the symptoms occur between 8 to 12 hours after the ingestion of contaminated food. The important symptoms are severe abdominal cramps and watery diarrhoea, vomiting is rare. The recovery is usually within 1' or 2 days.

The *C. perfringens* is the most common of all pathogenic bacteria, as it is widely distributed and usually can be isolated from soil, dust, marine sediments, human faeces and animal manure. They are also found in raw meat and poultry. The usual vehicles of infection are the dishes prepared from meat and poultry. The outbreaks have been reported from places where a large number of people eat, like the restaurants, institutional canteens, hospitals etc. The major reason for the cause of outbreaks is the time temperature abuse after the preparation of the dishes. The slow cooling process which sets in while the cooked food is held at room temperature, aids in the growth of spore germination and a large number of vegetative cells may develop within a few hours. *C. perfringens* causes a mild but common type of food poisoning. Its spores are heat resistant, surviving normal cooking. The organism usually forms the toxin, once it is in the human intestine and has started to sporulate. The toxin formed in food is not very heat resistant (destroyed by heating at 60°C for 10 minutes). *C. perfringens* food poisoning causes severe abdominal pain and prolific diarrhoea. Sometimes fever, 104 nausea and even vomiting may occur. Normally, a large number of cells need to be F1azart's Of Origin ingested to cause illness and the recovery is usually rapid (24-48 hours). The *C. perfringens* being widely distributed in nature and due to the danger of the presence of spores in raw and cooked foods due to contamination, the preventive approach should aim to restrict the germination of spores and inhibiting the multiplication of the vegetative cells. To achieve this, the

cooled foods have to be: (i) consumed as early as possible (ii) held at temperature above 56°C or higher, if there is a delay in consuming (iii) cooled rapidly (within 1 hour) and held below 7"C, and (iv) if the food is prepared far in advance, heating thoroughly above 74°C before serving.

10.5.3.2 Cholera

Cholera is one of the diseases which had caused epidemics all over the world. The organism which causes the disease is *Vibrio cholerae*. It is an aerobic, facultatively anaerobic, straight or slightly curved, short, gram-negative, oxidose-positive rod with a single flagellum as can be seen in Fig. 9 . The *V. cholerae* produces a heat-sensitive enterotoxin while growing in the human intestine. It causes the characteristic cholera symptoms including 'rice water stool'. The toxin is heat labile. The incubation period of cholera ranges from a few hours 1 to 5 days but usually between 2-3 days. The symptoms include abrupt onset of vomiting and watery diarrhoea. Diarrhoea has a rice water appearance, where the stools look like water with flecks of rice in it hence the name 'rice water stool'. Dehydration may be followed, causing death in some cases, if prompt electrolyte supplementation is not provided. Antibiotic treatment helps in reducing the duration of the disease by killing the organisms before they become bound to mucosa.

Fig:9*Vibrio cholerae*

The most common cause of cholera is consumption of food or drinking water that has been contaminated with the bacteria. It is important to note that after a disaster such as earthquake, flood etc. this is a very real danger, since regular and clean water and food supplies are often unavailable. The disease can be spread even further by infected people using already dirty water sources to clean themselves or dispose off the waste. Man is the main reservoir of V. cholerae, who is the carrier. The organisms are transmitted through contamination of food and water by faecal matter. Cholera can be prevented if there is safe disposal of sewage and also through supply of

protected water. The good personal hygiene of food handlers also helps in reducing the spread of the disease.

10.5.3.3 *Yersiniaenterocolitica*Gastroenteritis

Yersinia enterocolitica gastroenteritis is an infection caused by the bacterium Yersinia enterocolitica. *Yersinia enterocolitica* is a gram-negative bacterium that can cause gastrointestinal infections in humans. It is commonly found in animals, particularly pigs, and can contaminate food and water.

Fig:10*Yersinia enterocolitica*

Transmission: Yersinia enterocolitica gastroenteritis is primarily transmitted through the consumption of contaminated food, particularly raw or undercooked pork, unpasteurized milk, and contaminated water. Person-to-person transmission is possible, especially in close contact settings.

Symptoms: The symptoms of Yersinia enterocolitica gastroenteritis typically appear within 4 to 7 days after consuming contaminated food or water. They may include diarrhea, abdominal pain, fever, nausea, and vomiting. In some cases, the infection can mimic appendicitis with severe abdominal pain.

Risk factors: Certain populations are at a higher risk of developing severe symptoms from *Yersinia enterocolitica*infection, including young children, older adults, and individuals with weakened immune systems.

Prevention: To prevent Yersinia enterocolitica gastroenteritis, it is important to practice proper food safety measures. This includes cooking meat, especially pork, thoroughly to kill the bacteria, avoiding cross-contamination between raw and cooked foods, and practicing good hygiene, such as thorough handwashing.

Treatment: Most cases of Yersinia enterocolitica gastroenteritis do not require specific treatment and resolve on their own within a few weeks. It is important to stay hydrated by drinking plenty of fluids. In severe cases or for individuals with compromised immune systems, antibiotics may be prescribed.

By following proper food safety practices, such as cooking food thoroughly and practicing good hygiene, the risk of *Yersinia enterocolitica* gastroenteritis can be reduced. If you experience severe symptoms or prolonged illness, it is important to seek medical attention for proper diagnosis and management.

10.6 Investigation and detection of foodborne diseases

The investigation and detection of foodborne diseases involve a combination of surveillance, laboratory testing, epidemiological studies, and outbreak investigations. Here are the key steps involved in the process:

Surveillance: Public health agencies and organizations actively monitor and collect data on foodborne illnesses through various surveillance systems. This includes tracking reports of illness from healthcare providers, laboratories, and the general public. Surveillance helps identify patterns, trends, and potential outbreaks.

Case Identification: When individuals seek medical attention for suspected foodborne illnesses, healthcare providers play a crucial role in identifying and reporting cases to the appropriate authorities. Prompt reporting helps initiate investigations and control measures.

Epidemiological Studies: Epidemiological studies are conducted to determine the source and cause of foodborne diseases. This involves interviewing affected individuals about their symptoms, food consumption history, and potential exposures. Comparing the information from multiple cases can help identify commonalities and potential sources of contamination.

Laboratory Testing: Laboratory analysis is essential for confirming the presence of pathogens or toxins in food and biological samples. Samples from affected individuals, suspected food sources, and environmental samples are collected and tested to identify the specific causative agents. Techniques such as culture,

polymerase chain reaction (PCR), serological tests, and toxin detection methods may be employed.

Outbreak Investigations: When multiple cases of foodborne illness are identified, outbreak investigations are initiated. This involves identifying a common source or exposure and assessing the extent of the outbreak. Epidemiologists, environmental health specialists, and laboratory scientists collaborate to determine the root cause and implement appropriate control measures.

Traceback and Recall: If a specific food or product is identified as the source of contamination, traceback investigations are conducted to trace its origin and distribution. This information helps in implementing targeted recalls and preventing further exposure to contaminated products.

Collaboration and Communication: Throughout the investigation process, collaboration between public health agencies, healthcare providers, laboratories, and the food industry is crucial. Timely and effective communication of findings, warnings, and control measures helps protect public health and prevent further cases of foodborne diseases.

Continuous monitoring, analysis of data, and research also contribute to improving the detection and investigation of foodborne diseases. These efforts aid in identifying emerging pathogens, understanding new sources of contamination, and implementing preventive measures to reduce the risk of foodborne illnesses.

10.6.1 Detection of Salmonella:

Salmonella include Gram negative bacilli that are usually found in poultry, eggs, unprocessed milk, meat and water. These bacteria have been implicated in several food poisoning instances. Members of genus Salmonella are motile and possess two antigens that play an important role in the identification of these bacteria. These antigens are flagellar H antigen and somatic O antigen. The classical method of detecting Salmonella, the causative agent of salmonellosis, involves several steps.

Requirements: Selenite cystine (SC) broth, tetrathionate (TT) broth, Rappaport-Vassiliadis (RV), bismuth sulfite (BS) agar, xylose lysine desoxycholate (XLD) agar, Hektoen enteric (HE) agar, triple sugar Iron (TSI) agar, lysine iron (LI) agar, urea

broth, physiological saline, Spicer-Edwards flagellar (H) antisera, phenol red dulcitol broth, tryptone broth, polyvalent somatic (O) antisera, MR-VP broth, Koser citrate broth, -naphthol, Potassium hydroxide

Methodsof Detection

Classical Plate Count Methods

Sample Collection: A sample suspected to contain Salmonella, such as food, water, or clinical specimens (e.g., stool or blood), is collected using aseptic techniques. The sample should be representative and collected in a sterile container Fig 10..

Pre-enrichment: The collected sample is pre-enriched in a non-selective broth or medium that supports the growth of a wide range of microorganisms. This step allows for the revival of stressed or injured Salmonella cells.

Selective Enrichment: A portion of the pre-enriched sample is transferred to a selective enrichment broth or medium that specifically promotes the growth of Salmonella while inhibiting the growth of competing bacteria. Commonly used selective media include Rappaport-Vassiliadis (RV) broth or selenite broth.

Plating: After the enrichment step, a small volume of the selective enrichment broth is streaked or spread onto selective agar plates. Examples of selective agar media for Salmonella include Xylose Lysine Deoxycholate (XLD) agar, Brilliant Green agar (BGA), and Hektoen Enteric agar (HE).

Incubation: The plated agar plates are then incubated at the optimal temperature for Salmonella growth, usually around 37°C. Incubation times vary, but commonly range from 18 to 24 hours.

Colony Isolation: After incubation, characteristic Salmonella colonies appear on the agar plates. These colonies are often distinguished by their morphology, color, and other specific features. Suspected Salmonella colonies can be selected and subcultured onto fresh agar plates to obtain a pure culture.

Biochemical tests

1. Pick at least two suspected Salmonella colonies from each agar and carry out Triple sugar iron (TSI) agar and Lysine iron (LI) agar tests.

2. Inoculate the TSI slant by stab and streak method. Without heating the loop stab the butt twice and streak the slant of LI agar.
3. Incubate the tubes at 35oC for 24 h.
4. Examine the results obtained in the TSI and LI tests. Salmonella produces an acidic butt (yellow) and alkaline slant (red). The blackening of butt may or may not occur depending on the H2S production. In LI test Salmonella produce an alkaline butt (purple) with H2S production. All the cultures giving alkaline reaction (purple butt) for LI agar are used for confirmation. Cultures giving an acidic reaction (yellow butt) in LI agar but an acidic butt and alkaline slant in TSI are also used.
5. Examine a minimum of 6 TSI cultures for 25 g of sample, for identification of Salmonella.
6. Inoculate urea broth with the presumptive Salmonella isolates and incubate the tubes at 35oC for 24 h. Keep an uninoculated tube as a control. This broth contains the pH indicator phenol red. The phenol red is peach color at a pH of 6.8 and turns a hot pink at a pH of 8.4. Discard the cultures if the colour of medium changes to purple red indicating a positive test for urease production because Salmonella is known to be negative for urease test.
7. Carry out the serological polyvalent flagellar (H) test with the urease negative cultures. Inoculate BHI broth with the cultures from TSI slant and incubate at 35oC until visible growth occurs. Add 2.5 ml of formalinized physiological saline solution to 5 ml of BHI culture. Add 0.5 ml of polyvalent flagellar (H) antisera to 10X75 mm serological test tube. To this add 0.5 ml of formalinized broth. Prepare control by adding 0.5 ml of formalinized physiological saline to the formalinized antigen. Incubate the tubes at 48 – 50oC for 1 hr. Observe for agglutination. The test is positive if agglutination is observed and negative if there is no agglutination. In some cases the test may be nonspecific where agglutination is observed both in the test mixture and in control. Repeat this test with Spicer-Edwards flagellar (H) antisera for the cultures giving a negative result.
8. Perform the additional biochemical tests on the urease negative isolates by inoculating them in Phenol red dulcitol broth in Durhams tube. Development of yellow color and gas production indicates positive reaction. Salmonella is positive for this test.
9. Inoculate the growth from TSI slants into tryptone broth tube and incubate at 35oC for 24 h. Further inoculate potassium cyanide broth and malonate broth from the tube containing tryptone broth. Keep these tubes at 35oC for 48 h. Observe for growth in

potassium cyanide broth. Salmonella is not able to grow in this broth. Salmonella also give a negative test for malonate which is indicated by the green or unchanged color of the broth.

10. Transfer 5 ml of tryptone broth culture to an empty test tube and add 0.3 ml of Kovacs reagent. Development of deep red color is positive test. Salmonella is negative for this test.
11. Perform the polyvalent somatic (O) test for Salmonella by preparing an emulsion of growth obtained from TSI slant in 2 ml of 0.85% saline. Take a drop of this suspension on a clean slide and add a drop of polyvalent somatic (O) antisera. Mix them and tilt the slide back and forth for 1 min. For control add a drop of saline to the drop of culture suspension and mix as described above. Observe for agglutination. If agglutination is positive for test and negative for culture the result is taken as positive for Salmonella. If both are negative the test is also negative for Salmonella and if agglutination is positive then the test is nonspecific.
12. Perform additional biochemical tests including lactose fermentation, sucrose fermentation, MR-VP and citrate utilization.
13. Compare the results obtained in the above biochemical and serological tests with the standard reactions exhibited by Salmonella as shown in Table 3.7.
14. Biochemical tests can also be carried out using commercially available kits such as API 20E, Enterotube II, Enterobacteriaceae II, MICRO-ID, or Vitek GNI for presumptiveidentification of foodborne Salmonella.

15.

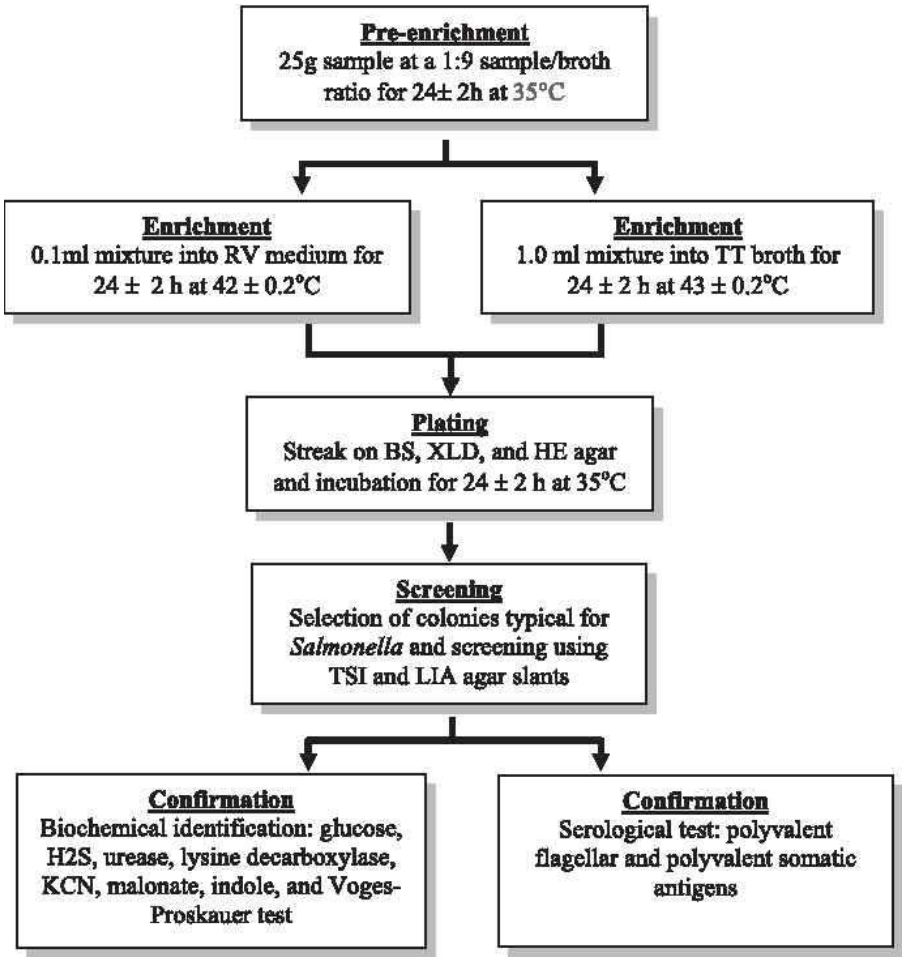

Fig: 10 Flow Chart for Identification of Salmonella by Classical Plant Count Method

(RV medium: Rappaport–Vassiliadis (RV) is enrichment medium for salmonellae, BS medium: Bismuth sulfite agar is a type of agar media used to isolate Salmonella species, XLD medium: Xylose Lysine Deoxycholate agar (XLD agar) is a selective growth medium used in the isolation of Salmonella, HE Agar: *Hektoen enteric* agar (HEK, *HE* or HEA) is a selective and differential agar primarily used to recover *Salmonella*

TSI Agar: Triple Sugar Iron Agar is a nonselective medium used for the differentiation of colonies of suspected Salmonella from other Enterobacteriaceae obtained on isolation media

LIA Agar: Lysine Iron Agar (LIA) is used to identify members of *Salmonella*)

10.6.2 Detection of*Staphylococcus aureus*

Staphylococcus aureus, a gram-positive bacterium, exhibits a spherical shape and is a facultative anaerobe, lacking spore-forming abilities. Notably, it is coagulase positive. Certain strains of S. aureus possess the capability to produce a highly heat-stable protein toxin that can cause illness in humans.Pyogenic infections are commonly attributed to Staphylococcus aureus. This bacterium is carried in the nasopharynx region of approximately 50-75% of healthy individuals. It is recognized for its production of various toxins such as haemolysins, fibrinolysin, coagulase, leucocidin, hyaluronidase, DNAase, epidermolytic toxins, enterotoxins, and toxic shock syndrome toxin (TSST-1). These toxins play a significant role in the pathogenesis of *S. aureus*.*Staphylococcus aureus* has been identified as the causal agent in numerous outbreaks of food poisoning. When ready-to-eat foods contain a count of S. aureus exceeding 102 cfu/g, they are deemed unsatisfactory for consumption. The condition caused by enterotoxins produced by certain strains of S. aureus is referred to as staphylococcal food poisoning (also known as staphyloenterotoxicosis or staphyloenterotoxemia).In addition to food poisoning, *Staphylococcus aureus* is a common cause of mastitis in dairy cattle and is often found as a contaminant in raw milk and dairy products. It resides in the nasopharyngeal cavity and on the skin, making contamination with staphylococci a possibility during food handling. Staphylococcal food poisoning can result from such contamination. The presence of these bacteria in food suggests inadequate sanitary conditions in the processing plant. However, since these bacteria can be effectively eliminated by heat treatment and sanitizers, their presence indicates post-processing contamination of the food product. Control measures for Staphylococcus aureus include pasteurization, low-temperature storage, proper hygiene, and sanitation practices. These measures help mitigate the risk of contamination and ensure food safety

METHOD OF DETECTION

Isolation, identification and enumeration of S. aureus

Isolation of S. aureus from food products is carried out by direct plate count method as follows:

1. Weigh 50 g of food sample in a sterile jar and add 450 ml of Butterfield's phosphate-buffered dilution water to it. Blend it at high speed. This is 10-1 dilution of the food sample.

2. Further prepare the decimal dilutions by adding 10 ml of previous dilution to 90 ml of sterile diluent. Mix the dilutions properly.
3. Transfer 1ml of each dilution aseptically on 3 plates of Baird Parker agar. 1 ml of inoculum should be equitably distributed on three plates.
4. Spread the inoculum evenly on the surface of the media by sterile glass spreader.
5. Incubate these plates at 35oC for 45-48 h in inverted position.
6. Consider the plates having typical *S. aureus* colonies (circular, smooth, convex, moist, 2-3 mm in diameter, gray to jet-black, frequently with light-coloured (off-white) margin, surrounded by opaque zone and frequently with an outer clear zone; colonies have buttery to gummy consistency (when touched with inoculating needle). Some other bacteria may also form colonies similar to that of *S. aureus* but these colonies lack opaque and clear zones as formed by *S. aureus* colonies. Rough colonies with dry texture may also be produced by *S. aureus* in certain cases. Plates having typical S. aureus colonies and counting in the range of 20 – 200 colonies (including other bacterial colonies) are used for determining cfu, however, when typical colonies are present at lower dilutions only, then plates with > 200 colonies may be used. In this case, colonies showing typical morphology of *S. aureus* are counted for determining cfu. Select >1 colony of each counted colony type and test for coagulase production.
7. Count all the colonies of particular types that give positive coagulase test. Add all such colonies obtained on triplicate plates and multiply with the sample dilution factor to report thecfu of *S. aureus* / g of sample
8. **Coagulase Test**
9. Transfer the suspect colonies to narrow tubes containing 0.3 ml of BHI broth and emulsify. Inoculate TSA medium with a loopful of BHI suspension and incubate them (BHI tubes and TSA slants) at 35^{o}C for 24 h.
10. Add 0.5 ml of reconstituted plasma with EDTA to BHI suspension and incubate at 35^{o}C for 6h. Examine periodically for clot formation during the incubation period.
11. Consider the tubes exhibiting firm clot as positive for *S. aureus*.
12. Keep the positive and negative controls for the coagulase test.
13. Carry out gram staining for all the suspect cultures and observe microscopically for presence of gram positive cocci in bunches.

10.7 Summary

Food contamination can occur through various means, including biological, chemical, and physical contaminants. Biological contaminants encompass pathogens like bacteria, viruses, parasites, and fungi that can infiltrate food during different stages of production, processing, storage, or handling. Common bacterial culprits include *Salmonella, E. coli*, *Campylobacter*, and *Listeria*.Molds or fungi, also can contaminate food through contaminated water, soil, improper pesticide usage, or mishandling. Additionally, improper storage and temperature misuse can cause rapid bacterial growth and contamination.

Foodborne infections and intoxications can have various symptoms, including nausea, vomiting, diarrhea, abdominal pain, fever, and dehydration. These illnesses can be transmitted through contaminated raw or undercooked meat, poultry, seafood, eggs, raw fruits and vegetables, unpasteurized dairy products, improperly processed or canned foods, and cross-contamination. To prevent such hazards, it is important to ensure proper cooking of food, maintain safe storage temperatures, practice good hygiene, prevent cross-contamination, consider food irradiation, and handle and store food properly. Detecting foodborne diseases often involves investigating outbreaks, tracing the source of contamination, and conducting laboratory tests on food samples and affected individuals to identify the causative agents.

To prevent contamination, it is crucial to implement good manufacturing practices, maintain hygiene and sanitation protocols, conduct regular inspections, employ hazard analysis and critical control points (HACCP) systems, and educate food handlers and consumers about safe food handling practices.

Key Word: Food contamination, Food borne diseases, Microbial detection technique,

Fill in the blank:

a) *Salmonella tyhimuruim* causes ……
b) Primary source of salmonellosis is ..... while ..... are the carriers.
c) The main mode of transmission of Shigellosis is ..... to .....
d) Two major sources of contamination leading to *parahaemolyticus* are ..... and ..... : .....
e) Food borne diseases are classified as food ..... , food ..... ; ..... and .....infections.

f) The most frequently reported food borne disease is ..... food poisoning.

g) Strains of Bacillus cereus produces two types of toxins, namely ..... and .....

h) Botulism is caused by the ingestion of ..... : .....

Give the symptoms, foods involved and preventive measures of the following diseases:

1. Salmonellosis

.....

2. Shigellosis

.....

.....

3. E. coli diarrhoea

.....

4. Hepatitis A

Answer to check up your progress

a) typhoid
b) Animal, human
c) water, food
d) through contaminated water, food
e) of raw or undercooked seafood
f) intoxication, infection, toxic
g) Staphlococcal
h) Diarrhoeagenic, emetic

Unit XI: Microbes: Used in biotechnology, fermented foods and their benefits

Content

structure

11.5.10 Enzyme Produced by Microbies

11.6 Function fermented Foods

11.6.1 Probiotics

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11.6.3 Symbiotic

11.7 Benefits of Fermentation

11.8 Summary

11. Objective:

- The aim of microbes in biotechnology is to utilize their metabolic capabilities for the production of valuable compounds, genetic engineering, enzyme production, and environmental management.

- Fermented foods offer benefits such as enhanced nutritive value, improved digestion, increased bioavailability of nutrients, and potential probiotic effects on gut health and immunity.

11.1Introduction

Microbial biotechnology is a branch of biotechnology that focuses on the utilization of microorganisms for various applications in fields such as medicine, agriculture, industry, and environmental management. Microbes, including bacteria, fungi, and viruses, possess unique biochemical and genetic characteristics that make them valuable tools in these areas. One of the most well-known applications of microbial biotechnology is in the production of pharmaceuticals and therapeutics. Microbes can be genetically engineered to produce a wide range of useful compounds, including antibiotics, vaccines, hormones, enzymes, and complex proteins. For example, the bacterium Escherichia coli has been modified to produce human insulin, a crucial hormone for managing diabetes.

In agriculture, microbial biotechnology plays a significant role in improving crop productivity and reducing the use of chemical fertilizers and pesticides. Certain bacteria and fungi can form beneficial associations with plant roots, known as symbiotic relationships, promoting nutrient uptake and disease resistance. These microorganisms can be applied as biofertilizers or biopesticides to enhance soil fertility and protect crops from pests and pathogens.

The industrial sector also benefits from microbial biotechnology. Microbes can be harnessed for the production of biofuels, bioplastics, and various chemicals. For instance, certain bacteria and yeasts can convert plant biomass into bioethanol through fermentation processes. This offers a more sustainable alternative to fossil fuels and reduces greenhouse gas emissions.

Environmental management is another area where microbial biotechnology has proven invaluable. Microbes play a crucial role in bioremediation, the process of using living organisms to degrade or remove pollutants from contaminated sites. Certain bacteria and fungi have the ability to break down toxic substances, such as oil spills or industrial waste, into harmless byproducts, aiding in the restoration of polluted ecosystems.

The field of microbial biotechnology continues to advance as scientists discover new microorganisms and develop innovative techniques for manipulating their genetic makeup. These advancements hold promise for addressing various global challenges, such as improving human health, ensuring food security, and mitigating environmental pollution.

11.2 Food Micro-biotechnology:

Food micro-biotechnology is a branch of biotechnology that focuses on the application of microorganisms in the production, processing, and preservation of food. It involves the use of beneficial microorganisms, genetic engineering, and fermentation techniques to improve the quality, safety, and nutritional value of food products.

Biotechnology plays a significant role in food microbiology by harnessing the power of microorganisms for various applications in the food industry. Important roles of biotechnology in food microbiology:

Microbial Strain Selection: Biotechnology enables the identification, selection, and modification of specific microbial strains for desired properties. This is important in food microbiology to identify beneficial microbial strains that can improve food quality, flavor, and safety.

Starter Cultures and Fermentation: Biotechnology is utilized to develop starter cultures consisting of specific microbial strains that initiate and control fermentation processes in food production. This includes the production of fermented dairy products, bread, sauerkraut, and other fermented foods. Starter cultures ensure consistent product quality, flavor development, and safety.

Probiotics: Biotechnology plays a crucial role in the development and production of probiotics. Probiotics are live microorganisms that, when consumed in adequate amounts, confer health benefits. Biotechnology allows for the selection, optimization, and production of probiotic strains that provide specific health benefits, such as promoting a healthy gut microbiota, enhancing immune function, and improving digestion.

Food Safety and Preservation: Biotechnology tools, such as DNA-based methods and rapid detection techniques, are used to monitor and ensure the safety of food products. This

includes the identification and detection of foodborne pathogens, spoilage microorganisms, and allergens, enabling timely interventions and preventive measures.

Biopreservation: Biotechnology is employed to develop and utilize natural antimicrobial compounds produced by microorganisms for food preservation. This includes the use of bacteriocins, antimicrobial peptides, and organic acids derived from microorganisms to inhibit the growth of pathogens and spoilage organisms, extending the shelf life of food products.

Genetic Modification: Biotechnology techniques, such as genetic engineering, allow for the modification of microorganisms to enhance their functionality in food production. This includes the introduction of genes that improve the production of enzymes, flavor compounds, and antimicrobial substances, as well as genes that confer resistance to environmental stressors.

Quality Control and Traceability: Biotechnology tools, such as molecular fingerprinting and DNA sequencing, are utilized for quality control and traceability in the food industry. These techniques help in identifying and tracking the origin of microbial contaminants, ensuring product authenticity, and preventing food fraud. Thus biotechnology plays a critical role in food microbiology by enabling the selection, modification, and application of microorganisms for improved food quality, safety, and preservation. It facilitates the development of innovative food products and processes that meet consumer demands for healthier, safer, and more sustainable food options.

11.3 Fermentation:

Fermented foods are foods that have undergone a process called fermentation, where microorganisms, such as bacteria or yeast, convert sugars or carbohydrates into other compounds, such as organic acids or alcohol. This process not only enhances the flavor, texture, and preservation of the food but also provides several potential health benefits. Fermentation has been used for centuries by various cultures, including Asian communities, as a method of food preservation. It offers several advantages and benefits in food processing and preservation. Fermentation is indeed a form of anaerobic respiration. Anaerobic respiration refers to the process of generating energy from glucose or other organic

compounds without the presence of oxygen. It occurs when oxygen is not available or in environments with low oxygen levels. During anaerobic respiration, in the absence of oxygen, cells undergo fermentation to produce energy. Fermentation involves the partial breakdown of glucose or other organic molecules and the subsequent production of ATP (adenosine triphosphate) without the involvement of the electron transport chain.

Carbohydrates are the common substrate of fermentation, and it can yield various products such as ethanol, lactic acid, and hydrogen. Fermentation can indeed produce a wide range of compounds, including butyric acid and acetone, depending on the microorganisms and conditions involved. Yeast is a well-known example of a microorganism that carries out fermentation, particularly in the production of ethanol in beverages like beer, wine, and other alcoholic drinks. Yeast consumes sugars and converts them into ethanol and carbon dioxide through the process of alcoholic fermentation. This process is utilized in the brewing and winemaking industries, where yeast plays a vital role in transforming the sugars present in grains or grapes into alcohol.

Louis Pasteur, the renowned French chemist, made significant contributions to the understanding of fermentation. In 1857, Pasteur connected yeast to the process of fermentation, linking the metabolic activity of yeast cells to the production of alcohol. He conducted experiments and observations that demonstrated the role of microorganisms in fermentation, disproving the prevalent belief at the time that fermentation was purely a chemical process.

Fermentation is an enzyme catalysed, metabolic process whereby organisms convert starch or sugar to alcohol or an acid anaerobically releasing energy. The science of fermentation is called “zymology”.The fermentation reaction described involving glucose as the substrate and ethanol as the product is known as alcoholic fermentation. It is commonly carried out by the yeast species *Saccharomyces cerevisiae*, which is widely used in food production, particularly in the brewing and baking industries.

The reaction can be summarized as follows:

$$C_6H_{12}O_6 + 2P_i + 2ADP- \rightarrow 2CH_3CH_2OH + 2CO_2 + 2\ ATP \text{ (energy released:118 kJ/mol)}$$

In this reaction, glucose, a six-carbon sugar, is metabolized by yeast through a series of enzymatic reactions. The process involves the conversion of glucose into two molecules of ethanol and the release of two molecules of carbon dioxide as a byproduct. This reaction occurs in the absence of oxygen, making it an anaerobic process.

The ability of *Saccharomyces cerevisiae* to carry out alcoholic fermentation is of great importance in the production of alcoholic beverages such as beer, wine, and spirits. The yeast consumes the sugars present in the ingredients (such as malted grains in beer or grapes in wine) and converts them into ethanol and carbon dioxide. The carbon dioxide generated during fermentation is responsible for the carbonation and characteristic bubbles in beverages like beer.

11.4 **Types of fermentation :**

Types of fermentation based on the number of end products formed:

11.4.1 **Homofermentation:**

Homofermentation is a type of fermentation where only one major end product is formed. The process involves the conversion of a substrate into a single primary product. Examples of homofermentative fermentations include lactic acid fermentation and ethanol fermentation.

11.4.1.1 **Lactic Acid Fermentation (homofermentative):**

This fermentation pathway primarily produces lactic acid as the main end product. It is commonly carried out by lactic acid bacteria, such as species of Lactobacillus and Streptococcus. Yogurt and sauerkraut production involve homofermentative lactic acid fermentation.

$$C_6H_{12}O_6(Glucose) \xrightarrow[Lactate.dehydrogenase]{Lactobacillus} 2CH_3CHOHCOOH(LacticAcid)$$

11.4.1.2.**Ethanol Fermentation (homofermentative**):

In this fermentation, the primary end product is ethanol (alcohol). It is predominantly carried out by yeast, such as *Saccharomyces cerevisiae*. Alcoholic beverage production, such as beer and wine, relies on homofermentative ethanol fermentation.

$C_6H_{12}O_6 + 2P_i + 2ADP- \rightarrow 2CH_3CH_2OH + 2CO_2 + 2\ ATP$ (energy released:118 kJ/mol)

11.4.2 Heterofermentation:

Heterofermentation is a type of fermentation where multiple end products are formed. The process involves the conversion of a substrate into different metabolic products. Heterofermentation is typically more diverse in terms of the products produced compared to homofermentation**.**

11.4.2.1

Acetic acid fermentation, also known as acetous fermentation, is a type of fermentation that produces acetic acid as the primary end product. It occurs through the oxidation of ethanol, typically in the presence of certain bacteria called acetic acid bacteria.The process of acetic acid fermentation involves two main stages:

11.4.2.2

Alcoholic Fermentation: First, ethanol is produced through the process of alcoholic fermentation. This occurs when yeast or other microorganisms convert sugars into ethanol and carbon dioxide. This step is similar to the fermentation process used in the production of alcoholic beverages.

11.4.2.3

Acetic Acid Production: In the second stage, acetic acid bacteria, such as species of *Acetobacter* and *Gluconobacter*, oxidize the ethanol to produce acetic acid. This oxidation process involves the conversion of ethanol to acetaldehyde, followed by the conversion of acetaldehyde to acetic acid. Oxygen is required for this stage, making it an aerobic process.

$$C_2H_5OH\,(ethanol) + O_2 \xrightarrow{acetobacter} CH_3COOH\,(acetic\ acid) + H_2O$$

11.4.2.4

Propionic Acid Fermentation: Propionic acid fermentation occurs in certain bacteria, such as *Propionibacterium*. It involves the conversion of sugars into propionic acid, along with the production of small amounts of acetic acid and carbon dioxide. This type of fermentation is important in the production of Swiss cheese, as it contributes to the characteristic flavor and texture of the cheese.

11.4.2.5

Butyric Acid Fermentation: Butyric acid fermentation is carried out by bacteria, such as *Clostridiumbutyricum*species. It involves the conversion of sugars into butyric acid and other volatile fatty acids. This type of fermentation is involved in the production of some fermented foods and can contribute to their flavor and aroma.

$$C_6H_{12}O_2 \xrightarrow[butyricum]{clostridium} C_4H_8O_2 + 2CO_2 + 2H_2$$

11.4.2.6

Mixed Acid Fermentation (heterofermentative): Mixed acid fermentation is a type of heterofermentation in which a variety of organic acids, alcohols, gases (such as carbon dioxide and hydrogen), and other compounds are produced. *Escherichia coli*is an example of a microorganism capable of mixed acid fermentation.

11.5 **Fermented foods:**

Microorganisms, such as bacteria, yeasts, and molds, are used to ferment food and beverage products. Fermentation can enhance flavors, textures, and nutritional profiles of foods. Examples include the fermentation of milk to produce yogurt, cheese, and kefir, the fermentation of cabbage to produce sauerkraut, and the fermentation of grapes to produce wine Fermentation is a widespread and diverse process used to create a wide variety of flavorful and nutritious foods. These are different category of fermentation on the basis of primary product used given below. Different fermented food product list given below

11.5.1 **Fermented Cereal Products:**

- Idli: Idli is a popular South Indian dish made by fermenting a batter consisting of rice and urad dal (split black lentils). The batter is fermented overnight, which leads to a fluffy and soft texture when steamed. Idlis are often served with chutney and sambar.
- Dosa: Dosa is another South Indian delicacy made from a fermented batter of rice and urad dal. The fermented batter is spread thin on a hot griddle and cooked to a crispy texture. Dosas can be served with various fillings or accompaniments, such as chutney and sambar.
- Injera: Injera is a sourdough flatbread that is a staple in Ethiopian and Eritrean cuisine. It is made from fermented teff flour, which is a gluten-free grain. The batter is fermented for several days, resulting in a tangy flavor and a spongy texture. Injera is traditionally used as a base for various stews and dishes.
- Sourdough Bread: Sourdough bread is made using a fermented dough starter called a sourdough culture or "starter." The starter is a mixture of flour and water that captures wild yeast and lactobacilli bacteria from the environment. The fermentation process imparts a distinct sour flavor and improves the texture and digestibility of the bread.

Table 1: Fermented Cereal Product

Product Name	Major Ingradients	Microorganism	Country of Use
Idli and Dosa	Rice, Black gram	*L. mensenteroides* *S. fecalis* *T. candida* *T.pullulans*	South India, Sri Lanka
Dhklaosa	Rice, Bengal gram	*L. mensenteroides* *S. fecalis* *T. candida* *T.pullulans*	India
Jalebies	Wheat flour	S. bayanus	India, Nepal
Mantou	Wheat Flour	*Saccharomyces*	China
Kichdok	Rice, Takju	*Saccharomyces*	Korea
Puto	Rice, Sugar	*L. mesenteroided* *S. facalis* *Yeast*	
Mungbean Starch Noodle	Mungbean	*L. mensenteroides* *L. casei* *L. cellobiosus*	China, Thailand, Japan

		L.fermenti	
Khanojeen	Rice	*Lectobaccillus Streptococcus sp*	Thailand

(Source: FAO 1999 fermented Cereals: A global perspective, FAO Agriculture Services Bulletin No. 138.)

11.5.2 **Fermented Dairy Products:**

- Yogurt: Yogurt is produced by fermenting milk with specific strains of bacteria, such as Lactobacillus bulgaricus and Streptococcus thermophilus. The bacteria convert lactose, the naturally occurring sugar in milk, into lactic acid, which gives yogurt its tangy taste and thick consistency. Yogurt is enjoyed on its own or used as an ingredient in various dishes and desserts.
- Cheese: Cheese is made by fermenting milk with the addition of specific bacteria or molds, which aid in the fermentation process. The fermentation causes the milk proteins to coagulate, forming curds. The curds are then drained, pressed, and aged, resulting in the creation of various types of cheese, each with its own flavor and texture.
- Buttermilk: Traditional buttermilk is a fermented dairy beverage that was historically made by collecting the liquid left over after churning butter. Nowadays, cultured buttermilk is commonly available, made by fermenting pasteurized milk with lactic acid bacteria. Buttermilk has a tangy flavor and is used in baking, marinades, and dressings.
- Labneh: Labneh is a Middle Eastern strained yogurt cheese. It is made by straining yogurt to remove the whey, resulting in a thicker, creamier consistency. Labneh can be enjoyed on its own, spread on bread or crackers, or used as a base for dips and spreads.

Table 2: Fermented Dairy Product

Product name	Major Ingradients	Microorganism	Country of Use
Lassi	Dairy based	LAB	Bangladesh, India and Pakistan
Doogh	Milk	LAB	Iran, Afghanistan,

			Armenia, Iraq, and Syria
Yogurt	Milk	*Lactobacillus delbrueckii*subsp. *bulgaricus*and *Streptococcus thermophilus*	Turkey an many Asian countries
Dahi	Milk (Cow/ Buffalo)	L. *lactis subsp. lactis, S. salivarius subsp. thermophilus, L. delbrueckii subsp. bulgaricus, plantarum, lactose* fermenting yeast, Mixed culture	India and turky
Kafir	Sheep, Cow, Goad mix milk and fermentation in Sking bag or wooden barrel	L lactis subsp. lactis, Leuconostoc spp., L.delbrueckii subsp. caucassiucu, Saccharomyces kefir, Torula kefir, micrococci, spore forming bacilli	Caucasus
Kumis	Mare's, camel's or ass's milk fermentation in skin bag	*L. delbrueckii subsp. bulgaricus, Torulakumiss, Saccharomyces lactis, micrococci, spore forming bacilli lactis, micrococci, spore forming bacilli*	

11.5.3 **Fermented Fish Products:**

- Fish Sauce: Fish sauce is a condiment commonly used in Southeast Asian cuisine. It is made by fermenting fish (usually anchovies or other small fish) with salt. The fermentation process breaks down the fish proteins and produces a savory, umami-rich liquid that is used as a flavor enhancer in cooking.
- Surströmming: Surströmming is a traditional Swedish fermented Baltic Sea herring. The herring is fermented in barrels for several months, resulting in a pungent odor. It is typically eaten by opening the can outdoors due to the strong smell. Surströmming is often enjoyed on flatbread with accompaniments like onions and potatoes.

- Bagoong: Bagoong is a fermented shrimp or fish paste commonly used in Filipino cuisine. It is made by fermenting shrimp or fish with salt. Bagoong adds a salty, savory, and umami flavor to dishes and is often used as a dipping sauce or ingredient in various Filipino recipes.

Table 3: Fermented Fish and Sea Food

Product name	Major Ingradients	Microorganism	Country of Use
Sikhae	Sea fish, cooked millet	L.mesentteroides Lb. pantarum	
Narezushi		L.mesentteroides Lb. pantarum	
Burongisda	Fresh water fish	Lb. brevis, Strptococcus Pediococcus sp.	Philippine
Balaobalao	Shrimp, Rice, salt	L. mesenteroides P.cerevisiae	Philippine
Kungchao	Shrimp, Rice	P.cerevisiae	Thailand
Nham	Pork, Cooked Rice	P.cerevisiae Lb. plantarum Lb.brevis	
Nem Chua	Pork, Cooked Rice	Pediococcus sp. Lactobacillus sp	Vietnam
Pla-ra	Fresh water fish roasted Rice	Pediococcussp	Thailand

11.5. 4 **Fermented Fruit and Vegetable Products:**

- Sauerkraut: Sauerkraut is a fermented cabbage dish that originated in Central Europe. It is made by finely shredding cabbage and fermenting it with salt. The fermentation

process produces lactic acid, giving sauerkraut its sour taste. It is commonly used as a condiment or side dish.

- Kimchi: Kimchi is a traditional Korean side dish made from fermented vegetables, usually including cabbage. The vegetables are seasoned with a mixture of ingredients like chili pepper, garlic, ginger, and salt before undergoing fermentation. Kimchi has a spicy, tangy flavor and is a staple in Korean cuisine.
- Pickles: Pickles are cucumbers or other vegetables that have been fermented in a brine solution. The fermentation process gives pickles their tangy and sour taste. They can be made with various vegetables and spices, resulting in a range of flavors and textures.
- Fermented Hot Sauce: Fermented hot sauces, such as sriracha or kimchi sauce, are made by fermenting chili peppers with salt and other ingredients. The fermentation enhances the flavor and complexity of the hot sauce while also providing a tangy and spicy kick.

Table 4 : **Fermented Fruit and Vegetable Products:**

Product name	Major Ingradients	Microorganism	Country of Use
Pickles	Vegetable	*L. mesenteroides*	India
Kimchi	Vegetable	LAB (*L. mesenteroides*, *Lb. brevis*, *Lb.plantarum)*	Korea
Suan-tsai	cabbage	LAB (Lactobacillus, Leuconostoc, and Pediococcus)	
Atchara	unripepapaya	LAB	Philippines

11.5. 5 **Fermented Legume Products:**

- Tempeh: Tempeh is a traditional Indonesian fermented soybean product. It is made by fermenting whole soybeans with a specific type of fungus called Rhizopusoligosporus. The fermentation process binds the soybeans together into a firm cake with a nutty flavor and a meaty texture. Tempeh is a popular plant-based protein source.
- Miso: Miso is a fermented soybean paste widely used in Japanese cuisine. It is made by fermenting soybeans with salt and a specific mold called koji. The fermentation process can vary in length, resulting in different types of miso with varying flavors and colors. Miso is commonly used in soups, marinades, and dressings.
- Natto: Natto is a traditional Japanese fermented soybean dish. It is made by fermenting soybeans with the bacterium Bacillus subtilis. Natto has a distinctive sticky texture and a strong flavor. It is often enjoyed with rice as a breakfast dish or used as an ingredient in other Japanese dishes.

Table 5: **Fermented Legume Products**

Product name	**Major Ingradients**	**Microorganism**	**Country of Use**
Tofu	Soyabeanwhey curd	Actinomucorelegans, Mucorhiemalis, M.silvaticus, M. subtilissimus	China, Taiwan
Sofu	Soyabeanwhey curd	Actinomucorelegans, Mucorhiemalis, M.silvaticus, M. subtilissimus	China, Taiwan
Soya Sauces	Soybeans and wheat	Aspergillusoryzae or A. soyae, Lactobacillus bacteria, Zygosaccharomyces rouxii	Japan, China, Philippines, other parts of Orient
Nattō	soybeans	• fermented with *Bacillus subtilis var. natto*.	Japan
Tempeh	soybeans	Rhizopus spp., principally R. oligosporus	Indonesia and vicinity, Surinam

11.5.6Fermented Meat Products:

- Salami: Fermented and air-dried sausage made from ground meat, typically pork, and seasoned with various spices.
- Pepperoni: Another type of fermented and air-dried sausage made from beef or pork and flavored with spices, especially pepper.
- Sausages: Various types of fermented sausages, such as chorizo, soppressata, and summer sausages, made by fermenting and curing ground meats with seasonings.

11.5.7 Fermented Beverages:

- Beer: Alcoholic beverage produced through the fermentation of cereal grains, mainly barley, using yeast. Various styles of beer exist worldwide, each with its own fermentation and flavor profiles.
- Wine: Alcoholic beverage made by fermenting grapes or other fruits. The fermentation process converts the fruit sugars into alcohol, resulting in different types and flavors of wine.
- Generally called shaosingjiu in China, cheongju in Korea, and sake in Japan, contain around 15% alcohol and are designated as rice- wine

- Kombucha: Fermented tea beverage made by fermenting sweetened tea with a SCOBY (symbiotic culture of bacteria and yeast). It has a slightly tangy taste and effervescence.
- Sake: Traditional Japanese rice wine made through the fermentation of polished rice with a specific type of yeast called koji-kin. It has a distinct flavor and is an integral part of Japanese culture.

Table 6:**Fermented Beverages:**

Product name	**Major Ingredients**	**Microorganism**	**Country of Use**

Tapuy	Rice, glutinousrice	Saccharomyces ,Mucer, Aspergilus, leuconostoc, Lb. plantarum	Philippine
Brembali		Glutinous Mucerindicus, Candida	Indonesia
Takju	Rice	LAB, Saccharomyces servecia	Korea

11.5.8 Microbial Primary Metabolities

Evidence is beginning to accumulate, describing their beneficial effects in a variety of GI (Gastro Intestinal) and non-GI disorders. They offer dietary means to support the balance of the intestinal flora. They may be used to counteract local immunological dysfunction, to stabilize the intestinal barrier function, to prevent infectious succession of pathogenic microorganisms and to influence intestinal metabolism. Looking ahead, this field holds immense promise for the future in delivering novel therapies in different fields

Table : 7 Microbial Primary Metabolities

Primary Metabolite	**Organism**	**Significance**
Xanthan gum	Xanthomonascampestris	
Vitamin B12	Pseudomonas	nutritional
Ethanol	Saccharomyces cerevisiae	alcoholic beverages
	Kluyzveromycesfragilis	
Citric acid	Aspergillusniger	food industry
Acetone and	Clostridium	
butanol	acetobutyricum	solvent
Lysine	Corynebacterium	nutritional additive
Dextran	Leuconostocmesenteroides	industrial

11.5.9 Secondary metabolites

Secondary metabolites Organisms produce a number of products, in addition to the primary metabolites. The microbial growth phase, during which products that have no obvious role in metabolism of the synthesizing culture organisms are produced, is called the idiophase, and these products are called secondary metabolites. In reality, the distinction between the primary and secondary metabolites is not a straight jacket situation. Many secondary metabolites are produced from intermediates and end products of secondary metabolism. Some like the Enterobacteriaceae do not undergo secondary metabolism.

Table 8: Secondary Metabolites

Primary Metabolite	**Organism**	**Significance**
Penicillin	Penicilliumchrysogenum	antibiotic
Erythromycin	Streptomyces erythreus	antibiotic
Ethanol	Saccharomyces cerevisiae	alcoholic beverages
Streptomycin	Streptomyces griseus	antibiotic
Cephalosporin	Cephalosporiumacrimonium	antibiotic
Acetone and	Clostridium	
butanol	acetobutyricum	solvent
Lysine	Corynebacterium	nutritional additive
Dextran	Leuconostocmesenteroides	industrial

11.5.10 Industrial Enzymesreduction

Production of Enzymes Industrial production of enzymes is needed for the commercial production of food and beverages. Enzymes are also used in clinical or industrial analysis and now they are even added to washing powders (cellulase, protease, lipase). Enzymes may be produced by microbial, plant or animal cultures. Even plant and animal enzymes can be produced by microbial fermentation through techniques of genetic manipulations. While most enzymes are produced in the tropophase, some like the amylases (by Bacillus stearothermophilus) are produced in the idiophase, and hence are secondary metabolites

Table 9: Enzyme produced by Microbes

Organism	Enzyme
Aspergillusoryzae	Amylases
Aspergillusniger	Glucoamylase
Trichodermareesii	Cellulase
Saccharomyces cerevisiae	Invertase
Kluyzveromycesfragilis	Lactase
Saccharomycopsislipolytica	Lipase
Aspergillus species	Pectinases and proteases
Bacillus species	Proteases
Mucorpusillus	Microbial rennet
Mucormeihei	Microbial rennet

Fig 1: Different Role of Food micro-biotechnology

11.6 Functional Fermented Food:

Dr. Elias Metchnikoff, a Nobel Laureate, around 100 years ago proposed that “Lactic acid bacteria can render a great service in the fight against intestinal putrefaction” and might “ postpone and ameliorate old age”. This concept was developed further through the decades, and today, the emerging probiotics, prebiotics and synbiotics era is a subject of scientific debate and intense research. The use of probiotics, prebiotics, and synbiotics is a promising area for the development of functional foods. Functional foods are generally characterized as foods similar in appearance to conventional foods, consumed as part of a usual diet, and providing health-related benefits beyond meeting basic nutritional needs. A food can be considered naturally ‘functional’ if it contains a food component that affects one or more targeted functions in a beneficial way. Dairy foods can be included in the functional food category because of their content of calcium, specific healthenhancing proteins, conjugated linoleic acid, sphingolipids, butyric acid, and probiotic cultures. Dairy foods appear to be the preferred medium for introducing probiotic bacteria such as human-derived species of lactic acid bacteria (e.g., *L. acidophilus, L. casei, L. gasseri, L. rhamnosus, L. reuteri, Bifidobacteriumbifidum, B. breve, B. infantis and B. longum*). Lactobacillus spp. (naturally found in the human small intestine) and various Bifidobacterium spp. (a major organism in the human large intestine) are the most commonly used probiotic cultures.

11.6.1 Probiotics

Probiotics are live microorganisms that provide health benefits when consumed in adequate amounts. These beneficial bacteria or yeasts are commonly associated with promoting a healthy gut microbiome and improving overall digestive health. Probiotics can be found in various food and supplement forms. The most commonly used probiotics belong to the genera Lactobacillus and Bifidobacterium and certain strains of L. casei or the L. acidophilus-group). However, other strains and species, such as Saccharomyces boulardii, are also used as probiotics.Among the numerous purported health benefits attributed to probiotic bacteria, the (transient) modulation of the intestinal microflora of the host and the capacity to interact with the immune system directly or mediated by the autochthonous microflora, are basic mechanisms. They are supported by an increasing number of in vitro and in vivo experiments using conventional and molecular biologic methods. In addition to these, a limited number of randomized, well-controlled human intervention trials have been reported.

11.6.2 Prebiotic :

A **prebiotic**The term Prebiotic is defined as “short chained carbohydrates that are indigestible by human enzymes in the GIT (Gastro-intestinal tract) and selectively stimulate the growth and activity of specific species of bacteria in the gut, usually *bifidobacteria* and *lactobacilli*, with benefits to health”. The most commonly used prebiotics in supplements are Fructo-oligosaccharides (FOS). Bifidobacteria, due to the presence of beta-fructofuranosidase enzyme are liable to break down and utilize FOS. This helps in stimulation of bifidobacterium growth in the GIT. FOS exhibits nutritional properties on colonic pH and stool bulking. It also increases bioavailability of essential minerals and decreases serum triglycerides. The other types of prebiotic substrates include-

• Xylitol, Sorbitol, Mannitol

• Disaccharides-Lactulose, Lactilol

• Oligosaccharides-Raffinose, Soybean, Palatinose, Isomaltose, Lactosucrose

• Polysaccharides-Insulin, resistant starch

11.6.3 Synbiotic: The term synbiotic is used when a product contains both probiotics and

prebiotics. Since the word alludes to synergism, this term should be reserved for products in which the prebiotic compound selectively favors the probiotic compound, e.g., FOS in combination with strains such as *Bifidobacterium B. infantis, B. longum* etc. Combining probiotics with prebiotics could improve the survival of the bacteria crossing the upper part of the GIT, thus enhancing their effects in the large bowel. Moreover, the local and the systemic beneficial effects of probiotics and prebiotics might be additive or even synergistic.

Require Dosage:

Dosage According to Earl Mindell, an internationally recognized expert on nutrition, healthy person should take 2-5 billion CFUs (colony stimulating units) of probiotics a day and those with GI conditions can take up to 10 billion CFUs per day. In acute infectious diarrhoea, lactobacillus is most effective at a dose of 10 billion CFUs

during the first 48 hours, which translates to 5 billion CFUs per day. For prescription probiotics, the current daily intake recommended is 5-10 billion CFUs per day. Capsules and Sachets of Probiotic plus Prebiotic combination (Pro-wel) and Probiotic alone (Darolac) are commercially available. Benefits offered by Probiotic and Prebiotic combination formula are :-

• Maximum Colony Forming Units (CFUs): ensure complete action.

• Fructo-oligosaccharide (FOS): offers nutrition to the probiotics and normal intestinal flora.

• Acid-resistant cells: reach intestine in full force.

• Freezed-dried and nitrogen-flushed cells: offers excellent stability

• Vegetable capsules: ensure universal appeal.

Benefits of Prebiotic, Probitic and Synbiotics: The well-established effects are given below:

1. Prevention and/or reduction of duration and complaints of rotavirus-induced or antibiotic-associated diarrhea as well as alleviation of complaints due to lactose intolerance.

2. Reduction of the concentration of cancer-promoting enzymes and/or putrefactive (bacterial) metabolites in the gut.

3. Prevention and alleviation of unspecific and irregular complaints of the gastrointestinal tracts in healthy people.

4. Beneficial effects on microbial aberrancies, inflammation and other complaints in connection with: inflammatory diseases of the gastrointestinal tract, Helicobacter pylori infection or bacterial overgrowth.

5. Normalization of passing stool and stool consistency in subjects suffering from obstipation or an irritable colon.

6. Prevention or alleviation of allergies and atopic diseases in infants.

7. Prevention of respiratory tract infections (common cold, influenza) and other infectious diseases as well as treatmentof urogenital infections. Insufficient or at most preliminary

evidence exists with respect to cancer prevention, a so-called hypocholesterolemic effect, improvement of the mouth flora and caries prevention or prevention or therapy of ischemic heart diseases or amelioration of autoimmune diseases (e.g. arthritis).

11.7 Benefits of Fermentation

Improved Digestion: Fermented foods are rich in probiotics, which are beneficial bacteria that support a healthy gut microbiome. Probiotics help to break down food components, aid in the absorption of nutrients, and promote a balanced digestive system. They can be particularly beneficial for individuals with digestive disorders like irritable bowel syndrome (IBS) or lactose intolerance.

Enhanced Nutrient Absorption: Fermentation can increase the bioavailability and digestibility of nutrients in food. It can break down complex molecules into simpler forms that are easier for the body to absorb. For example, fermentation of soybeans in the production of soy sauce or tempeh increases the availability of nutrients like protein and minerals.

Strengthened Immune System: The gut microbiome plays a crucial role in immune function, and consuming fermented foods can support a healthy balance of gut bacteria. Probiotics found in fermented foods have been shown to stimulate the immune system, enhance the production of antibodies, and help defend against harmful pathogens.

Increased Nutritional Value: Fermentation can increase the levels of certain vitamins and minerals in foods. For instance, fermented dairy products like yogurt and kefir can have higher levels of B vitamins and vitamin K compared to their non-fermented counterparts. Fermentation can also break down anti-nutrients present in some foods, making their nutrients more accessible to the body.

Gut Health and Mental Health Connection: Emerging research suggests that the gut microbiome may influence mental health and well-being. Consuming fermented foods and probiotics has been associated with improvements in mood and cognitive function. The gut-brain axis, a bidirectional communication pathway between the gut and the brain, may be influenced by the presence of beneficial gut bacteria.

Preservation of Food: Fermentation has been used for centuries as a method of food preservation. The fermentation process creates an acidic environment, inhibiting the growth

of spoilage-causing bacteria and increasing the shelf life of the food without the need for artificial preservatives.

Reduction of Undesirable Components: Fermentation can help reduce or eliminate certain undesirable components in food. For example, fermentation of soybeans in the production of soy sauce helps break down anti-nutrients and improve the digestibility and nutritional profile of the soybeans. Similarly, the fermentation of cabbage in sauerkraut production reduces the goitrogens present in raw cabbage, making it safer and more nutritious.

Nutritive Value and Appearance: Fermentation can enhance the nutritional value of food by increasing the availability and bioavailability of certain nutrients. For instance, fermentation of grains and legumes can increase the levels of vitamins, minerals, and amino acids. Additionally, fermentation can improve the texture, flavor, and appearance of food, making it more appealing to consumers.

Energy Efficiency in Cooking: Fermented foods often require less cooking time and energy compared to non-fermented foods. The fermentation process partially breaks down complex carbohydrates and proteins, making them easier to cook and digest. This not only saves energy but also retains the nutritional value of the food.

Safety: Fermentation can contribute to food safety by reducing the risk of pathogenic bacterial growth. The production of organic acids and other compounds during fermentation creates an inhospitable environment for harmful bacteria, thereby reducing the chances of food contamination and foodborne illnesses.

11.8 Summary

Fermented foods are popular throughout the world and in some regions, make a significant contribution to the diet of millions of individuals. Although fermentation of foods has been in use for thousands of year, it is likely that the underlying microbial and enzymatic processes responsible for the transformations were largely unknown. Fermentation is a relatively efficient, low energy preservation process which increases the shelf life and decreases the need for refrigeration or other forms of food preservation technologies. In Asia the preparation of fermented foods is a widespread tradition. The fermented products supply protein, minerals and other nutrients that add variety and nutritional fortification to otherwise starchy and bland diets. For instance Soy sauce is consumed

throughout the world and is a fundamental ingredient in diets from Indonesia to Japan. It is only recently that there has been a development in the understanding of these processes and their adaptation for commercialization. There is tremendous scope and potential for the use of micro-organisms towards meeting the growing world demand for food, through efficient utilization of available natural food and feed stocks and the transformation of waste materials. Genetic improvement of the organism is fundamental to the success of fermentation technology. Mutation and recombination are the two ways to meet this end Evidence is beginning to accumulate, describing their beneficial effects in a variety of GI (Gastro Intestinal) and non-GI disorders. They offer dietary means to support the balance of the intestinal flora. They may be used to counteract local immunological dysfunction, to stabilize the intestinal barrier function, to prevent infectious succession of pathogenic microorganisms and to influence intestinal metabolism. Looking ahead, this field holds immense promise for the future in delivering novel therapies in different fields

Check your improvement

Fill in the Blank

a)……..are the common substrate of fermentation

b)……. the renowned French chemist, made significant contributions to the understanding of fermentation.

c) Probiotics belong to the genera ……. And……

d)Butyric acid fermentation is carried out by bacteria, ……..species.

e) Ethanol fermentation, is predominantly carried out by ……..

f) Dosage, internationally recognized expert on nutrition, healthy person should take……. of probiotics a day and those with GI conditions can take up to 10 billion CFUs per day.

Lactic acid Fermentation

……

……

……
……

Role of Biotechnology in food science

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Benefit of Fermentation

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……
……

Answer:

a) Carbohydrate

b) Pasture

c) *Lactobacillus* and *Bifidobacterium*

d) *Clostridiumbutyricum*

e) Saccharomyces cerevisiae

f) 2-5 billion CFUs (colony stimulating units) of probiotics a day

Unit XII: The HACCP System and Food Safety Used in Controlling Microbiological Hazards

12. Objective

After studying this unit, you will be able to:

I. define HACCP, discuss the need, relevance of HACCP in the context of food safety,
II. enumerate the principles of HACCP, and
III. explain the guidelines for application of HACCP principles.

12.1 Introduction

Hazard Analysis and Critical Control Point (HACCP) is a comprehensive food safety program that originated from the need to ensure the well-being of astronauts during space missions. It is a proactive approach that emphasizes the prevention of hazards that could potentially lead to food-borne illnesses. Unlike the traditional method of relying on sporadic inspections and random sampling of finished products, HACCP employs a science-based methodology to implement controls throughout the entire food production process, from the sourcing of raw materials to the delivery of finished goods.The conventional approach of reactive measures, while inspecting manufacturing conditions and testing final products, has limitations in terms of efficiency and effectiveness. HACCP, on the other hand, provides a systematic framework that focuses on identifying and eliminating potential hazards before they can pose risks to consumers. By taking a preventive rather than reactive approach, HACCP aims to ensure the highest level of food safety. The HACCP system is built

on several key principles that guide its implementation. These principles include conducting a thorough hazard analysis to identify potential risks, determining critical control points (CCPs) where hazards can be controlled or prevented, establishing critical limits for each CCP, implementing monitoring procedures to ensure these limits are met, developing corrective actions for deviations from established limits, establishing verification procedures to validate the effectiveness of the system, and implementing a record-keeping system to document all aspects of the HACCP plan. Implementing the principles of HACCP requires adherence to specific guidelines. These guidelines outline the step-by-step process of developing and implementing a HACCP plan, including assembling a multidisciplinary team, conducting a detailed analysis of the production process, identifying potential hazards, determining CCPs, establishing critical limits, implementing monitoring procedures, defining corrective actions, and establishing verification and record-keeping procedures.

The below comparative table provides a clear overview of the advantages offered by HACCP in comparison to the previous food safety system.

Table 1: comparative table provides a clear overview of the advantages offered by HACCP in comparison to the previous food safety system.

Advantages of HACCP	**Previous Food Safety System**
Focuses on identifying and preventing hazards	Relied on spot-checks and random sampling of final products
Based on sound science	Relied on reactive measures and inspections
Enables more efficient and effective government oversight	Limited effectiveness and efficiency in oversight
Emphasizes continuous compliance with food safety laws and practices	Reactive approach to compliance
Places responsibility for ensuring food safety on manufacturers and distributors	Shared responsibility between industry and regulators
Enhances competitiveness in the global market	Limited ability to compete effectively internationally
Reduces barriers to international trade	Potential for trade barriers due to safety concerns

12.2 Importance of food safety and microbiological hazards

Food safety and microbiological hazards play a crucial role in protecting public health and ensuring the well-being of consumers. Some important point highlighting their importance:

Public Health Protection: Food safety measures are essential to safeguard consumers from foodborne illnesses caused by harmful microorganisms, such as bacteria, viruses, parasites, and fungi. Contaminated food can lead to severe health consequences, including gastrointestinal infections, food poisoning, and even life-threatening conditions.

Preventing Disease Outbreaks: Microbiological hazards in food can lead to disease outbreaks, affecting not only individuals but also communities. Prompt identification, control, and prevention of these hazards are vital in reducing the incidence of foodborne diseases and preventing large-scale outbreaks.

Consumer Confidence: A robust food safety system helps build trust and confidence among consumers. When people trust that the food they consume is safe, it enhances their satisfaction and promotes a positive perception of food products, leading to repeat purchases and brand loyalty.

Legal and Regulatory Compliance: Food safety is governed by strict regulations and standards implemented by national and international bodies. Compliance with these regulations is necessary for food businesses to operate legally, ensuring the safety and quality of their products.

Economic Impact: Food safety incidents can have significant economic consequences for businesses, including costly product recalls, lawsuits, damaged reputation, and loss of consumer trust. Adhering to food safety practices mitigates

these risks and contributes to the overall sustainability and profitability of the food industry.

Global Trade and Market Access: International trade in food products requires compliance with food safety standards and regulations established by importing countries. Meeting these requirements allows food businesses to access global markets, expand their customer base, and participate in international trade opportunities.

Foodborne Illness Costs: Foodborne illnesses impose substantial economic burdens on individuals, healthcare systems, and society as a whole. The costs associated with medical treatments, productivity losses, and decreased quality of life highlight the importance of preventing microbiological hazards and ensuring food safety.

12.3 Introduction to the Hazard Analysis and Critical Control Points (HACCP) system:

Hazard Analysis and Critical Control Point (HACCP) is an established methodology that ensures food safety through a systematic and science-based approach in food production. Originally developed in the 1960s by the Pillsbury Company in collaboration with NASA and the U.S. Army Laboratories, HACCP was initially designed to address microbiological safety concerns in food. However, its scope has expanded to include chemical and physical hazards as well. Recognizing its effectiveness, the Codex Alimentarius Commission endorsed HACCP as a cost-effective method for ensuring food safety in 1993. HACCP functions as a preventive system of food control, involving comprehensive examination and analysis of every stage in a food-related operation to identify and assess potential hazards. Critical control points, where action is required to control identified hazards, are determined. Critical limits that must be met at each control point are established, along with procedures to monitor these points effectively. Corrective procedures are put in place to address any deviations identified through monitoring. Additionally, the HACCP plan is documented, and procedures are implemented to verify its proper functioning. It is important to note that HACCP goes beyond end product testing and inspection. It takes a proactive and continuous approach to food safety, systematically identifying,

examining, analyzing, evaluating, and implementing corrective measures to control hazards at every stage of a food-related operation. This preventive nature makes HACCP effective and distinct in ensuring food safety.Within the definition of HACCP, several terms are encountered, such as critical control points and critical limits. Let's explore the meanings and definitions of these terms, as well as other relevant terminology that you may encounter during the study of HACCP.

12.4 Definition and principles of HACCP: HACCP, therefore, is a preventive system of food control. It involves examining and analysing every stage of a food-related operation to identify and assess hazards, determining the 'critical control points' at which action is required to control the identified hazards, establishing the critical limits that must be met at, and procedures to monitor, each critical control point, establishing corrective procedures when a deviation is identified by monitoring, documenting the HACCP plan and verifying procedures to establish that it is working correctly. From the definition above, the following points require consideration. HACCP is a food-related operation to: identify and assess hazard at every stage of operation, right from start to finish determine the critical control points establish the critical limit and procedures to monitor each critical control point, establish corrective procedures. It is obvious, therefore, that HACCP is not just based on end product testing and inspection. It is a preventive and a continuous approach to food safety identifying examining, analyzing/evaluating and establishing correctives measures and controlling hazards at every stage of a food-related operation. The principles of HACCP are applicable to all phases of food production (Fig1).

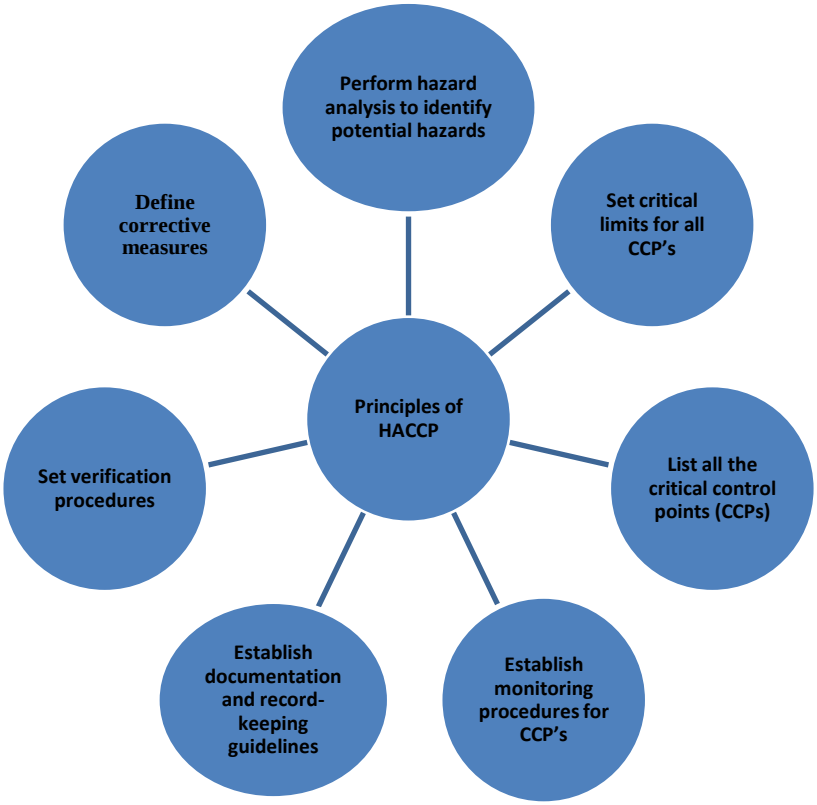


Fig: 1 Seven Important Principles of HACCP

- **Perform hazard analysis to identify potential hazards**

  To establish guidelines for your food business, it is imperative to begin with a hazard analysis to identify potential risks. This entails conducting a thorough examination of all aspects of your operations, testing and analyzing various stages, and documenting any potential hazards that may arise. For example, in the context of a restaurant, a hazard analysis would involve scrutinizing every step involved in food production, from the sourcing of raw materials to the final serving of meals. This analysis aims to pinpoint potential hazards, such as inadequate cooking temperatures, the risk of contamination during storage, or any other factors that may compromise food safety. By conducting a comprehensive hazard analysis, businesses can effectively identify areas of concern and develop appropriate guidelines and control measures to mitigate risks and ensure the safety of their food products.

- **List all the critical control points (CCPs)**

In order to create a HACCP food safety plan, it is crucial to identify and list all the critical control points (CCPs). CCPs are specific steps in the processes where appropriate measures can be implemented to control or eliminate potential hazards.By identifying CCPs, your business can effectively minimize the risk of hazards that may lead to food contamination or other safety issues.When determining your CCPs, it is important to consider the unique layout, processes, and operations of your business. CCPs are not standardized guidelines but rather specific to each process's characteristics.Here are some examples of common CCPs:

- Cooking temperature: Specifying the minimum or maximum temperature at which a particular food item should be cooked to ensure safety.
- Cooking time: Setting the maximum duration for which a dish should be cooked to prevent overcooking or undercooking, which may impact safety.
- Hand-washing procedures: Establishing criteria for proper hand hygiene practices before handling or plating food for customers.

Remember, your CCPs should be tailored to your specific operations and reflect the potential hazards and risks associated with your food production processes.

- **Set critical limits for all CCP's:**

  After identifying CCPs, you need to set critical limits for all of them. Critical limits define the maximum or minimum value that a CCP or hazard can reach beforeunacceptable levels. Going past the critical limit means that the food is not safe for consumption and thus, cannot be served to the end customer. Some standard criteria of critical limits that are set in HACCP systems are:

  - Temperature
  - pH level
  - Time

For example, creating critical limits for cooking a certain type of meat, there would be a certain temperature range that it should be cooked at. If the temperature falls below or exceeds this range, this would be crossing the critical limits which mean the meat is unsafe for further consumption.

- **Establish monitoring procedures for CCP's**

  To maintain food safety, it is essential to implement monitoring procedures to ensure that all processes and food items adhere to the established critical limits. This involves developing systematic protocols for monitoring each individual critical control point (CCP) to verify that they remain within safe limits.While there is no one-size-fits-all approach to creating monitoring procedures, many businesses find it useful to employ checklists as a practical tool for routine safety verification. These checklists serve as a structured framework to assess and document compliance with the defined critical limits and allow for regular monitoring of CCPs.By utilizing monitoring procedures and checklists, businesses can effectively track and evaluate the safety of their processes and food items, identifying and addressing any deviations from the established critical limits promptly. This proactive approach ensures on-going food safety and enables timely corrective actions to maintain the highest standards of quality and safeguard consumer health.

- Define corrective measures

  Maintaining food safety within critical limits can be challenging, even with continuous monitoring. Therefore, HACCP food safety plans necessitate the establishment of effective corrective measures to be implemented when a process exceeds the critical limits. These corrective measures are tailored to address the identified food hazards and aim to either immediately eliminate the hazard or prevent its recurrence in the future. Here are a few examples of corrective measures:

- Discarding overcooked food: If a cooking process exceeds the specified critical limit, the corrective measure would involve promptly discarding the overcooked food to prevent potential safety risks.

- Changing supplies: If ingredients such as spices, sauces, or other food items are found to be stale or expired, the corrective measure would involve replacing them with fresh supplies to ensure the safety and quality of the final product.
- Training and reprimanding employees: In cases where employees fail to adhere to hygiene guidelines or proper food handling practices, the corrective measure may involve providing additional training to enhance their understanding of food safety protocols. Additionally, appropriate disciplinary action or reprimands may be administered to reinforce the importance of following established guidelines.

These corrective measures are crucial in rectifying deviations from critical limits and preventing potential hazards from compromising food safety. By promptly implementing appropriate corrective actions, businesses can minimize risks, maintain compliance with food safety standards, and ensure the delivery of safe and high-quality food products to consumers.

- Set verification procedures

Verification processes play a vital role in ensuring the proper functioning of a Hazard Analysis and Critical Control Points (HACCP) system. These processes involve various activities such as auditing, inspections, feedback forms, and other assessment methods to validate the effectiveness and efficiency of the preventive measures implemented within the HACCP system.By conducting regular audits, businesses can thoroughly review the HACCP system, assess its compliance with established procedures, and identify any areas that may require improvement or adjustment. Inspections provide on-site evaluations to verify that critical control points (CCPs) are being properly monitored and controlled as intended.

Feedback forms and other feedback mechanisms enable stakeholders to provide valuable input regarding the HACCP system's performance and identify any potential areas of concern. This feedback helps in fine-tuning the system and addressing any emerging issues promptly.The verification processes within the HACCP system aim to ensure that all preventive measures are functioning correctly, minimizing the risk of hazards and ensuring food safety. By regularly verifying the HACCP system through various assessment methods, businesses can maintain a robust and effective food safety management system, providing confidence that their operations are in compliance with the necessary standards and regulations.

- **Establish documentation and record-keeping guidelines**

Even if your HACCP food safety system is operating smoothly, it is important to recognize that errors can still occur. To mitigate risks and ensure accountability, proper documentation and record-keeping guidelines must be established for all food-related processes.Documentation serves as a critical tool for capturing essential information and maintaining a clear record of activities. For instance, maintaining an employee hygiene record enables the documentation of hand washes or other hygiene practices to ensure compliance with food safety standards. Similarly, a food temperature record allows for the monitoring and recording of the temperatures at which food is served, ensuring it is within safe limits.While the process of documentation may seem tedious, it plays a crucial role in identifying issues and facilitating problem-solving when needed. By having a comprehensive record-keeping system in place, businesses can promptly identify any deviations or irregularities, investigate the causes, and take appropriate corrective actions.Documentation also assists in regulatory compliance, as it provides evidence of adherence to food safety regulations and guidelines. In the event of an audit or inspection, proper documentation demonstrates a commitment to maintaining food safety standards and can help build trust with customers, regulatory authorities, and stakeholders.Ultimately, effective documentation and record-keeping practices contribute to the overall effectiveness of your HACCP system by ensuring traceability, facilitating continuous improvement, and enabling timely response to any food safety concerns that may arise.

12.5 Guidelines for applying the principles of Hazard Analysis and Critical Control Points (HACCP):

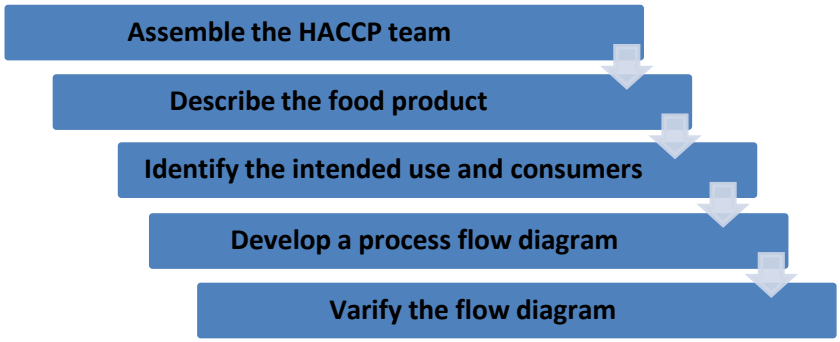
- Conduct a thorough hazard analysis: Identify and evaluate potential hazards at each stage of the food production process. Consider biological, chemical, and physical hazards that could pose risks to food safety.
- Determine critical control points (CCPs): Identify specific points in the process where control measures can be applied to prevent, eliminate, or reduce hazards to an acceptable level. Focus on critical steps that are essential for ensuring food safety.

- Establish critical limits: Define measurable criteria for each CCP, such as temperature, time, pH level, or visual inspection, to ensure the control measures are effective and the food is safe.
- Implement monitoring procedures: Develop procedures to regularly monitor the CCPs to ensure they remain within the established critical limits. Monitor and record data consistently to identify any deviations and take corrective actions promptly.
- Establish corrective actions: Define appropriate actions to be taken when monitoring reveals a deviation from the critical limits. Clearly outline the steps to bring the process back under control and prevent the distribution of unsafe food.
- Establish verification procedures: Regularly verify the effectiveness of the HACCP system through internal audits, inspections, and evaluations. Ensure that all control measures are functioning as intended and in compliance with food safety regulations.
- Maintain comprehensive record-keeping: Document all aspects of the HACCP system, including hazard analyses, CCPs, critical limits, monitoring results, corrective actions, and verification activities. Proper documentation provides evidence of adherence to food safety protocols and aids in traceability.
- Establish a review process: Regularly review and reassess the HACCP plan to ensure its continued effectiveness. Consider changes in processes, ingredients, equipment, regulations, and emerging hazards. Update the plan accordingly to maintain its relevance and efficacy.
- Provide employee training: Ensure that all personnel involved in the food production process receive appropriate training on HACCP principles, procedures, and their responsibilities. Foster a culture of food safety awareness and accountability throughout the organization.

By following these guidelines, businesses can effectively implement and maintain a robust HACCP system, leading to enhanced food safety, reduced risks, and compliance with regulatory requirements.

12.5.1Key Steps in Developing an HACCP Plan:

In the development of a HACCP plan, there are five preliminary tasks that need to be accomplished. These tasks serve as a foundation for the effective implementation of the HACCP system. The five preliminary tasks are as follows:



By completing these five preliminary tasks, businesses can lay the groundwork for the successful implementation of the HACCP plan. These tasks help in building a comprehensive understanding of the food production process, identifying potential hazards, and setting the stage for effective control measures to ensure food safety

- **Assemble the HACCP team**:Assembling the HACCP team is a crucial preliminary task in the development of a HACCP plan. The team should consist of individuals with diverse expertise and knowledge relevant to the specific food production process. team:

Identify team members	•Determine the roles and responsibilities needed for the HACCP team. This may include individuals from different departments such as production, quality assurance, food safety, engineering, and management.
Select team leader	•Designate a team leader who will oversee the development and implementation of the HACCP plan. The team leader should have strong leadership skills, knowledge of HACCP principles, and the ability to coordinate team activities.
Define team member responsibilities	•Clearly define the responsibilities of each team member based on their areas of expertise. identification, monitoringprocedures, record-keeping, and verification activities
Ensure training and competency	•Ensure that team members have the necessary knowledge and training in HACCP principles and relevant food safety regulations. If needed, provide additional training to team members to enhance their understanding of HACCP concepts.
Foster effective communication	•Establish clear lines of communication within the team. Encourage open dialogue, collaboration, and the sharing of information and ideas. Regular team meetings should be scheduled to discuss progress, address challenges, and make decisions collectively**.**
Encourage cross-functional collaboration	•Promote collaboration among team members from different departments or areas of expertise. This interdisciplinary approach ensures a comprehensive understanding of the food production process and facilitates the identification of potential hazards and control measures.
Provide resources and support	•Ensure that the HACCP team has access to the necessary resources, including relevant documents, data, and equipment. Provide ongoing support to the team, addressing any obstacles or challenges they may encounter during the HACCP plan development and implementation.

Fig: 2 Key steps involved in assembling the HACCP

By assembling a knowledgeable and dedicated HACCP team, businesses can leverage the collective expertise and ensure effective implementation of the HACCP plan. The team's collaborative efforts will lead to the development of a robust and tailored food safety system that addresses potential hazards and ensures the production of safe and high-quality food products.

- **Describe the food product**: Once the HACCP team is assembled, their first task is to provide a comprehensive description of the food product. This entails capturing essential details regarding the food's composition, ingredients, and processing methods. The team should compile a detailed description that includes relevant safety information to ensure effective hazard analysis and control.

  - **General Description**: Begin by providing a general overview of the food product, including its name, category, and intended use. This description should outline the purpose and context of the food item within the market or industry.
  - **Ingredients**: List all the ingredients used in the food product, including both primary components and any additives or preservatives. Specify the source, quality, and potential allergenic properties of each ingredient, as applicable.
  - **Processing Methods**: Describe the specific techniques and processes employed during the production of the food product. This includes steps such as cooking, heating, cooling, mixing, blending, or any other relevant procedures. Highlight any critical steps or parameters that may impact the safety or quality of the final product.
  - **Composition and Physical/Chemical Structure**: Provide detailed information about the composition and physical/chemical characteristics of the food product. This may include nutritional composition, pH levels, moisture content, texture, and other relevant properties that influence food safety and quality.
  - **Microbial/Static Treatments**: Document any treatments applied to the food product to control microbial growth or ensure product stability. This may involve heat treatment, freezing, brining, smoking, or other preservation

methods. Include specific parameters, such as time, temperature, and pressure, used during these treatments.

- **Packaging:** Specify the type of packaging materials used to store and transport the food product. This includes information about packaging integrity, barrier properties, and any additional features such as modified atmosphere packaging (MAP) or vacuum sealing.
- **Durability and Storage Conditions**: Outline the expected shelf life or durability of the food product under recommended storage conditions. Provide guidance on temperature requirements, humidity levels, light exposure, and any other factors critical to maintaining product safety and quality.
- **Distribution Method**: Describe the intended method of distribution for the food product. Specify whether it will be distributed frozen, refrigerated, or at ambient temperature. Consider any specific handling or transportation requirements to ensure the preservation of food safety during distribution.

By creating a comprehensive description of the food product, the HACCP team establishes a clear understanding of its characteristics, processing methods, and potential safety risks. This information serves as a foundation for conducting an effective hazard analysis and implementing appropriate control measures throughout the food production process.

- **Identify the intended use and consumers:** As part of developing a HACCP plan, it is important to clearly identify the intended use of the food product and the target consumers. This information helps in assessing potential hazards and implementing appropriate control measures to ensure food safety. The key aspects to be consider:
  - ✓ **Intended Use:** Describe the purpose or intended use of the food product. This includes whether it is meant to be consumed directly by individuals, used as an ingredient in other food products, or utilized in a specific culinary application. Understanding the intended use provides insights into the potential risks associated with the product.
  - ✓ **Target Consumers:** Identify the specific group of consumers for whom the food product is intended. This may include general consumers, infants, children, elderly individuals, pregnant women, or individuals with specific dietary needs or allergies. Different consumer groups may have varying

sensitivities or susceptibilities to certain hazards, which should be taken into account during the hazard analysis.

- ✓ **Special Considerations:** If the food product is intended for specific dietary needs or preferences, such as vegetarian, vegan, gluten-free, or allergen-free, highlight these considerations. This helps in identifying potential hazards related to allergens, cross-contamination, or specific dietary restrictions.
- ✓ **Consumption Patterns:** Determine the expected consumption patterns of the food product. This includes information on the frequency of consumption, portion sizes, and potential storage practices by consumers. Understanding how consumers typically handle and consume the product allows for better assessment of potential hazards and appropriate control measures.
- ✓ **Distribution Channels:** Identify the distribution channels through which the food product reaches consumers. This may include retail stores, restaurants, food service providers, online platforms, or direct sales. Each distribution channel may have unique handling, storage, and transportation practices that can impact food safety.

By clearly defining the intended use and target consumers of the food product, the HACCP team can assess potential hazards specific to the product's consumption, handling, and distribution. This information enables the team to implement control measures tailored to ensure the safety and quality of the product for its intended consumers**.**

- **Develop a process flow diagram:** The process flow diagram for a food production process involves several key steps. It begins with the procurement of raw materials, followed by their inspection and storage. The raw materials are then prepared, mixed, and cooked/processed to create the desired food product. After cooking, the product is cooled and undergoes packaging and labeling. It is then stored before being distributed to various points of sale. The process flow diagram helps visualize the sequential steps involved in food production, ensuring a systematic approach to identifying potential hazards and implementing control measures in accordance with the HACCP system.

Development of process flow diagram provides a visual representation of the steps involved in the development and distribution of a food product. It helps in identifying

critical control points, potential hazards, and the overall flow of the process, serving as a foundation for the implementation of HACCP principles to ensure food safety

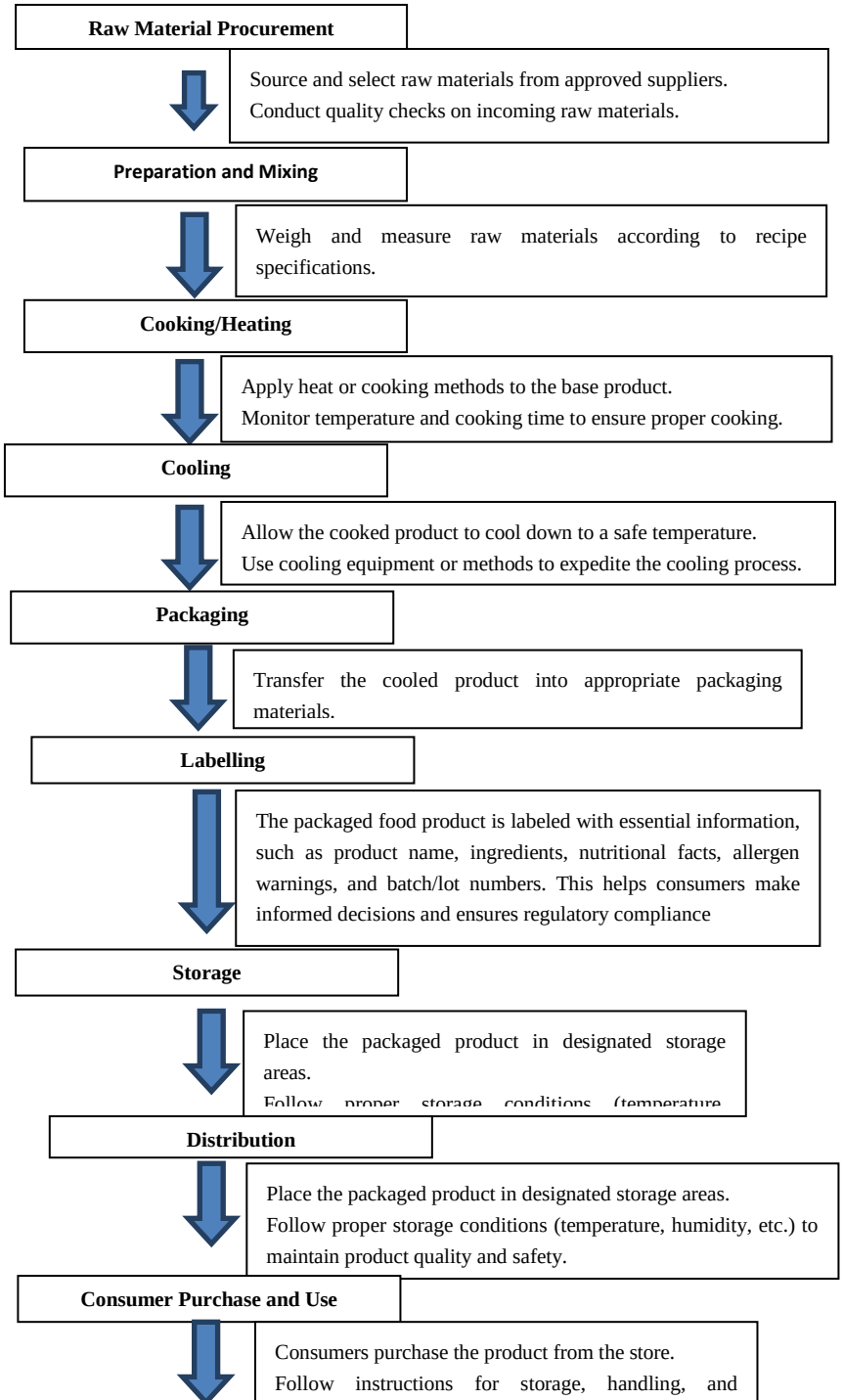


Fig:3Flow Diagram of General Process Development

- **Verify the flow diagram on spot:**To confirm the accuracy of a process flow diagram on-site, several steps can be taken. Firstly, conduct a thorough review of the actual production process, comparing it step-by-step with the depicted flow diagram. Engage with employees involved in the process to gather their insights and verify the alignment of the diagram with on-site operations. Analyze the equipment and layout to ensure they correspond to the flow diagram. Document any discrepancies or variations encountered during the review. Seek feedback from stakeholders such as supervisors and quality control personnel to validate and enhance the accuracy of the diagram. Finally, update the flow diagram based on on-site observations and stakeholder input to ensure it accurately represents the actual operations.

12.6 Discuss a case Study - Haccp in the Cheese Manufacturing Process

Based on the principle of the HACCP in this chapter, give the detailed the real situation of the cheese plant to boost the quality control system in order to produce safe and quality end products. The manufacturer used the all the seven principles of HACCP to improve the cheese product quality.

- **Perform hazard analysis to identify potential hazards (**Principles 1**)**

The hazard analysis for soft cheese manufacture is to identify different hazards in the various raw materials and steps of processing and consideration of control measures for the hazards. Raw materials are considered the main source of biological hazards and the raw milk is the most important source of pathogenic bacteria. The

average of microbiological analysis of raw materials, where the raw milk contained high load of total bacterial count 2.5x108 cfu/ml and contained 1.2x103 and 8.5x105 cfu/ml sporeforming bacteria and total fungi. Also, total and faecal coliforms were found 4.5x103 and 2.5x103 /ml, this result indicate that the production of milk lack hygienic practices. Equipments, utensils, work surfaces and food handlers are very important sources for microbial contamination of cheese product during processing steps, so swabs from containers, utensils, food handlers, tables, walls, packaging material and refrigerator were microbiologically investigated.

Table:2 Hazards in Ingredient and Incoming Material Analysis Chart

Ingredient & material	Preventative measure
Milk	Store < 4 ºC Proper transfer equipments Sanitize equipment Proper personal hygiene and handling
Starter culture	Qualified product supply, store < -40 ºC
Rennet	Qualified product supply, store < 4 ºC
Salt	Qualified product supply, store at Room temperature Proper personal hygiene and handling
Water	Supply quality water

Table:3 Microbiological examination of raw materials used in cheese manufacture (X±SD)

Microbiological tests Water	**Raw Materials**			
	Raw milk	Salt	Rennet enzyme	Water
Total bacterial count (cfu/ml or g)	2.5x108 ±2.1x108	8.7x103 ±1.5x103	8.3x106 ±1.7x106	5.2x10 ±4.1x10
Spore-forming bacteria (cfu/ml or g)	1.2x103 ±1.1x103	4.0x103 ±3.5x103	1.0x102 ±0.5x102	1.0x10 ±0.7x10

Total Fungi (cfu/ml or g)	8.5x105 ±1.5x105	ND	1.2x106 ±0.9x106	1.0x10 ±0.5x10
Total coliforms /ml or g	4.5x103 ±3.2x103	ND	2.5x102 ±1.7x102	ND
Pseudomonas aeruginosa /ml or g	ND	ND	ND	ND
Bacillus cereus (cfu/ml or g)	ND	ND	ND	ND
Staphylococcus aureus (cfu/ml or g)	ND	ND	ND	ND
Yersinia enterocolitica (cfu/ml or g)	ND	ND	ND	ND
Salmonella spp	-ve	-ve	-ve	-ve
Listeria monocytogenes	-ve	-ve	-ve	-ve
Campylobacter spp	+ve	-ve	-ve	-ve

List all the critical control points (CCPs) (Principles 2)

Determine Critical Control Points (CCPs) CCPs are points or areas in a process that are required to control the identified hazards and lack of control is likely to result in an unacceptable health hazards (FDA, 1995). CCPs in raw materials and processing line steps**(Fig 3**) of fresh cheese were determined according to CCP decision tree (Figure 4). The CCP decision tree will help to identify appropriate CCPs in the process. Using a CCP Decision Tree promotes structured thinking and ensures a consistent approach at every process step and for each hazard identified. It is a flow of three questions. All three questions focus on analyzing the hazards in the raw material and determining whether or not each hazard is a critical control point. Using the decision tree will allow the producer to identify the potential critical hazards in raw materials. For the raw materials, it is clear that the raw milk was very important CCP; on the other hand,raw milk receiving at 4 – 6ºC, pasteurization at 72ºC for 15 sec and storage of cheese product at 4 - 6ºC steps were the main CCPs in the processing line of fresh cheese. Pasteurization of milk according to standard procedure (at least 72°C for minimally 15 sec) reduces the probability of vegetative pathogens survival by a factor of 10^6. However, spores of pathogens, including those of *Clostridium botulinum* and *B. cereus*, are not eliminated by pasteurization, but disease incidences with pasteurized milk are rare. Milk that has been pasteurized correctly is, therefore, unlikely to cause disease.

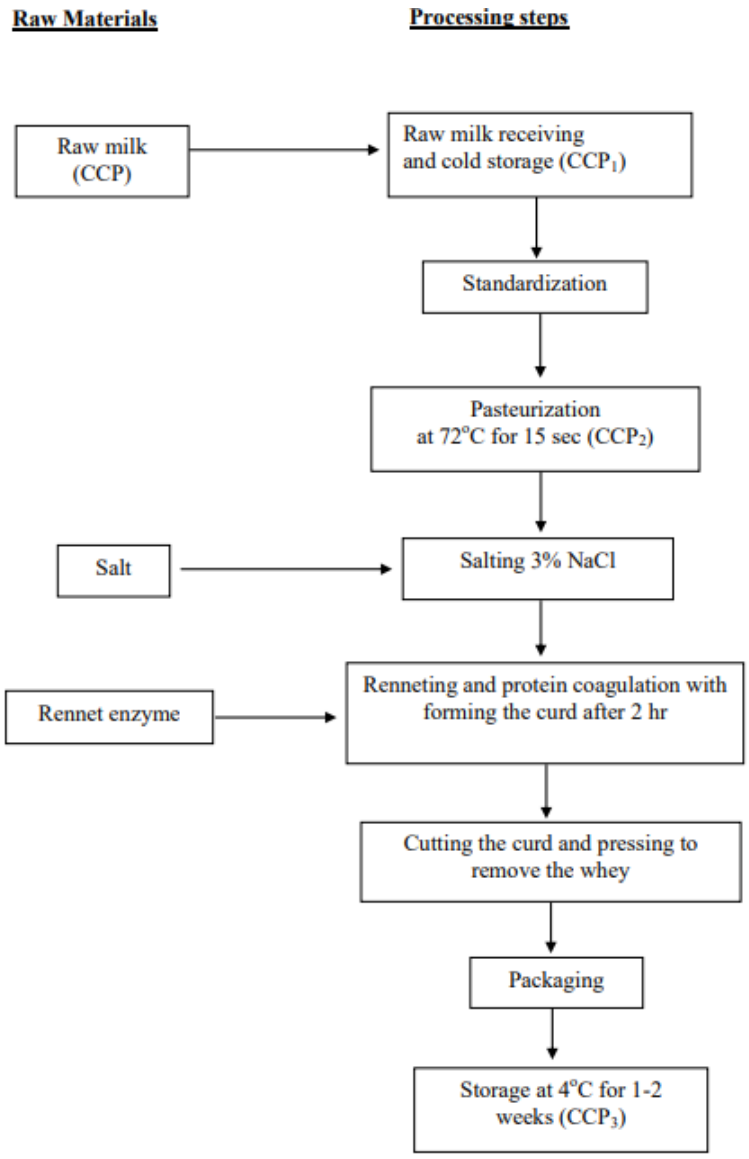


Fig:3 Flow diagram of the fresh Cheese manufacturing steps and CCPs for controlling hazards

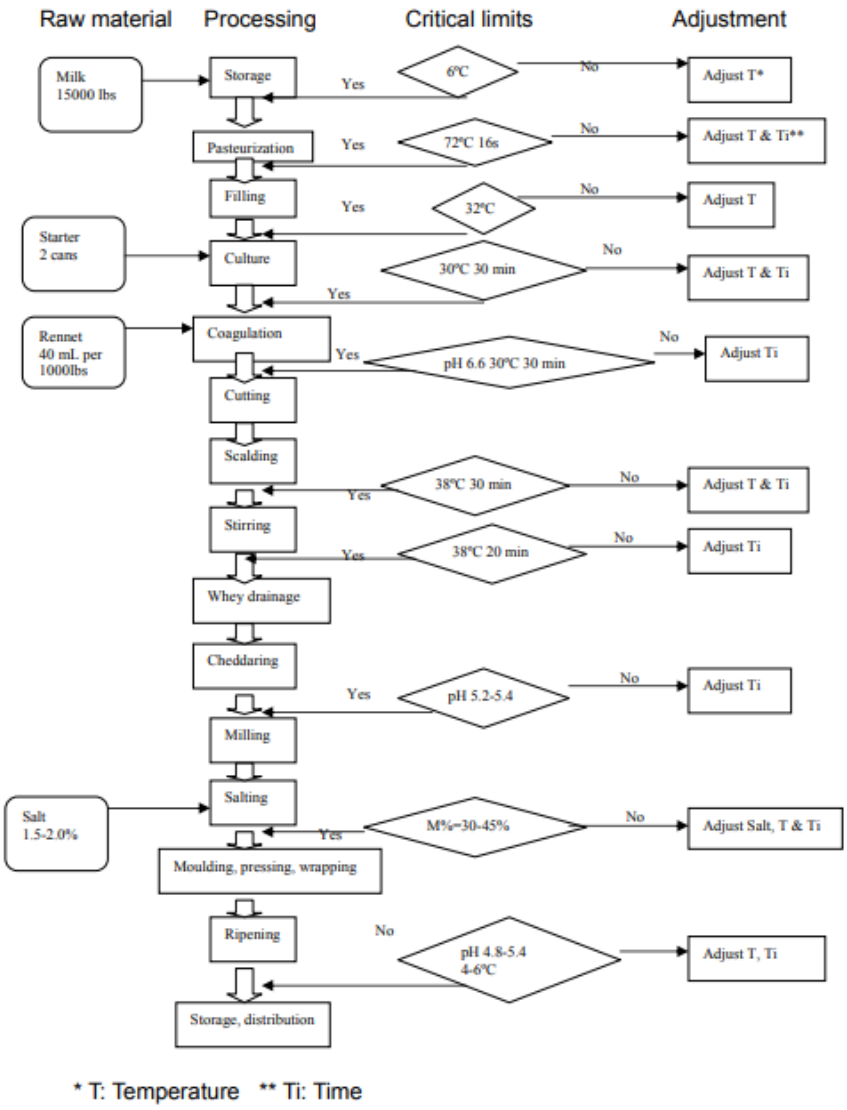


Fig 4: Detailed flow diagram is specific for the cheese production. It is made of four parts: raw material, processing, critical limits and adjustment. The reason is the producer needs to check the condition of each step during processing. If it is inside the critical limits, the process continues; otherwise the process is stop and the proper adjustment is made. The adjustment is determined based on the temperature, time and salt change. If the condition cannot be controlled the product will be reject.

(**3) Establish Critical limits (Principles 3)**: Critical limits serve as fundamental benchmarks for evaluating the efficacy of food safety protocols. These limits are crucial in minimizing or eradicating significant food safety hazards. In Figure 5, specific critical limits are outlined for each processing step. For instance, during the raw milk receiving step, the critical limit necessitates adherence to raw milk standards and receiving milk at a temperature below 6ºC. Similarly, for the pasteurization step, the critical limit requires a minimum heat temperature of 72ºC for 15 seconds. Lastly, in the storage of cheese products, the critical limit stipulates that the storage temperature must not exceed 6ºC.

(4) **Establish Monitoring procedures (Principles 4)**: Temperature monitoring should be carried out using a calibrated thermometer at each stage, particularly during critical steps like pasteurization where monitoring the duration is also crucial. Various non-continuous monitoring procedures can be employed, such as rapid platform tests for the raw milk receiving step and microbiological and chemical tests for raw materials and packaging. Advances in microbiological detection methods have the potential to enhance food safety measures and can be leveraged to strengthen food safety programs.

(**5) Establish Corrective Actions ((Principles 5)**: Corrective actions should be taken when monitoring system indicate that, any of the critical limits was out of control. Corrective actions with every step were summaries in Fig 5. Such as rejection of contaminated raw materials, correction and resetting of pasteurizer temperature and time, maintenance or repairing of separator and pasteurizer and discarding the product if contamination was evident.

(6) Record keeping: Documentation is needed to record measurements that show standards are being monitored. Effective HACCP record-keeping plan contains; listing of the HACCP team and responsibilities, description of the food and its intend use, list all regulations that must be met, temperature monitoring logs, flow chart from receiving to consumption and corrective actions. Accurate record keeping is essential part of successful HACCP plan.

(7) Verification of HACCP system working (Principles 6): Verification of HACCP system includes routine calibration of CCPs monitoring system, testing of finished product and random collection of raw materials and end product, and then testing them chemically and microbiologically. The overall HACCP process must be verified periodically to be sure that, the HACCP system is effective and work well. The microbiological analysis during processing steps of cheese after HACCP application, the result indicate that, *Pseudomonas*

aeruginosa, *Bacillus cereus*, *Yersinia enterocolitica*, *Salmonella spp* and *Listeria monocytogenes* were not detected at all, while, fungi, total and faecal coliform and C*ampylobacter* were not detected after pasteurization step and in the final cheese product which become acceptable after HACCP application Fig 5. The most important motive for implementing HACCP system for dairy producers was to increase and improve safety of their products. Secondly most important incentive was the quality increase of their products and customer confidence. Although it was logical to assume that HACCP is a system that minimizes testing by focusing on critical control points. Cleaning and sanitation control of health and hygiene of employees, equipment maintenance and calibration, pest, water and temperature control together with traceability were very important for implementing HACCP system in dairy plants.

Process step	Hazards	Preventative measure	Critical limits	Monitoring procedure	Monitoring frequency	Corrective action	Responsibility
Raw & packaging material CCP # 1	Microbiological chemical & physical contamination	Qualified starter & rennet supply Qualified cryvoac supply	No unqualified material be used	Apply supply quality assurance	Each supply	Change supplier Operator training	
Pasteurization CCP #2	Survival of pathogens such asE.coli, *Staphylococcus aureus, Bacillus cereus, etc.*	Pasteurizer checks: -check the heat plate -check the temperature controller -check the flow diversion	Temperature set at 72ºC Time set at 16 sec	Check thermometer and time check equipment is properly running Supervisor managing and record keeping	Each batch Routinely Each batch	Adjust the temperature And time by setting the equipment well Call the engineer to repair	
Filling CCP #3	Microbiological contamination	Proper temperature setting	Temperature set at 32ºC	Check thermometer Record keeping	Each batch Each batch	Adjust the heater to change temperature	
Adding starter & rennet CCP #4	Microbiological contamination Physical contamination	Proper additional rate Agitate properly	Starter: 2cans, Rennet: 40 mL per 1000 lbs milk pH is measured at 6.6 before adding rennet Agitator set at medium	Check the additional rate of the starter and rennet & pH check the rate of the agitator Record keeping	Each batch Each batch	Applying more testing on pH Use active starter culture Adjust agitate rate Operator training	
Coagulation CCP #5	Microbiological contamination Physical contamination	Proper time setting and recording Take the stirring tools out of the tank	Time is set at 30min Tools prevent coagulation	Check the time and the stirring tools Record keeping	Each batch Each batch	Reject product Operator training	
Cutting, scalding & stirring CCP #6	Microbiological contamination	Proper time & temperature setting	Temperature is set at 38ºC, scalding for 30min and stirring for 20min	Check the temperature and the time Record keeping	Each batch Each batch	Adjust the heater to change temperature Operator training	
Milling CCP #7	Microbiological contamination	More cheddaring time control the pH Use of an active starter culture at the correct addition	pH is measured at 5.2-5.4	Consistently monitor pH during cheddaring Supervisor's managing and record keeping	Each batch	Reject product Applying more testing on pH Operator training	
Salting CCP #8	Microbiological contamination	Correct level of salt Correct mixing	Salt%=1.5-2.0%	Records and testing	Each batch	Incorrectly salted curd must not be	

Fig 5: The HACCP control chart shows all the potential critical hazards that can occur during processing in this small scale cheese plant. It is the most essential part of the whole HACCP plan, which is the organization analysis and documentation of the CCPs

12.7 Emphasizing the importance of implementing HACCP for ensuring food safety and protecting public health in India.

Implementing HACCP is of paramount importance when it comes to ensuring food safety and safeguarding public health. In a world where foodborne illnesses and contamination pose significant risks, a reactive approach to food safety is no longer sufficient. HACCP provides a proactive and preventive framework that identifies potential hazards, establishes critical control points, and implements effective control measures to mitigate those hazards.By implementing HACCP, we shift our focus from mere inspection and testing of end products to a comprehensive system that encompasses the entire food production process. This proactive approach allows us to identify and address potential hazards at critical stages, from sourcing raw materials to processing, packaging, and distribution. As a result, we can prevent the occurrence of foodborne illnesses and contamination before they pose a threat to consumers.

Furthermore, HACCP not only enhances food safety but also proves to be cost-effective in the long run. By implementing preventive measures and addressing potential hazards proactively, we can reduce the occurrence of costly recalls, product wastage, and damage to brand reputation. This approach saves both time and resources while ensuring the production of safe and high-quality food. In addition to protecting public health, implementing HACCP also helps us fulfill our international obligations. Agreements such as the Sanitary and Phytosanitary Measures (SPS) and Technical Barriers to Trade (TBT) require us to adopt stringent food safety standards. By implementing HACCP, we

demonstrate our commitment to ensuring food safety and meeting these international obligations, thereby enhancing trade opportunities and promoting consumer confidence in our products.

The Food Safety and Standards Authority of India (FSSAI) is the regulatory body responsible for ensuring food safety and quality in the country. FSSAI has implemented the Food Safety and Standards (Food Safety Management Systems) Regulations, 2020, which incorporate the HACCP system as a mandatory requirement for certain food businesses. The regulations outline the requirements for implementing and maintaining a food safety management system based on HACCP principles. Many food businesses in India, especially larger establishments, have adopted and implemented HACCP as a means to ensure food safety and comply with regulatory requirements. HACCP is particularly important in industries such as dairy, meat processing, seafood, and packaged food manufacturing, where potential hazards need to be controlled to safeguard public health.

It's worth noting that food safety regulations and their implementation may evolve over time, so it's recommended to consult the latest information from the FSSAI or other relevant authorities in India for the most up-to-date status of HACCP implementation in the country.

In conclusion, the implementation of HACCP is vital for ensuring food safety, protecting public health, and meeting international standards. By adopting a preventive and proactive approach, we can effectively identify and control hazards, reduce the risk of foodborne illnesses, and ensure the production of safe and high-quality food. The benefits of HACCP extend beyond consumer protection, offering cost savings, improved brand reputation, and increased trade opportunities. As we navigate an increasingly complex food landscape, HACCP stands as a crucial tool in safeguarding public health and ensuring the safety of our food supply.

Summary: This module introduced us to HACCP, which stands for Hazard Analysis Critical Control Point. We acquired knowledge about HACCP, which provides a proactive and cost-effective approach to ensuring food safety. It is now widely recognized that relying solely on end product inspection, testing, or even 100 percent inspection does not guarantee food safety. These methods have limitations as they may overlook potential hazards present in raw materials or food products. Moreover, with the

increasing presence of contaminants in the environment and the food chain, consumer concerns regarding the safety of the food they consume have grown. In this context, HACCP emerges as a solution that instills greater confidence in the food chain by establishing a preventive food safety assurance system. Implementing HACCP is crucial for us as a nation due to our international obligations, such as the Agreements on Sanitary and Phytosanitary Measures (SPS) and Technical Barriers to Trade (TBT). Apart from meeting these obligations, the unit emphasized several benefits associated with the HACCP system. It effectively overcomes the limitations of traditional approaches to food safety control, which mainly rely on intermittent inspection and end product testing. Furthermore, HACCP offers numerous advantages for consumers, industry, and government stakeholders alike.

Terms used in the context of HACCP:

Control: a) To manage the conditions of an operation to maintain compliance with established criteria.

b) The state where correct procedures are being followed and criteria are being met.

Control Measure: Any action or activity that can be used to prevent, eliminate or reduce a significant hazard.

Control Point: Any step at which biological, chemical or physical factors can be controlled.

Corrective Action: Procedures followed when a deviation occurs.

Criterion: A requirement on which a judgment or decision can be based.

Critical Control Point: A step at which control can be applied and is essential to prevent or eliminate a food safety, hazard or reduce it to an acceptable level.

Critical Limit: A maximum and/or minimum value to which a biological, chemical or physical parameter must be controlled at a CCP to prevent, eliminate or reduce to an acceptable level the occurrence of a food safety hazard.

CCP Decision Tree: A sequence of questions to assist in determining whether a control point is a CCP.

Deviation: Failure to meet a critical limit.

HACCP: A systematic approach to the identification, evaluation and control of food safety hazards.

HACCP Plan: The written document which is based upon the principles of HACCP and which delineates the procedures to be followed.

HACCP System: The result of the implementation of the HACCP Plan.

HACCP Team: The group of people who are responsible for developing, implementing and maintaining the HACCP system.

Hazard: A biological, chemical or physical agent that is reasonably likely to cause illness or injury in the absence of its control.

Hazard Analysis: The process of collecting and evaluating information on hazards associated with the food under consideration to decide which are significant and must be addressed in the HACCP plan.

Monitor: To conduct a planned sequence of observations or measurements to assess whether a CCP is under control and to produce an accurate record for future use in verification.

Prerequisite Programs: Procedures, including good manufacturing practices that address operational conditions providing the foundation for the HACCP system.

Severity: The seriousness of the effect(s) of a hazard. Step: A point, procedure, operation or stage in the food system from primary production to final consumption.

Validation: That element of verification focused on collecting and evaluating scientific and technical information to determine if the HACCP plan, when properly implemented, will effectively control the hazards.

Verification: Those activities, other than monitoring, that determine the validity of the HACCP plan and that the system is operating according to the plan

Check your Knowledge

What do you understand by the term 'HACCP'? Enlist the benefits of

HACCP:

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2) Explain why HACCP is a better and effective method over traditional food safety assurance programmes?

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3) Why there is a need for HACCP in maintaining international standards in food trade?

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List thethree common hazards encountered in liquid milk process.

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3) What are the critical control points and the critical limits in the liquid milk process?

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4) Why should we establish a monitoring system in a HACCP plan?

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