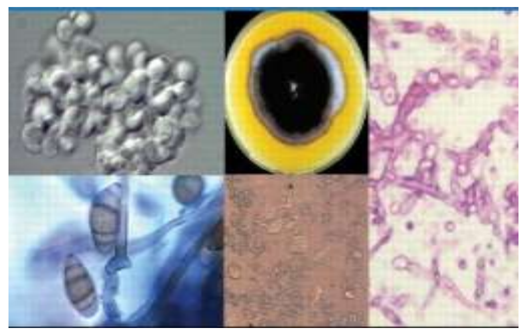
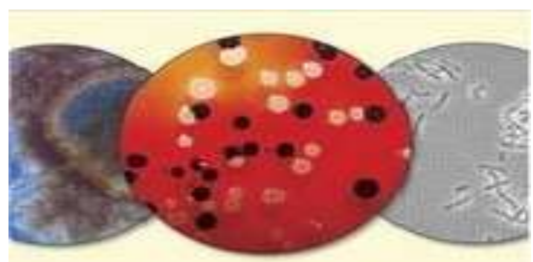
Kingdom of Saudi Arabia
Ministry of Education
Northern Border University
Faculty of Science and Arts
Department of Biology
(Microbiology)

FOOD MICROBIOLOGY COURSE (3303-425)





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1. Introduction to Food Microbiology

❖ Glossary of terms

No.	Term	Meaning (Arabic)
1	Food microbiology	ميكروبيولوجيا الأغذية
2	Fresh foods	أغذية طازجة
3	Frozen foods	أغذية مُجمدة
4	Canned foods	أغذية مُعلبة
5	Food contamination	تلوث الغذاء
6	Food spoilage	فساد الأغذية
7	Physical food spoilage	الفساد الفيزيقي (الطبيعي) للغذاء
8	Chemical food spoilage	الفساد الكيميائي للغذاء
9	Microbial food spoilage	الفساد الميكروبي للغذاء
10	Microbial toxins	السموم الميكروبية
11	Food poisoning	تسمم غذائي
12	Foodborne illnesses	الأمراض المنقولة بالغذاء
13	Toxic food poisoning	التسمم الغذائي بالتوكسين
14	Infectious food poisoning	التسمم الغذائي بالعدوى
15	Staphylococcal intoxication	التسمم بالمكورات العنقوية
16	Botulism	التسمم البوتشوليني
17	Mycotoxins	الميكوتوكسينات (السموم الفطرية)
18	Mycotoxicosis	التسمم الميكوتوكسيني
19	Food protection	حماية الغذاء
20	Food preservation	حفظ الغذاء
21	Pasteurization	البسترة
22	Probiotics	المُتممات الغذايئة (كائنات حية)
23	Prebiotics	المكونات التغذوية (المُغذية) للبروبيوتك

❖ What is Food Microbiology?

Food Microbiology: is the study of the microorganisms that inhabit (create or contaminate) food.

Or

Study of microorganisms causing food spoilage (pathogens that may cause disease especially if food is improperly cooked or stored) and microorganisms used to create some foods such as cheese, yogurt, bread, beer and wine.

Unfortunately, microorganisms also can be detrimental. They are the cause of many diseases in humans, animals, and plants. Disease-causing microorganisms are called pathogens. A pathogen, or the substances it produces, must invade the human, animal, or plant body to cause illness.

While many diseases are transmissible from person to person or from animals to humans, only a few are transmitted through foods. Diseases that are caused by eating food are usually referred to as food poisoning or foodborne illnesses.

Except for a few sterile foods, all foods harbor one or more types of microorganisms. Some of them have desirable roles in food, such as in the production of naturally fermented food, whereas others cause food spoilage and foodborne diseases.

❖ History and Development of Food Microbiology

As a discipline, Food Microbiology does not have a precise beginning. Actually the meaning of food Microbiology always growth time by the time. Events which stretched over several centuries ultimately led to the recognition of the significance and role of microorganisms in foods.

Food borne disease and food spoilage have been part of the human experience since the dawn of our race. Although the actual cause of these problems is due to thousands of years.

A brief history about the food microbiology is discussed here:

(A) Food Fermentation

1822 - **C.J. Person** named the microscopic organism found on the surface of wine during vinegar production as *Mycoderma mesentericum.*

1837 - **Theodor Schwann** named the organism involved in sugar fermentation as *Saccharomyces* (sugar fungus).

1838 - **Charles Cogniard-Latour** suggested that growth of yeasts was associated with alcohol fermentation.

1860 - **Louis Pasteur** showed that fermentation of lactic acid and alcohol from sugar was the result of growth of specific bacteria and yeasts, respectively.

1883 - **Emil Christian Hansen** used pure cultures of yeasts to ferment beer.

(B) Food Spoilage

1804 - **Francois Nicolas Appert** developed methods to preserve foods in sealed glass bottles by heat in boiling water. He credited this process to Lazzaro Spallanzani (1765), who first used the method to disprove the spontaneous generation theory.

1819 - **Peter Durand** developed canning preservation of foods in steel cans. Charles Mitchell introduced tin lining of metal cans in 1839.

1870 - **L. Pasteur** recommended heating of wine at 145rF (62.7˚C) for 30 min to destroy souring bacteria. F. Soxhlet advanced boiling of milk for 35 min to kill contaminated bacteria. Later, this method was modified and named pasteurization, and used to kill mainly vegetative pathogens and many spoilage bacteria.

1895 - **Harry Russell** showed that gaseous swelling with bad odors in canned peas was due to growth of heat-resistant bacteria (spores).

(C) Foodborne Diseases

1820 - **Justin Kerner** described food poisoning from eating blood sausage (due to botulism).

1849 - **John Snow** suggested the spread of cholera through drinking water contaminated with sewage. In 1854, Filippo Facini named the cholera bacilli as *Vibrio cholera*.

1856 - **William Budd** suggested that water contamination with feces from infected person spread typhoid fever and advocated the use of chlorine in water supply to overcome the problem.

1885 - **Theodor Escherich** isolated *Bacterium coli* (later named *Escherichia coli*) from the feces and suggested that some strains were associated with infant diarrhea.

1888 - **A.A. Gartner** isolated *Bacterium* (later *Salmonella*) *enteritidis* from the organs of a diseased man as well as from the meat the man ate. In 1896, Marie von Ermengem proved that *Salmonella enteritidis* caused a fatal disease in humans who consumed contaminated sausage.

1894 - **J. Denys** associated pyogenic *Staphylococcus* with death of a person who ate meat prepared from a diseased cow.

❖ What is the importance of food microbiology?

Studying of food microbiology is important to know:

1. Food borne diseases of microbial origin
2. Microbial food spoilage
3. Beneficial uses of microbes in food
4. Control of microbial growth in foods
5. Destruction of microbes in foods
6. Probiotic bacteria
7. Microbial food fermentation
8. Regulatory aspects to ensure consumers related to Microbial hazards in food.

2. Important microbes in food and their characteristics

The microbial groups important in foods consist of several species and types of bacteria, yeasts, molds, and viruses. Although some algae and protozoa as well as some worms (such as nematodes) are also important in foods, they are not included among the microbial groups in this course.

Bacteria, molds, and viruses are important in food for their ability to cause foodborne diseases and food spoilage and to produce food and food ingredients. Many bacterial species and some molds and viruses, but not yeasts, are able to cause foodborne diseases.

Most bacteria, molds, and yeasts, because of their ability to grow in foods (viruses cannot grow in foods), can potentially cause food spoilage.

Among the four major groups, bacteria constitute the largest group and considered the most important in food spoilage and foodborne diseases, because:

(1) Their ubiquitous presence.

(2) Rapid growth rate.

(3) Even under conditions where yeasts and molds cannot grow.

Prion or proteinaceous infectious particles have recently been identified to cause transmissible spongiform encephalopathies (TSEs) in humans and animals. However, their ability to cause foodborne diseases is not clearly understood.

❖ Important Microorganisms in Food

A. Important Mold Genera

Molds are important in food because they can grow even in conditions in which many bacteria cannot grow, such as:

(1) Low pH.

For example: Acidic food products are more contaminated with molds than bacteria.

(2) Low water activity (A_w).

For example: Dried food products are more contaminated with molds than bacteria.

(3) High osmotic pressure.

For example: Highly salted or sugary food products are more contaminated with molds than bacteria.

Many types of molds are found in foods. They are important spoilage microorganisms. Many strains also produce mycotoxins and have been implicated in foodborne intoxication. Many are used in food bioprocessing. Finally, many are used to produce food additives and enzymes.

Some of the most common genera of molds found in food are listed below:

(1) *Aspergillus*

It is widely distributed and contains many species important in food. Members have septate hyphae and produce black-colored asexual spores on conidia.

Many are xerophilic (able to grow in low A_w) and can grow in grains, causing spoilage. They are also involved in spoilage of foods such as: jams, fruits and vegetables (rot). Some species or strains produce mycotoxins (e.g., *Aspergillus flavus* produces aflatoxin).

Aspergillus niger is used to process citric acid from sucrose and to produce enzymes such as b-galactosidase.

(2) *Alternaria*

Members are septate and form dark-colored spores on conidia. They cause rot in tomatoes and rancid flavor in dairy products. Some species or strains produce mycotoxins. Species: *Alternaria tenuis*.

(3) *Fusarium*

Many types are associated with rot in citrus fruits, potatoes, and grains. Species: *Fusarium solani*.

(4) *Mucor*

It is widely distributed. Members have nonseptate hyphae and produce sporangiophores. They produce cottony colonies. Some species are used in food fermentation and as a source of enzymes. They cause spoilage of vegetables. Species: *Mucor rouxii*.

(5) *Penicillium*

It is widely distributed and contains many species. Members have septate hyphae and form conidiophores on a blue-green, brushlike conidia head. Some species are used in food production, such as *Penicillium roquefortii* and *Penicillium camembertii* in cheese.

Many species cause fungal rot in fruits and vegetables. They also cause spoilage of grains, breads, and meat. Some strains produce mycotoxins (e.g., Ochratoxin A).

(6) *Rhizopus*

Hyphae are aseptate and form sporangiophores in sporangium. They cause spoilage of many fruits and vegetables. *Rhizopus stolonifer* is the common black bread mold.

B. Important Yeasts Genera

Yeasts are important in food because of their ability to cause spoilage. Many are also used in food bioprocessing. Some are used to produce food additives.

Several important genera are briefly described next:

(1) *Saccharomyces*

Cells are round, oval, or elongated. It is the most important genus and contains heterogenous groups.

Saccharomyces cerevisiae are used in baking for leavening bread and in alcoholic fermentation. They also cause spoilage of food, producing alcohol and CO_2.

(2) *Pichia*

Cells are oval to cylindrical and form pellicles in beer, wine, and brine to cause spoilage. Some are also used in oriental food fermentation. Species: *Pichia membranaefaciens*.

(3) *Candida*

Many species spoil foods with high acid, salt, and sugar and form pellicles on the surface of liquids. Some can cause rancidity in butter and dairy products (e.g., *Candida lipolyticum*).

C. Important Viruses

Viruses are important in food because some of them are able to cause enteric disease but not food spoilage because they cannot grow in foods, and thus, if present in a food, can cause foodborne diseases.

Hepatitis A viruses have been implicated in foodborne infections. Several other enteric viruses, such as poliovirus, echo virus, and Coxsackie virus, can cause foodborne diseases. In some countries where the level of sanitation is not very high, they can contaminate foods and cause disease.

Some bacterial viruses (bacteriophages) are used to identify some pathogens (*Salmonella* spp., *Staphylococcus aureus* strains) on the basis of the sensitivity of the cells to a series of bacteriophages. Bacteriophages are used to transfer genetic traits in some bacterial species or strains by a process called transduction (e.g., in *Escherichia coli* or *Lactococcus lactis*).

Finally, some bacteriophages can be very important because they can cause fermentation failure. Many lactic acid bacteria, used as starter cultures in food fermentation, are sensitive to different bacteriophages. They can infect and destroy starter-culture bacteria, causing product failure.

D. Important Parasites Genera

There are several intestinal and tissue helminths (roundworms, flatworms, and tapeworms) and protozoa are known to cause human illness when present in food.

(1) *Trichinella*

Trichinella is a roundworm and can be present in high frequencies in pigs feeding on garbage. A person gets infected by consuming raw or insufficiently cooked meat of infected animals. The infected meat contains encysted larvae. Once the meat is consumed, the cysts dissolve in the GI tract, releasing the larvae. Species: *Trichinella spiralis.*

(2) *Anisakis*

The nematode *Anisakiasis* is a parasite in many fish, particularly of marine origin. Human infection results from the consumption of raw infected fish. Inadequately cooked, brined, or smoked fish has also been implicated in some cases. Species: *Anisakiasis* simplex.

(3) *Taenia*

Taeniasis is a tapeworm disease caused by *Taenia saginata* from beef. The illness results from the ingestion of raw or improperly cooked meat contaminated with larvae of the tapeworms. Species: *Taenia saginata*

(4) *Toxoplasma*

Toxoplasmosis is a tissue protozoan disease transmitted to humans from the consumption of undercooked and raw meat and raw milk contaminated with oocysts of *Toxoplasma.* Contamination of food with feces, especially from cats, can also be involved. In many people, it does not cause any problems. Species: *Toxoplasma gondii.*

(5) *Giardia*

Giardiasis is produced by the intestinal protozan *Giardia.* Giardiasis has been considered as food- and waterborne disease in many parts of the world. The incidence is much higher in countries with inadequate sanitation facilities and improper water supplies.

Contaminated raw vegetables; foods such as salads and sandwiches, contaminated with water containing the causative agent; and poor personal hygiene are considered the major causes of the disease. Species: *Giardia lamblia.*

E. Important Bacterial Genera

Among the microorganisms found in foods, bacteria constitute major important groups. This is not only because many different species can be present in foods but also because of their:

- Rapid growth rate.
- Ability to utilize food nutrients.
- Ability to grow under a wide range of temperatures, aerobiosis, pH, and water activity.
- Better survive adverse situations, such as survival of spores at high temperature.

For convenience, bacteria important in foods have been arbitrarily divided into several groups on the basis of similarities in certain characteristics. This grouping does not have any taxonomic significance. Some of these groups and their importance in foods are listed here.

1. Lactic Acid Bacteria

They are bacteria that produce relatively large quantities of lactic acid from carbohydrates. Species mainly from genera *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus thermophilus* are included in this group.

2. Acetic Acid Bacteria

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

3. Propionic Acid Bacteria

They are bacteria that produce propionic acid and are used in dairy fermentation. Species such as *Propionibacterium freudenreichii* are included in this group.

4. Butyric Acid Bacteria

They are bacteria that produce butyric acid in relatively large amounts. Some *Clostridium* spp. such as *Clostridium butyricum* are included in this group.

5. Proteolytic Bacteria

They are bacteria that can hydrolyze proteins because they produce extracellular proteinases. Species in genera *Micrococcus, Staphylococcus, Bacillus, Clostridium, Pseudomonas, Alteromonas, Flavobacterium, Alcaligenes,* some in Enterobacteriaceae, and *Brevibacterium* are included in this group.

6. Lipolytic Bacteria

They are bacteria that are able to hydrolyze triglycerides because they produce extracellular lipases. Species in genera *Micrococcus, Staphylococcus, Pseudomonas,* *Alteromonas*, and *Flavobacterium* are included in this group.

7. Saccharolytic Bacteria

They are bacteria that are able to hydrolyze complex carbohydrates. Species in genera *Bacillus*, *Clostridium*, *Aeromonas*, *Pseudomonas*, and *Enterobacter* are included in this group.

8. Thermophilic Bacteria

They are bacteria that are able to grow at 50rC and above. Species from genera *Bacillus*, *Clostridium*, *Pediococcus*, *Streptococcus*, and *Lactobacillus* are included in this group.

9. Psychrotrophic Bacteria

They are bacteria that are able to grow at refrigerated temperature (£5˚C). Some species from *Pseudomonas*, *Alteromonas*, *Alcaligenes*, *Flavobacterium*, *Serratia*, *Bacillus*, *Clostridium*, *Lactobacillus*, *Leuconostoc*, *Carnobacterium*, *Brochothrix*, *Listeria*, *Yersinia*, and *Aeromonas* are included in this group.

10. Thermoduric Bacteria

They are bacteria that are able to survive pasteurization temperature treatment. Some species from *Micrococcus*, *Enterococcus*, *Lactobacillus*, *Pediococcus*, *Bacillus* (spores), and *Clostridium* (spores) are included in this group.

11. Halotolerant Bacteria

They are bacteria that are able to survive in food products of high salt concentrations (10%). Some species from *Staphylococcus*, *Bacillus*, *Micrococcus*, *Pediococcus*, *Vibrio*, and *Corynebacterium* are included in this group.

12. Aciduric Bacteria

They are bacteria that are able to survive at low pH (<4.0). Some species from *Lactobacillus*, *Pediococcus*, *Lactococcus*, *Enterococcus*, and *Streptococcus* are included in this group.

13. Osmophilic Bacteria

They are bacteria that can grow at a relatively higher osmotic environment than that needed for other bacteria. Some species from genera *Staphylococcus*, *Leuconostoc*, and *Lactobacillus* are included in this group. They are much less osmophilic than yeasts and molds.

14. Gas-Producing Bacteria

They are bacteria that produce gas (CO_2, H_2, H_2S) during metabolism of nutrients. Species from genera *Leuconostoc*, *Lactobacillus*, *Propionibacterium*, *Escherichia*, *Enterobacter*, *Clostridium,* and *Desulfotomaculum* are included in this group.

15. Slime Producers

They are bacteria that produce slime because they synthesise polysaccharides. Some species or strains from *Xanthomonas, Leuconostoc*, *Alcaligenes*, *Enterobacter*, *Lactococcus*, and *Lactobacillus* are included in this group.

16. Spore Formers

They are bacteria having the ability to produce spores. Species from *Bacillus*, *Clostridium*, and D*esulfotomaculum* are included in this group. They are further divided into aerobic sporeformers, anaerobic sporeformers, flat sour sporeformers, thermophilic sporeformers, and sulfide-producing sporeformers.

17. Aerobes

They are bacteria that require oxygen for growth and multiplication. Species from *Pseudomonas*, *Bacillus*, and *Flavobacterium* are included in this group.

18. Anaerobes

They are bacteria that cannot grow in the presence of oxygen. Species from *Clostridium* are included in this group.

19. Facultative Anaerobes

They are bacteria that are able to grow in both the presence and absence of oxygen. *Lactobacillus*, *Pediococcus*, *Leuconostoc*, enteric pathogens, and some species of *Bacillus*, *Serratia*, and coliforms are included in this group.

20. Coliforms

Species from *Escherichia*, *Enterobacter*, *Citrobacter*, and *Klebsiella* are included in this group. They are used as an index of sanitation.

21. Fecal Coliforms

Mainly *Escherichia coli* is included in this group. They are also used as an index of sanitation.

22. Enteric Pathogens

Pathogenic *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Escherichia*, *Vibrio*, *Listeria,* and others that can cause gastrointestinal infection are included in this group.

3. Sources of Food Contamination

The internal tissues of healthy plants (fruits and vegetables) and animals (meats and animal products) are essentially sterile. Yet raw and processed (except sterile) foods contain different types of molds, yeasts, bacteria, and viruses.

Microorganisms get into foods from both internal and external sources, from the time of production until the time of consumption. Natural sources for foods of plant origin include the surfaces of fruits, vegetables, and grains, and the pores in some tubers (e.g., radish and onion). Natural sources for foods of animal origin include skin, hair, feathers, gastrointestinal tract, urinogenital tract, respiratory tract, and milk ducts (teat canal) in udders of milk animals.

An understanding of the sources of microorganisms in food is important to:

1. To control access of the microorganisms in the food.
2. To kill them in food.
3. Determination the microbiological quality of food.
4. Set up microbiological specifications of foods and food ingredients.

❖ Predominant Microorganisms in Foods of Different Sources

(A) Foods of plant origin (Fruits and Vegetables)

The inside tissue of foods from plant sources are essentially sterile, except for a few porous vegetables الخضروات التي يسهل اختراقها (e.g., radishes and onions) and leafy vegetables (e.g., cabbage).

Some plants produce natural antimicrobial metabolites that can limit the presence of microorganisms. Fruits and vegetables harbor microorganisms on the surface; their type and level vary with soil condition, type of fertilizers and water used, and air quality.

Molds, yeasts, lactic acid bacteria, and bacteria from genera *Pseudomonas*, *Alcaligenes*, *Micrococcus*, *Erwinia*, *Bacillus*, *Clostridium*, and *Enterobacter* can be expected from this source.

Increasing the microbial numbers of foods of plant origin is due to many reasons such as:

(1) Diseases of the plants.

(2) Damage of the surface (before, during, and after harvest).

(3) Long delay between harvesting and washing.

(4) Unfavorable storage and transport conditions after harvesting and before processing.

To reduce the microbial load in foods of plant origin must be:

(1) Using of proper methods during growing (such as use of treated sewage or other types of fertilizers).

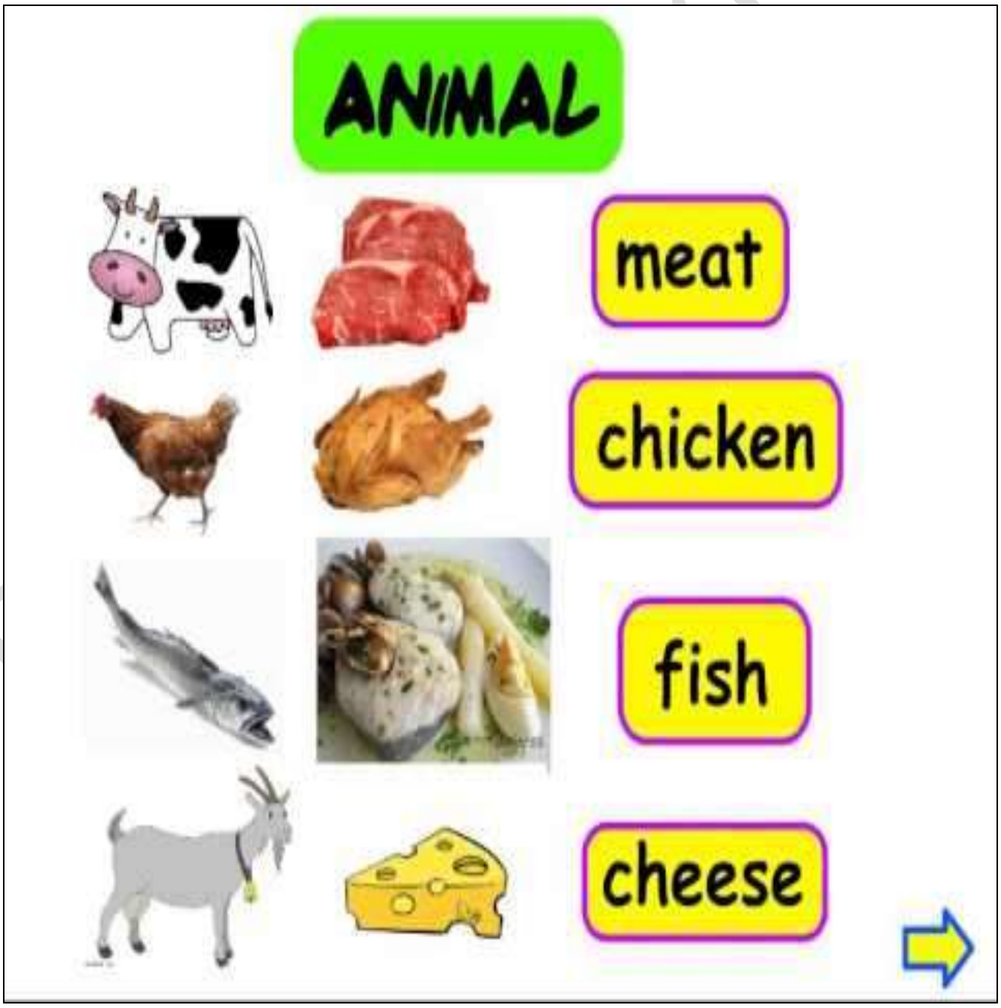
(2) Damage reduction during harvesting.

(3) Quick washing with good quality water to remove soil and dirt.

(4) Storage at low temperature before and after processing.

(B) Food of Animal origin (Animals, Birds, Fish, and Shellfish)

Food animals and birds normally carry many types of indigenous microorganisms in the digestive, respiratory, and urinogenital tracts, the teat canal in the udder, as well as in the skin, hooves, hair, and feathers. Their numbers, depending on the specific organ, can be very high (large intestinal contents can have as high as 10^{10} bacteria/g).



Many, as carriers, can harbor pathogens such as *Salmonella serovars*, pathogenic *Escherichia coli*, *Campylobacter jejuni*, *Yersinia enterocolitica*, and *Listeria monocytogenes* without showing symptoms.

Disease situations, such as mastitis in cows and intestinal, respiratory, and uterine infections, as well as injury can change the ecology of normal microflora. Similarly, poor husbandry resulting in fecal contamination on the body surface (skin, hair, feathers, and udder) and supplying contaminated water and feed (e.g., contaminated with salmonellae) can also change their normal microbial flora. Fish and shellfish also carry normal microflora in the scales, skin, and digestive tracts. Water quality, feeding habits, and diseases can change the normal microbial types and level. Pathogens such as *Vibrio parahaemolyticus*, *Vib. vulnificus*, and *Vib. cholerae* are of major concern from these sources.

Many spoilage and pathogenic microorganisms can get into foods of animal origin (milk, egg, meat, and fishery products) during production and processing. **Milk** can be contaminated with fecal materials on the udder surface, **Egg** shells with fecal material during laying, **Meat** with the intestinal contents during slaughteringالذبح والسلخ , and **Fish** with intestinal contents during processing, **Birds** can be contaminated with several spoilage and pathogenic microorganisms from skin, hair, and feathers, namely *Staphylococcus aureus*, *Micrococcus* spp., *Propionibacterium* spp., *Corynebacterium* spp., and molds and yeasts.

TO reduce the incidence of pathogenic microorganisms in foods of animal origin must be:

(1) Good housing for the animals and birds.

(2) Testing animals and birds for pathogens and culling the carriers.

(3) Hair removal; feather removal; careful removal of digestive, urinogenital, and respiratory organs without contaminating tissues; removal of contaminated parts.

(4) Proper cleaning of the udder before milking, cooling milk immediately after milking processing as soon as possible, and sanitization at all stages are important to keep microbial levels low in milk.

(5) Eggs should be collected soon after laying and washed and stored as recommended procedures.

(6) Fish and marine products should be harvested from unpolluted and recommended water.

Other sources for presence of microorganism in foods include:

- Air
- Soil
- Water
- Humans
- Food ingerdients

(1) Air

Microorganisms are present in dust and moisture droplets in the air. They do not grow in dust, but are transient and variable, depending on the environment. Their level is controlled by the degree of humidity, size and level of dust particles, temperature and air velocity, and resistance of microorganisms to drying.

Generally, dry air with low dust content and higher temperature has a low microbial level. Spores of *Bacillus* spp., *Clostridium* spp., and molds, and cells of some Gram positive bacteria (e.g., *Micrococcus* spp. and *Sarcina* spp.), as well as yeasts, can be predominantly present in air.

Microbial contamination of food from the air can be reduced by:

(1) Removing the potential sources.

(2) Reducing humidity level.

(3) Controlling dust particles in the air (using filters).

(4) Using positive air pressure.

(5) Installing UV light.

(2) Soil

Soil, especially the type used for growing agricultural, animals and birds, contains several varieties of microorganisms. Because microorganisms can multiply in soil, their numbers can be very high (billions/g). Many types of molds, yeasts, and bacterial genera can enter foods from the soil.

Soil contaminated with fecal materials can be the source of enteric pathogenic bacteria and viruses in food. Sediments where fish and marine foods are harvested can also be a source of microorganisms, including pathogens, in those foods. Different types of parasites can also get in food from soil.

Microbial contamination of food from the soil can be reduced by:

Removal of soil and sediments by washing and avoiding soil contamination.

(3) Water

Water is used to produce, process, and under certain conditions, store foods (ice). It is used for irrigation of crops, drinking by animals and birds, raising fishery and marine products, washing foods, processing (pasteurization, canning) and storage of foods (e.g., fish on ice). Water is also used as an ingredient in many processed foods. Thus, water quality can greatly influence microbial quality of foods.

Contamination of foods with pathogenic bacteria, viruses, and parasites from water has been recorded. Wastewater can be recycled for irrigation. However, chlorine-treated potable water (drinking water) should be used in processing, washing, sanitation, and as an ingredient.

Although potable water does not contain coliforms and pathogens (mainly enteric types), it can contain other bacteria capable of causing food spoilage, such as *Pseudomonas*, *Alcaligenes*, and *Flavobacterium*. Improperly treated water can contain pathogenic and spoilage microorganisms.

To overcome the problems, many food processors must use water has a higher microbial quality than that of potable water.

(4) Humans

Between production and consumption, foods come in contact with different people handling the foods. They include not only people working in farms and food processing plants, but also those handling foods at restaurants, catering services and at home.

Human carriers have been the source of pathogenic microorganisms in foods that later caused foodborne diseases, especially with ready to-eat foods.

Improperly cleaned hands, lack of 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 generalized diseases (e.g., flu, strep throat, or hepatitis A in an early stage) can amplify the situation.

The incidence of pathogenic microorganisms in foods from water can be reduced by:

(1) Proper training of personnel in personal hygiene.

(2) Regular checking of health.

(5) Food Ingredients

In prepared or fabricated foods, many ingredients or additives can be the source of both spoilage and pathogenic microorganisms. Various spices generally have very high populations of mold and bacterial spores.

Starch, sugar, and flour might have spores of thermophilic bacteria. Pathogens have been isolated from dried coconut, egg, and chocolate.

To reduce the microorganisms in food from this source

(1) The ingredients should be produced under sanitary conditions.

(2) Given antimicrobial treatments.

(3) In addition, setting up acceptable microbial specifications for the ingredients.

4. Different Methods of Food Preservation from Microbes

Food protection and food preservation have one aim in common they are intended to prevent contamination and spoilage of foods. Many of the methods of food protection and preservation used today are of ancient origin.

(1) Food protection

Food protection is defined as: measures taken to protect food from being contaminated by any agent. All food must be protected at all times during storage and preparation. The contaminants from which the food must be protected include:

(1) Any water that is not known to be safe.
(2) Dirty hands.
(3) Dust.
(4) Flies.
(5) Insecticides and other chemicals.
(6) Unclean utensils and work surfaces الأواني والأسطح الغير نظيفة.

(2) Food preservation

Food preservation is defined as: variety of techniques that allow food to be kept for extended periods of time without losing nutritional quality and avoiding the growth of unwanted microorganisms.

There are three basic objectives for the preservation of foods:

(1) Prevention of contamination of food from damaging agents.

(2) Delay or prevention of growth of microorganisms in the food.

(3) Delay of enzymic spoilage, i.e. self-decomposition of the food by naturally occurring enzymes within it.

❖ Control of Microorganisms in Foods

To control growth and kill microorganisms in a food, it is important to recognize that a control method is more effective when a food has fewer microbial cells and when the cells are in the exponential growth phase and are injured.

Bacteria, yeasts, molds, phages, and viruses differ in sensitivity to the methods used to control them. Different species and strains of the same species also differ in sensitivity to these control methods.

❖ Methods of food preservation:

(1) Preservation by control of access (Cleaning and Sanitation).

(2) Preservation by physical removal.

(3) Preservation by heat.

(4) Preservation by low temperature.

(5) Preservation by reduced A_w.

(6) Preservation by low pH and organic acids.

(7) Preservation by antimicrobial preservatives.

(8) Preservation by irradiation.

(1) Preservation of Foods by Control of Access (Cleaning and Sanitation)

The internal tissues of plants and animals used as foods are essentially sterile. However, many types of microorganisms capable of causing food spoilage and foodborne diseases enter foods from different sources.

It is impossible to prevent access of microorganisms in food from these sources. However, it is possible to control their access to food in order to reduce the initial load and minimize microbial spoilage and health hazard. To minimize the access of microorganisms in such foods, the microbiological quality of the environment to which a food is exposed (food contact surfaces) and the ingredients added to a food should be of good microbiological quality.

To minimize the access of microorganisms in foods, several factors need to be considered:

(A) Quality of Water

The microbiological quality of the water, especially if the foods are ready-to-eat, should be free from any spoilage bacterial pathogens such as *Pseudomonas* spp. To reduce this water, brine and curing solutions should be made fresh and used daily. Storing brine for extended periods before use may reduce the concentration of nitrite through formation and dissipation of nitrous oxide and may reduce shelf life of the products.

(B) Quality of Air

Some food-processing operations, such as spray drying of nonfat dry milk, require large volumes of air that come into direct contact with the food. Although the air is heated, it does not kill all the microorganisms present in the dust of the air and thus can be a source of microbial contamination of foods. The installation of air inlets to obtain dry air with the least amount of dust and filtration of the air is important to reduce microbial contamination from this source.

(C) Training of Personnel

Any operation system should have an active program to teach the personnel the importance of sanitation and personal hygiene to ensure product safety and stability.

(D) Sanitation of Food-Processing Equipment

Efficient cleaning can remove some microorganisms along with the soil from the food contact surfaces, but cannot ensure complete removal of pathogens. To achieve this goal, food contact surfaces are subjected to sanitation after cleaning. The methods should effectively destroy pathogenic microorganisms as well as reduce total microbial load. Several physical and chemical methods are used for sanitation of food-processing equipment.

(2) Preservation of Foods by Physical Removal

Microorganisms can be physically removed from solid and liquid foods by several methods. In general, these methods can partially remove microorganisms from food, and by doing so they reduce the microbial level and help other antimicrobial steps that follow to become more effective. They are generally used with raw foods before further processing.

Physical methods used for food preservation are:

(A) Centrifugation

Centrifugation is used in some liquid foods, such as milk, fruit juices, and syrups, to remove suspended undesirable particles.

The heavier particles move outward and are separated from the lighter liquid mass. Although this is not intended to remove microorganisms, spores, large bacterial rods, bacterial clamps and chains, yeasts and molds can be removed because of their heavier mass. Under high force, as much as 90% of the microbial population can be removed.

(B) Filtration

Filtration is used in some liquid foods, such as soft drinks, fruit juices, beer, wine, and water, to remove undesirable solids and microorganisms and to give a sparkling clear appearance. As heating is avoided or given only at minimum levels, the natural flavors of the products and heat-sensitive nutrients (e.g., vitamin C in citrus juices) are retained to give the products natural characteristics. The filtration process can also be used as a step in the production of concentrated juice with better flavor and higher vitamins.

Many types of filtration systems are available. In many filtration processes, coarse filters are used initially to remove large components; this is followed by ultrafiltration. Ultrafiltration methods, depending on pore size of the filter materials (0.45 to 0.7 mm), are effective in removing yeasts, molds, and most bacterial cells and spores from liquid products. Filtration of air is also used in some food-processing operations, such as spray drying of milk, to remove dust from air used for drying. The process also removes some microorganisms with dust, and this reduces the microbial level in food from air.

(C) Trimming

Fruits and vegetables showing damage and spoilage are generally trimmed. In this manner, areas heavily contaminated with microorganisms are removed. Trimming the outside leaves in cabbage used for sauerkraut production also helps to reduce microorganisms coming from soil.

Trimming is also practiced for removing the visible mold growth from hard cheeses, bread, and some food products.

Although this method helps to remove highly contaminated areas, it does not ensure complete removal of the causative microorganisms.

(D) Washing

Fruits and vegetables are washed regularly to reduce temperature (which helps reduce the metabolic rate of a produce and microbial growth) and to remove soil. Washing also used for shell eggs to remove fecal materials and dirt. Although these treatments are expected to reduce microbial load, they can spread contamination of undesirable microorganisms, particularly enteric pathogens.

Carcasses of food animals, such as beef, pork, and lamb, are washed to remove hair, soil particles, and microorganisms. Instead of hand washing, automated machine-washing at a high pressure is currently used to effectively remove undesirable materials and microorganisms from the carcass. In addition to high pressure, the effectiveness of hot water, steam, ozonated water, and water containing chlorine, acetic acid, propionic acid, lactic acid, tripolyphosphates, or bacteriocins (nisin and pediocin) of lactic acid bacteria has been studied for removing microorganisms, particularly enteric pathogens such as *Salmonella* spp.

(3) Preservation of Foods by Heat

The main objective (microbiological) of heating food is to destroy vegetative cells and spores of microorganisms. Although very drastic heat treatment (sterilization) can be used to kill all the microorganisms present in a food, most foods are heated to destroy specific pathogens and some spoilage microorganisms, which are important in a food. This is necessary in order to retain the acceptance and nutritional qualities of a food.

To control growth of surviving microorganisms in the food, other control methods are used following heat treatment. Heating of foods also helps destroy undesirable enzymes (microbial and food) that would otherwise adversely affect the acceptance quality of food. Some microorganisms also produce heat-stable proteinases and lipases in food. Heating a food to a desired temperature for a specific time can help destroy or reduce the activity of these enzymes. This is especially important in foods stored for a long time at room temperature.

Some microorganisms can release toxins in food; also, some foods can have natural toxins. If a toxin is heat sensitive, sufficient heating will destroy it and consumption of such a food will not cause health hazards. It is also important to recognize that microbial (and natural) heat-stable toxins are not completely destroyed even after high heat treatment.

Heating (warming) of ready-to-eat foods before serving is also usually used to prevent growth of pathogenic and spoilage microorganisms. A temperature above 50ºC, preferably 60ºC, is important to control growth of many microorganisms in such foods during storage before serving. Finally, heating of raw materials, such as milk, is done before adding starter culture bacteria for fermentation to kill undesired microorganisms (including bacteriophages) and to allow growth of the starter cultures without competition.

- Based on type of food to be preserved and microorganisms to be destroyed, there are three main methods of heat:

(a) Low-Heat Processing (Pasteurization)

Pasteurization is defined as: a food preservation method in which moderately high (62˚C to 100˚C) temperatures are used (for about 15 to 30 minutes) to inactivate certain enzymes and kill certain other microorganism specially in milk.

The temperature used for low-heat processing or pasteurization is below 100ºC. The objectives of pasteurization are to destroy all the vegetative cells of the pathogens and a large number (~90%) of associative microorganisms (yeasts, molds, bacteria, and viruses). In certain foods, pasteurization destroys some natural enzymes (e.g., phophatase in milk).

The temperature and time are set to the lowest level to meet the microbiological objectives and to minimize thermal damage of the food, which otherwise could reduce the acceptance quality (such as heated flavor in milk) or pose processing difficulties (such as coagulation of liquid egg).

Depending on the temperature used, thermoduric cells of spoilage bacteria and spores of pathogenic and spoilage bacteria still survive after the treatment. Thus, additional methods need to be used to control the growth of survivors (as well as post pasteurization contaminants) of pasteurized products, unless a product has a natural safety factor (e.g., low pH in some acid products).

(b) High-Heat

The process involves heating foods at or above 100°C for a desired period of time. The temperature and time of heating are selected on the basis of product characteristics and the specific microorganisms to be destroyed. Most products are given a commercially sterile treatment to destroy microorganisms growing in a product under normal storage conditions. Low-acid or high-pH (pH > 4.6) products are given 12D treatment to destroy *Clo. botulinum* Type A and B spores (the most resistant spores of a pathogen). However, the products can have viable spores of thermophilic spoilage bacteria (e.g., *Bacillus stearothermophilus*, *Bac. coagulans*, *Clo. thermosaccharolyticum*, and *Desulfotomaculum nigrificans*.

As long as the products are stored at or below 30°C, these spores will not germinate. If the products are temperature abused to 40°C and above even for a short time, the spores will germinate. Subsequent storage at or below 30°C will not prevent outgrowth and multiplication of these thermophiles to cause food spoilage. The time and temperature required for commercial sterility of a particular food are determined by actual pack inoculation studies.

(c) Microwave Heating

Heating or cooking foods by the microwave at home is quite common in both developed and developing countries. Frozen foods can be thawed and heated very rapidly, in a few minutes, depending on the size of a product.

However, the method has not been well accepted as a source of rapidly generated high heat for commercial operations. In a microwave oven, the waves change their polarity very quickly. Oppositely charged water molecules in a food rapidly move to align along the waves. The movement of the water molecules generates frictional heat, causing the temperature of the food to rise very rapidly. Depending on the exposure time and intensity of the wave, the temperature can be very high.

Microwave treatment is quite lethal to microorganisms and the destruction is caused by the high temperature. At present, microwave-heated foods cannot be considered safe from pathogens. Generally, when a food is heated in a microwave oven, it is not heated uniformly and some areas can remain cold. If a food harbors pathogens, there are chances that they will survive in the cold spots.

- **Mechanism of Antimicrobial Action of Heat Preservation**

Depending on the temperature and time of heating, microbial cells and spores can be heat-shocked which led to:

(1) Injury in the cell membrane and cell wall

(2) DNA (strand break)

(3) Degradation of Ribosomal RNA

(4) Denaturation of some important enzymes.

(4) Preservation of Foods by Low Temperature

The main microbiological objective in low-temperature preservation of food is to prevent or reduce growth of microorganisms. Low temperature also reduces or prevents catalytic activity of microbial enzymes, especially heat-stable proteinases and lipases.

Germination of spores is also reduced. Low-temperature storage, especially freezing (and thawing), is also lethal to microbial cells, and, under specific conditions, 90% or more of the population can die during low-temperature preservation. However, the death rate of microorganisms at low temperature, as compared with that at heat treatment, cannot be predicted (as D and Z values in heating). Also, spores are not killed at low temperature. Thus, foods are not preserved at low temperature in order to kill microbial cells. Freezing is also used to preserve starter cultures for use in food bioprocessing.

- Based on type of food to be preserved and microorganisms to be destroyed, there are three main methods of low temperature:

(a) Icing التثليج

This is used in retail stores where the foods are kept over ice; the surface in contact with the ice can reach between 0 and 1°C.

Fresh fish, seafood, meats, cut fruits, vegetable salads, different types of ready-to-eat salads are stored by this method.

(b) Refrigeration التبريد

The temperature specification for refrigeration of foods has changed over time. Previously, 7 °C was considered a desirable temperature. However, technological improvements have made it economical to have domestic refrigeration units at 4 to 5 °C.

For perishable products, £4.4°C (40°F) is considered a desirable refrigeration temperature. Commercial food processors may use as low as ≈1°C for refrigeration of perishable foods (such as fresh meat and fish). For optimum refrigeration in commercial facilities along with low temperature, the relative humidity and proper spacing of the products are also controlled.

Raw and processed foods of plant and animal origin, as well as many prepared and ready-to-eat foods, are now preserved by refrigeration. Their volume is increasing rapidly because consumers prefer such foods. Some of these foods are expected to have a storage life of 60 d or more.

(c) Freezing التجميد

The minimum temperature used in home freezers (in the refrigerator) is –20°C, a temperature at which most of the free water in a food remains in a frozen state. Dry ice (–78°C) and liquid nitrogen (–196°C) can also be used for freezing; they are used for rapid freezing (instant freezing) and not for only freezing a food to that low temperature. Following freezing, the temperature of the foods is maintained –20 to –30ºC. Depending on the type, foods can be stored at refrigerated temperature for months or even more than a year.

Raw produce (vegetables, fruits), meat, fish, processed products, and cooked products (ready-to-eat after thawing and warming) are preserved by freezing. Microorganisms do not grow at –20ºC in frozen foods. Instead, microbial cells die during frozen storage. However, the survivors can multiply in the unfrozen foods. Accidental thawing or slow thawing can facilitate growth of survivors (spoilage and pathogenic microorganisms). Enzymes, released by the dead microbial cells can reduce the acceptance quality of the food.

- **Mechanisms of Antimicrobial Action**

The metabolic activities, enzymatic reactions, and growth rates of microorganisms are maximum at the optimum growth temperature. As the temperature is lowered, microbial activities associated with growth slow down. Normally, the generation time, within a certain range, is doubled for every 10˚C reduction in temperature. Thus, a species dividing every 60 min in a food at 22˚C will take 120 min to divide if the temperature is reduced to 12˚C.

(1) At lower range, generation time can be even higher than double.

(2) The rate of catalytic activity of some enzymes also decreases as the temperature of an environment is reduced. Water is present in a food as free water and bound (with the hydrated molecules) water.

As the temperature in a food system drops to –2°C, free water in the food starts freezing and forming ice crystals (pure water freezes at 0°C, but in a food with solutes it freezes below 0°C).

(3) components of the cell wall (or outer membrane) and cell membrane (or inner membrane) are injured. DNA strand break, ribosomal RNA degradation, and activation and inactivation of some enzymes have also been reported in some studies. In sublethally injured cells, the structural and functional injuries are reversible. In lethally injured (or dead) cells, the damages are irreversible.

(5) Preservation of Foods by Reducing the Water Activity (A_w)

The ability of dried seeds, grains and fruits to resist spoilage was probably recognized by humans even before their discovery of agriculture.

Subsequently, this simple method (drying) was practiced to preserve the large volume of foods produced during growing seasons to make them available during nongrowing seasons. Reduced A_w was also extended to preserve other foods (e.g., meat, fish, and milk).

Reducing of water content can be carried out not only by drying but also by adding solutes (e.g., salt, honey, and starch) to bind water.

The main objectives of reducing A_w in food are to prevent or reduce the growth of vegetative cells and germination and outgrowth of spores of microorganisms.

Prevention of toxin production by toxigenic molds and bacteria is also an important consideration. Microbial cells (not spores) also suffer reversible injury and death in foods with low A_w, although not in a predictable manner as in heat treatment. Finally, reduced A_w is also used to retain viability of starter-culture bacteria for use in food bioprocessing.

The water activity of foods can be reduced by using one or more of these three basic principles:

(a) Removing water by dehydration

(b) Removing water by crystallization

(c) Adding solutes to bind water

- **Methods of reduction the water activity**

(a) Natural Dehydration

Natural dehydration is a low-cost method in which water is removed by the heat of the sun. It is used to dry grains as well as to dry some fruits (raisins), vegetables, fish, meat, milk, and curd (from milk), especially in warmer countries. The process is slow; depending on the conditions used, spoilage and pathogenic bacteria as well as yeasts and molds (including toxigenic types) can grow during drying.

(b) Mechanical Drying

Mechanical drying is a controlled process, and drying is achieved in a few seconds to a few hours. Some of the methods used are tunnel drying (in which a food travels through a tunnel against flow of hot air that removes the water), roller drying (in which a liquid is dried by applying a thin layer on the surface of a roller drum heated from inside), and spray drying (in which a liquid is sprayed in small droplets, which then come in contact with hot air that dries the droplets instantly).

Vegetables, fruits, fruit juices, milk, coffee, tea, and meat jerky are some foods that are dried by mechanical means. Depending on the temperature and time of exposure, some microbial cells can die during drying, whereas other cells can be sublethally injured. Also, during storage, depending on the storage conditions, microbial cells can die rapidly at the initial stage and then at a slow rate. Spores generally survive and remain viable during storage in a dried food.

(c) Freeze-Drying

The acceptance quality of food is least affected by freeze-drying, as compared with both natural and mechanical drying. However, freeze-drying is a relatively costly process. It can be used for both solid and liquid foods. The process initially involves freezing the food, preferably rapidly at a low temperature, and then exposing the frozen food to a relatively high vacuum environment. The water molecules are removed from the food by sublimation (from solid state to vapor state) without affecting its shape or size.

The method has been used to produce freeze-dried vegetables, fruits, fruit juices, coffee, tea, and meat and fish products, some as specialty products. Microbial cells are exposed to two stresses — freezing and drying — that reduce some viability as well as induce sublethal injury. During storage, especially at a high storage temperature and in the presence of oxygen, cells die rapidly initially and then more slowly. Spores are not affected by the process.

(d) Smoking

Many meat and fish products are exposed to low heat and smoke for cooking and depositing smoke on the surface at the same time. The heating process removes water from the products, thereby lowering their A_w. Many low-heat-processed meat products (dry and semidry sausages) and smoked fishes are produced this way. Heat kills many microorganisms. The growth of the survivors is controlled by low *Aw* as well as the many types of antimicrobial substances present in the smoke.

- **Mechanism of Antimicrobial Action of A_w Preservation**

Microorganisms need water for transport of nutrients, nutrient metabolism, and removal of cellular wastes. In a food, the total water (moisture) is present as free water and bound water; the latter remains bound to hydrophilic colloids and solutes (it can also remain as capillary water or in a frozen state as ice crystals).

Thus, only the free water (which is related to A_w) is important for microbial growth. Microorganisms also retain a slightly lower A_w inside the cells than the external environment to maintain turgor pressure, and this is important for cell growth. The loss of water causes an osmotic shock and plasmolysis, during which the cells do not grow.

(6) Preservation of Foods by Low pH and Organic Acids

During the early stages of human history when food was scarce, our ancestors probably recognized that some foods from plant sources, especially fruits, resisted spoilage. Later, they observed that the fermented foods and beverages prepared from fruits, vegetables, milk, fish, and meat were much more shelf stable than the raw materials from which they were produced develop large varieties of fermented foods, especially in tropical areas where, unless preserved, many foods spoil rapidly.

The major antimicrobial objective of using weak organic acids is to reduce the pH of food to control microbial growth. As the pH drops below 5.0, some bacteria become injured and die. However, the death rate in low pH is not predictable as in the case of heat. Thus, it could not be used with the objective of destroying a predictable percentage of a microbial population in the normal pH range of foods.

- **Acids Used**

(a) Acetic Acid

Acetic acid is used usually as vinegar (5 to 10% acetic acid) or as salts of sodium and calcium in pickles, salad dressings, and sauces. It is more effective against bacteria than yeasts and molds. The inhibitory action of acetic acid is produced through neutralizing the electrochemical gradient of the cell membrane as well as denaturing proteins inside the cells.

(b) Citric Acid

Citric acid is used at 1% (or more) in nonalcoholic drinks, jams and jellies, baking products, cheeses, canned vegetables, and sauces. It is less effective than lactic acid against bacteria as well as yeasts and molds. Its antibacterial effect is probably by a mechanism different than that for lipophilic acids. The antibacterial effect is partially due to its ability to chelate divalent cations. However, many foods can have sufficient divalent cations to neutralize this effect.

(c) Benzoic Acid

Benzoic acid is used as an acid or sodium salt at 500 to 2000 ppm (0.05 to 0.2%) in many low-pH products, such as nonalcoholic and alcoholic beverages, pickles, mayonnaise and salad dressings, mustards, and cottage cheese. It is more effective against yeasts and molds than bacteria. The inhibitory effect is produced by both the undissociated and dissociated acids. The inhibitory action is produced in several ways.

It inhibits the functions of many enzymes necessary for oxidative phosphorylation. Like other acids, it also destroys the membrane potential. In addition, it inhibits functions of membrane proteins.

(d) Lactic Acid

Lactic acid is used as acid or sodium and potassium salts up to 2% in carbonated drinks, salad dressings, pickled vegetables, low-heat processed meat products, and sauces. It is less effective than acetic, propionic, benzoic, or sorbic acids, but more effective than citric acid. It is more effective against bacteria but quite ineffective against yeasts and molds. It produces an inhibitory effect mainly by neutralizing the membrane proton gradient. It has also been recommended at a 1 to 2% level to wash carcasses of food animals to reduce microbial load.

A. Propionic Acid

Propionic acid is used as salts of calcium and sodium at 1000 to 2000 ppm (0.1 to 0.2%) in bread, bakery products, cheeses, jam and jellies, and tomato puree. It is effective against molds and bacteria but almost ineffective against yeasts at concentrations used in foods. The inhibitory concentration of undissociated acid is 0.05% against molds and bacteria. The antimicrobial action is produced through the acidification of cytoplasm as well as destabilization of membrane proton gradients.

B. Sorbic Acid

It is an unsaturated acid and used either as acid or as salts of sodium, potassium, or calcium. It is used in nonalcoholic drinks, some alcoholic drinks, processed fruits and vegetables, dairy desserts, confectioneries, mayonnaise, salad dressings, spreads, and mustards. The concentrations used vary from 500 to 2000 ppm (0.05 to 0.2%). It is more effective against molds and yeasts than against bacteria. Among bacteria, catalase-negative (e.g., lactic acid bacteria) are more resistant than catalase-positive.

- **Mechanisms of Antimicrobial Action of Low pH and Organic Acids Preservation**

The antimicrobial action of a weak organic acid is produced by the combined actions of the undissociated molecules and the dissociated ions. Microorganisms that are important in food tend to maintain an internal cytoplasmic pH 6.5 to 7.0 in acidophiles and 7.5 to 8.0 in neutrophiles.

The low pH can also act on the cellular components (such as proteins) and adversely affect their structural and functional integrity. These changes can interfere with the nutrient transport and energy generation, and in turn interfere with microbial growth. In addition, low pH can reversibly and irreversibly damage cellular macromolecules, which can subsequently inflict sublethal as well as lethal injury to cells. Low pH can alter the ionic environment of the spore coat by replacing its ions (e.g., Ca^{2+}) with H^+ and make the spores unstable toward other environmental stresses, such as heat and low A_w.

(7) Preservation of Foods by Antimicrobial Preservatives

Many chemical compounds, either present naturally, formed during processing, or legally added as ingredients, can kill microorganisms or control their growth in foods. They are, as a group, designated as antimicrobial inhibitors or preservatives.

Some of the naturally occurring preservatives can be present in sufficient amounts in foods to produce antimicrobial action, such as lysozyme in egg white and organic acids in citrus fruits. Similarly, some of the antimicrobials can be formed in enough quantities during food processing to control undesirable microbial growth, such as lactic acid in yogurt fermentation.

Antimicrobial chemicals are used in food in relatively small doses either to kill undesirable microorganisms or to prevent or retard their growth. They differ greatly in the abilities to act against different microorganisms (**broad spectrum**). Some are effective against many microorganisms, whereas others are effective against either molds and yeasts or only bacteria (**narrow spectrum**). Similarly, some compounds are effective against either Gram-positive or Gram-negative bacteria, or bacterial spores, or viruses. Those capable of killing microorganisms are designated as germicides (kill all types), fungicides, bactericides, sporicides, and viricides, depending on their specificity of killing actions against specific groups. Those that inhibit or retard microbial growth are classified as fungistatic or bacteriostatic.

Foods can have antimicrobial compounds in three ways:

(1) Present naturally.

(2) Formed during processing.

(3) Added as ingredients.

- **Examples Of Antimicrobial Preservatives**

(a) Nitrite ($NaNO_2$ and KNO_2)

Curing agents that contain nitrite, and together with NaCl, sugar, spices, ascorbate, and erythorbate, are permitted for use in heat-processed meat, poultry, and fish products to control growth and toxin production by *Clostridium botulinum*.

The mechanisms of antibacterial action of nitrite are not properly understood, but the inhibitory effect is probably produced in several ways, such as reactions with some enzymes in vegetative cells and germinating spores, restriction of the bacterial use of iron, and interference with membrane permeability, thereby limiting transport. In addition to clostridial species, nitrite is inhibitory, to some extent, to *Staphylococcus aureus*.

(b) Sulfur Dioxide (SO_2) and Sulfites (SO_3)

Sulfur dioxide, sodium sulfite (Na_2SO_3), sodium bisulfide ($NaHSO_3$), and sodium metabisulfite ($Na_2S_2O_5$) are used to control microorganisms (and insects) in soft fruits, fruit juices, lemon juices, beverages, wines, sausages, pickles, and fresh shrimp.

Currently, these additives are not permitted in the U.S. in meat, as they destroy vitamin B1. They are more effective against molds and yeasts than bacteria; among bacteria, the aerobic Gram-negative rods are the most susceptible. The antimicrobial action is produced by the undissociated sulfurous acid that rapidly enters the cell and reacts with the thiol groups in structural proteins, enzymes, and cofactors, as well as with other cellular components.

However, people with respiratory problems can be mildly to severely allergic to sulfites. The products need to be labeled to show the presence of sulfites.

(c) H_2O_2

A solution of H_2O_2 (0.05 to 0.1%) is recommended as an antimicrobial agent in raw milk to be used in cheese processing (to control growth of psychrotrophic Gram negatives that produce heat-stable enzymes).

In raw milk and liquid egg, catalase is used before pasteurization to hydrolyze H_2O_2 to water and oxygen. H_2O_2 is a strong oxidizing agent, and the germicidal action is associated with this property. Recently, H_2O_2 has been used to produce modified plant fiber flour from straws for use in low-calorie foods and for bleaching and color improvement of grains, chocolate, instant tea, fish, sausage casings, and many others, and to reduce sulfite in wines. In the future, the use of H_2O_2 in foods is expected to increase.

(d) Antibiotics (Tetracyclines, Natamycin, and Tylosin)

Several classical antibiotics were studied as antimicrobial food preservatives. Tetracyclines (10 ppm) were approved by the Food and Drug Administration (FDA) to extend the refrigerated shelf life of seafood and poultry in the 1950s.

However, because of the possible increase in antibiotic-resistant bacteria, the use of these antibiotics in food was later banned. Natamycin, a microlid produced by *Streptomyces natalensis*, is an antifungal agent. Its use as a dip or spray to prevent growth of molds and formation of mycotoxins on the surface of some cheeses, sausages, and in raw peanuts has been approved by the Expert Committee of the World Health Organization (WHO).

It is customarily used at 500 ppm, which leaves detectable but safe levels of the antibiotic on the product surface. Tylosin, a microlid that inhibits protein synthesis, is a bactericidal antibiotic that is more effective against Gram-positive than Gram-negative bacteria and also inhibits outgrowth of germinated endospores. Because of its high heat resistance, it has been studied at a low concentration (1 ppm) to determine its effectiveness in controlling the growth of spore formers in low-acid canned products.

(e) Spices

Many spices, condiments, and plant extracts are known to contain antimicrobial compounds. Some of these include cinnamic aldehyde in cinnamon; eugenol (2methoxy-4-allyl phenol) in cloves, allspice, and cinnamon; and paramene and thymol in oregano and thyme. Their bacteriostatic and fungistatic properties depend on the active agent. Because of the small amounts used as spices in foods, they probably do not produce any antimicrobial effects.

However, the antimicrobial components can be used in higher concentrations as oleoresins or essential oils. The antimicrobial properties of garlic, onion, and ginger, as well as cabbage, carrots, and others have generated interest for their possible use as natural preservatives. It is expected that in the future the antimicrobial compounds from plants, especially food plants, will be studied more effectively and thoroughly.

(8) Preservation of Foods by Irradiation

In the electromagnetic spectrum, energy exists as waves and the intensity of the energy increases as the waves get shorter. On either side of visible rays (~400 to 800 nm) are invisible long waves (>800 nm; IR and radio waves for radio, TV, microwave, and radar) and invisible short waves (<300 nm; UV rays, x-rays, b-rays, g-rays, and cosmic rays). Exposure to long waves, visible light waves, and UV rays does not cause any change in the atomic structures.

A food is irradiated because of the destructive power of ionization on microorganisms a food harbors. Depending on the method used, it can either completely or partially destroy molds, yeasts, bacterial cells and spores, and viruses. In addition, irradiation can destroy worms, insects, and larvae in food. It also prevents sprouting of some foods, such as potatoes and onions. However, irradiation cannot destroy toxins or undesirable enzymes in a food, in which respect it differs from heat treatment (heat also does not destroy heat-stable toxins and enzymes).

Irradiation is a cold sterilization process inasmuch as the temperature of a food does not increase during treatment, and thus irradiated foods do not show some of the damaging effects of heat on food quality. However, irradiation can cause oxidation of lipids and denaturation of food proteins, especially when used at higher doses.

- **Mechanisms Of Antimicrobial Action of Radioactivity Preservation**

When an object (food or microorganism) is exposed to high-energy `Y-rays (10^{-1} to 10^{-2} nm), the energy is absorbed by thousands of atoms and molecules in a fraction of a second, which strips electrons from them. This produces negative and positive ion pairs. The released electrons can be highly energized and thus can remove electrons from other atoms and convert them into ions. This energization and ionization can adversely affect the normal characteristics of biological systems.

Ionizing radiation produces both direct and indirect effects on microorganisms. The direct effect is produced from the removal of electrons from the DNA, thereby damaging them. The direct effect is produced from the ionization of water molecules present in the cell. The hydrogen and hydroxyl radicals formed in this process are highly reactive and cause oxidation, reduction, and the breakdown of C–C bonds of other molecules, including DNA. Studies have shown that both single- and doublestrand breaks in DNA at the sugar-phosphate backbone can be produced by the hydroxyl radical. In addition, the radicals can change the bases, such as thymine to dihydroxydihydrothymine. The consequence of these damages is the inability of microorganisms to replicate DNA and reproduce, resulting in death.

In addition to DNA damage, ionizing radiation also causes damage in the membrane and other structures, causing sublethal injury. Some microorganisms can repair the damage to the DNA strands (especially single-strand breaks) and in the bases, and are designated as radiation-resistant microorganisms.

5. Different Food Spoilage

Food spoilage can be defined as: a disagreeable change in a food's normal state such changes in color, texture, flavor (smell and taste) and shape of food.

A food is considered spoiled when it loses its acceptance qualities. The factors considered in judging the acceptance qualities of a food include color, texture, flavor (smell and taste), shape, and absence of abnormalities. Loss of one or more normal characteristics in a food is considered to be due to spoilage. Food spoilage causes not only economic loss, but also loss of consumable foods.

Types of food spoilage:

(1) **Physical spoilage**: such as dehydration of fresh vegetables (wilting).

(2) **Chemical spoilage**: such as oxidation of fat, browning of fruits and vegetables, and autolytic degradation of some vegetables (by pectinases) and fishes (by proteinases).

(3) **Microbial Spoilage:** results either as a consequence of microbial growth in a food or because of the action of some microbial enzymes present in a food. e.g: Black bread mold.

I. Spoilage of Fresh Foods

(a) Fresh and Ready-To-Eat Meat Products

Fresh meats from food animals and birds contain a large group of potential spoilage bacteria that include species of *Pseudomonas*, *Acinetobacter*, *Alcaligenes*, *Aeromonas*, *Escherichia*, *Enterobacter*, *Serratia*, *Proteus*, *Brochothrix*, *Micrococcus*, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Carnobacterium*, and *Clostridium*, as well as yeasts and molds.

The predominant spoilage flora in a meat is determined by nutrient availability, oxygen availability, storage temperature, pH, storage time of the product, and generation time of the microorganisms present in a given environment.

To delay microbial spoilage, fresh meats are stored at refrigerated temperature (≈5°C). unless the facilities are not available. Thus, normally psychrotrophic bacteria are the most predominant types in raw meat spoilage. Under aerobic storage at low temperature, growth of psychrotrophic aerobes and facultative anaerobes is favored. In retail-cut meats, because of a shorter generation time, *Pseudomonas* spp. grows rapidly.

Comminuted meats spoil more rapidly than retail cuts because they have more surface area. Under aerobic storage, growth of aerobic bacteria (predominantly *Pseudomonas* spp.) causes changes in odor, texture, color, and sliminess. The inside is initially microaerophilic (due to dissolved oxygen from trapped air), which then changes to anaerobic, and growth of facultative bacteria predominates.

To reduce spoilage of fresh meats, initial microbial level should be reduced. In addition, storage at low temperatures (close to 0 to –1°C), modified atmosphere packaging, and vacuum packaging should be done. Also, the addition of small amounts of organic acids to lower the pH of meat (slightly above pH 5.0), drying of meat surfaces (to reduce A_w) control the microbial load in the meat products.

(b) Eggs and Egg Products

(1) Shell Eggs

The pores in the eggshell and inner membrane do not prevent entrance of bacteria and hyphae of molds, especially when the pore size increases during storage. The presence of moisture enhances the entrance of motile bacteria. The albumen (egg white) and yolk have 0.5 to 1.0% carbohydrate and are high in proteins but low in nonprotein nitrogen. During storage, the pH can shift to the alkaline side (pH 9 to 10). In addition, lysozyme (causes lysis of bacterial cell wall mucopeptide), conalbumin (chelates iron), antivitamin proteins (avidin binds riboflavin), and protease inhibitors in eggs inhibit microbial growth.

The most predominant spoilage of shell eggs is caused by Gram-negative motile rods from several genera that include *Pseudomonas*, *Proteus*, *Alcaligenes*, *Aeromonas*, and the coliform group. The different types of spoilage are designated as rot. Some examples are green rot, causing greening of albumen because of growth of *Pseudomonas fluorescens*; black rot, causing muddy discoloration of yolk because of H_2S production by *Proteus vulgaris*; and red rot by *Ser. mercescens*, caused by production of red pigment. On some occasions, molds from genera *Penicillium*, *Alternaria*, and *Mucor* can grow inside eggs, especially when the eggs are oiled, and produce different types of fungal rots.

(2) Egg Products

Liquid eggs consisting of whole, yolk, or white are generally pasteurized or frozen, or both, to prevent microbial growth. If the liquid products are held at room temperature following breaking prior to pasteurization, spoilage bacteria can grow and cause off-flavor (putrid), sourness, or fish flavor (due to formation of trimethylamine). Pasteurized eggs at refrigerated temperature have a limited shelf life unless additional preservatives are added.

The predominant bacteria in pasteurized products are some Gram-positive bacteria that survive pasteurization, but spoilage is mainly caused by psychrotrophic Gram-negative bacteria getting into products after heat treatment. Dried eggs are not susceptible to microbial spoilage, because of low A_w.

(c) Fish, Crustaceans, and Mollusks

Fish harvested from both fresh and saltwater are susceptible to spoilage through autolytic enzyme actions, oxidation of unsaturated fatty acids, and microbial growth.

Protein hydrolysis by autolytic enzymes (proteinases) is predominant if the fish are not gutted following catch. Microbial spoilage is determined by the microbial types, their level, fish environment, fish types, methods used for harvest, and subsequent handling.

Gram-negative aerobic rods, such as *Pseudomonas* spp., *Acinetobacter*, *Moraxella*, and *Flavobacterium*, and facultative anaerobic rods, such as *Shewanella*, *Alcaligenes*, *Vibrio*, and coliforms, are the major spoilage bacteria.

Proteolytic bacterial species also produce extracellular proteinases that hydrolyze fish proteins and supply peptides and amino acids for further metabolism by spoilage bacteria. The volatile compounds produce different types of off-odors, namely, stale, fishy (due to trimethylamine), and putrid. Bacterial growth is also associated with slime production, discoloration of gills and eyes (in whole fish), and loss of muscle texture (soft due to proteolysis).

Under refrigeration, products have relatively long shelf life due to slower growth of spoilage bacteria. Salted fish, especially lightly salted fish, are susceptible to spoilage by halophilic bacteria such as *Vibrio* (at lower temperature) and *Micrococcus* (at higher temperature). Smoked fish, especially with lower A_w, inhibit growth of most bacteria.

However, molds can grow on the surface. Minced fish flesh, surimi, and seafood analogs prepared from fish tissues generally have high initial bacterial levels due to extensive processing (ca. 105–6/g). The types include those present in fish and those that get in during processing. These products, such as fresh fish, can be spoiled rapidly by Gram-negative rods unless frozen quickly or used soon after thawing.

Canned fish (tuna, salmon, and sardines) are given heat treatment to produce commercially sterile products. They can be spoiled by thermophilic spore formers unless proper preservation and storage conditions are used.

(d) Milk and Milk Products

(1) Raw Milk

Raw milk contains many types of microorganisms coming from different sources. The average composition of cow's milk is protein 3.2%, carbohydrates 4.8%, lipids 3.9%, and minerals 0.9%. Besides casein and lactalbumin, it has free amino acids, which can provide a good N source (and some C source, if necessary.

Milk fat can be hydrolyzed by microbial lipases, with the release of small-molecular volatile fatty acids (butyric, capric, and caproic acids).

Microbial spoilage of raw milk can potentially occur from the metabolism of lactose and the hydrolysis of triglycerides. If the milk is refrigerated immediately following milking and stored for days, the spoilage will be predominantly caused by Gram-negative psychrotrophic rods, such as *Pseudomonas*, *Alcaligenes*, *Flavobacterium* spp., and some coliforms. *Pseudomonas* and related species, being lactose negative, metabolize proteinaceous compounds to change the normal flavor of milk to bitter, fruity, or unclean. They also produce heat-stable lipases (producing rancid flavor) and heatstable proteinases that have important implications.

The growth of lactose-positive coliforms produces lactic acid, acetic acid, formic acid, CO_2, and H_2 (by mixed-acid fermentation) and causes curdling, foaming, and souring of milk. Some

Alcaligenes spp. (*Alc. faecalis*) and coliforms can also cause ropiness (sliminess) by producing viscous exopolysaccharides.

However, if the raw milk is not refrigerated soon, growth of mesophiles, such as species of *Lactococcus*, *Lactobacillus*, *Enterococcus*, *Micrococcus*, *Bacillus*, *Clostridium*, and coliforms, along with *Pseudomonas, Proteus*, and others, predominates. But lactose-hydrolyzing species, such as *Lactococcus* spp. and *Lactobacillus* spp., generally predominate, producing enough acid to lower the pH considerably and prevent or reduce growth of others. In such situations, curdling of milk and sour flavor is the predominant spoilage. If other microorganisms also grow, gas formation, proteolysis, and lipolysis become evident. Yeast and mold growth, under normal conditions, is generally not expected.

(2) Pasteurized Milk

Raw milk is pasteurized before it is sold for consumption as liquid milk.

Microorganisms present: Thermoduric bacteria (*Micrococcus*, *Enterococcus*, some *Lactobacillus*, *Streptococcus*, *Corynebacterium*, and spores of *Bacillus* and *Clostridium*) survive the process. In addition, coliforms, *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, and similar types can enter as post pasteurization contaminants.

Microbial action: some microorganisms produce enzyme lecithinase, which hydrolyzes phospholipids of the fat globule membrane, causing aggregation of fat globules that adhere to the container surfaces. Other microorganisms produce rennin-like enzymes by psychrotrophic *Bacillus* spp. and others can cause sweet curdling of milk.

Spoilage pattern: is the same as described for raw milk spoilage.

(e) Vegetables and Fruits

(1) Vegetables

Fresh vegetables contain microorganisms coming from soil, water, air, and other environmental sources, and can include some plant pathogens.

Microbial pathogens: The most common spoilage is caused by different types of molds, some of those from genera *Penicillium*, *Phytopthora*, *Alternaria*, *Botrytis*, and *Aspergillus*. Among the bacterial genera, species from *Pseudomonas*, *Erwinia*, *Bacillus*, and *Clostridium* are important.

Spoilage pattern: Microbial vegetable spoilage is generally described by a common term rot, along with the changes in the appearance, such as black rot, gray rot, pink rot, soft rot, stem-end rot. In addition to changes in color, microbial rot causes off-odor and loss of texture.

Preservation: Refrigeration, vacuum or modified atmosphere packaging, freezing, drying, heat treatment, and chemical preservatives are used to reduce microbial spoilage of vegetables.

(2) Fruits

Fresh fruits are high in carbohydrates (generally 10% or more), very low in proteins (£≈1.0%), and have a pH 4.5 or below.

Microbial pathogens: microbial spoilage of fruits and fruit products is confined to molds, yeasts, and aciduric bacteria (lactic acid bacteria, *Acetobacter*, *Gluconobactor*). Like fresh vegetables, fresh fruits are susceptible to rot by different types of molds from genera *Penicillium*, *Aspergillus*, *Alternaria*, *Botrytis*, *Rhizopus*, and others. Yeasts from genera *Saccharomyces*, *Candida*, *Torulopsis*, and *Hansenula* are associated with fermentation of some fruits, such as apples, strawberries, citrus fruits, and dates.

Spoilage pattern: According to the changes in appearance, the mold spoilage is designated as black rot, gray rot, soft rot, brown rot, or others.

Preservation: To reduce spoilage, fruits and fruit products are preserved by refrigeration, freezing, drying, reducing A_w, and heat treatment. Spoilage of fruit juices, jams, and wine is discussed later.

Spoilage of Canned Foods

Canned foods include those packed in hermetically sealed containers and given high heat treatment.

Canned food spoilage is both due to nonmicrobial (chemical and enzymatic reactions) and microbial reasons. Production of hydrogen (hydrogen swell), CO_2, browning, corrosion of cans due to chemical reactions, and liquification, gelation, and discoloration due to enzymatic reactions are some examples of non-microbial spoilage.

Microbial spoilage is due to three main reasons:

(1) Inadequate cooling after heating or high-temperature storage, allowing germination and growth of thermophilic spore formers.

(2) Inadequate heating, resulting in survival and growth of mesophilic microorganisms (vegetative cells and spores).

(3) Leakage (can be microscopic) in the cans, allowing microbial contamination from outside following heat treatment and their growth.

Also of importance in diagnosing the cause of canned food spoilage is the appearance of the unopened can or container. The ends of a can of food are normally flat or slightly concave where the microorganisms grow and produce gases.

6. Food Poisoning

Foodborne intoxication or food poisoning of microbial origin is <u>defined as</u> a poisoning occurs by ingesting a food containing a preformed toxin.

Two of bacterial origin, staphylococcal intoxication and botulism, and mycotoxicosis of mold origin will be discussed.

Some general characteristics of food poisoning are:

1. The toxin is produced by a pathogen while growing in a food.
2. A toxin can be heat labile or heat stable.
3. Ingestion of a food containing active toxin, not viable microbial cells, is necessary for poisoning (except for infant botulism, in which viable spores need to be ingested).
4. Symptoms generally occur quickly, as early as 30 min after ingestion.
5. Symptoms differ with type of toxin; enterotoxins produce gastric symptoms and neurotoxins produce neurological symptoms.
6. Febrile symptom may be not present.

(a) Staphylococcal Intoxication

Staphylococcal food poisoning caused by toxins of *Staphylococcus aureus*, is considered one of the most frequently occurring foodborne diseases worldwide.

Enterotoxigenic strains of *Sta. aureus* produce seven different enterotoxins: A, B, C1, C2, C3, D, and E (also designated as SEA, SEB, etc.).

Staphylococcal toxins are enteric toxins and cause gastroenteriti. The symptoms occur within 2 to 4 h, and are directly related to the potency and amounts of toxin ingested.

The primary symptoms, from stimulation of the autonomic nervous system by the toxins, are salivation, nausea and vomiting, abdominal cramps, and diarrhea. Some secondary symptoms are sweating, chills, headache, and dehydration.

(b) Botulism

Botulism results following consumption of food containing the potent toxin botulin of *Clostridium botulinum*.

The toxins of *Clo. botulinum* are neurotoxic proteins. In general, toxins associated with food intoxication in humans (Types A, B, E, and F) are extremely potent, and only a small amount of toxin is required to produce the symptoms and cause death.

This toxin produces neurological symptoms along with some gastric symptoms. Unless prompt treatment is administered, it is quite fatal. Infant botulism occurs when an infant ingests *Clo. botulinum* spores, which germinate, grow, and produce toxins in the GI tract and cause specific symptoms.

(c) Mycotoxicosis

Mycotoxins: secondary metabolites produced by different toxigenic species and strains of molds.

Many strains of molds, while growing in a suitable environment (including in foods), produce metabolites that are toxic to humans, animals, and birds, and are grouped as mycotoxins. Consumption of foods containing mycotoxins causes mycotoxicosis. They are secondary metabolites and not proteins or enteric toxins. Many are carcinogens and, when consumed, can cause cancer in different tissues in the body.

Mycotoxins include a large number of toxins produced by different toxigenic species and strains of molds. Many have not yet been identified. They are small-molecular-weight heterocyclic organic compounds and some have more than one chemical type. An example is aflatoxin, which has two major types, B1 and G1, and each has several subtypes. Aflatoxin B1 is considered the most potent, produced by *Asp. flavus*.

7. Important microbes in dairy products and changes that causes

Theoretically, milk that is secreted to the udder of a healthy cow should be free of microorganisms. However, freshly drawn milk is generally not free of microorganisms. Numbers of several hundred to several thousand CFU/ml are often found in freshly drawn milk, and they represent the movement up the teat canal of some and the presence of others at the lower ends of teats.

❖ Pathogens of Milk

Since milk act as an excellent nutrient source and because milk-producing animals may harbor organisms that cause human diseases, it is not surprising that raw milk can be a source of diseases.

Some of the most common diseases to which humans are susceptible and which may occur in milk of cows:

- Brucellosis الحمى المالطية
- Anthrax الجمرة الخبيثة
- Tuberculosis مرض السل الرئوي
- Salmonellosis مرض التيفود
- Staph./Strep. Mastitis التهاب الثدي

Prior to the general use of mechanical milking devices, raw milk was the source of both human respiratory diseases (e.g., diphtheria) as well as enteric infections (e.g., typhoid fever).

When milking a cow by hand where the milk is collected in an open pail, an infected person (or carrier) may contaminate the milk by coughing or by hand contact.

❖ Spoilage of Milk

Milk undergoes microbial spoilage in a way that is unique because of its nutritional content. Only a relatively small number of milk-borne bacteria can obtain energy from the disaccharide lactose (especially at refrigerator temperatures) in contrast to the disaccharides sucrose and maltose, and the lactic acid bacteria are well suited to this task. The thermoduric *Streptococcus salivarius* subsp. *thermophilus* strains preferentially use the glucose moiety of lactose and excrete galactose, which is a ready substrate for non-lactose users.

❖ Probiotics and Prebiotics

Probiotics: it is a consumable product that contains live organisms that are or are believed to be beneficial to the consumer.

Or: the term has been applied to bacteria that act as control agents in an aquaculture environment.

Yogurt appears to be the most widely consumed of probiotic products (especially in the United States) and while it is consumed by some because it is a fermented dairy product, it is consumed by others because of its real or presumed health benefits.

Prebiotics: are not microorganisms; they are substrates for the indigenous probiotic-type bacteria that reside in the colon.

These substrates are non-digestible as they pass through the small intestine, and they consist of oligosaccharides such as fructo oligosaccharides of which inulin is an example.

Inulin is a type of carbohydrate called an oligosaccharide, which means its chemical composition consists of several simple sugars linked together to form what's known as a fructan. This composition makes inulin a non-digestible prebiotic, which allows it to pass through humans' small and large intestines unabsorbed. During this process, inulin naturally ferments and feeds the healthy intestinal microflora (bacterial organisms, including *Lactobacillus* & *bifidobacterium*) that populate the gut hence, stimulate their antagonistic activity to aerobic pathogens.

Unlike probiotics, these substrates can be added to a number of food types that do not support cell viability over long periods of time. Their use obviates the need for cultures that can persist in the small intestines.