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THE CONTENTS

History and development of food microbiology

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 other cause food spoilage and food born disease. To study the role of microorganisms in food and to control them, when necessary, it is important to isolate them in pure culture and study their morphological, physiological, biochemical, and genetic characteristics.

Discovery of microorganisms

- Antony van Leeuwenhook observed bacteria in rainwater, vinegar, and other materials; and also described some to be motile. He called them *animalcules*, and between 1676 and 1683 he reported his observations.
- Ehrenberg (1838) introduced the term bacteria.
- Ferdinand Cohen was the first to discover that some bacteria produced spores.
- The existence of submicroscopic viruses was recognized in the mid-19th century, they were observed only after the invention of electron microscope in the 1940.

Where are they come from?

- Redi (1665) disproved the theory of spontaneous generation by showing that the maggots in spoilage meat and fish could only appear if flies were allowed to contaminate them.
- Turbevill Needham (1749) showed that boiled meat and meat broth, following storage in covered flasks, could have the presence of animalcules within a short time (used to prove the spontaneous generation theory).
- Lazzaro Spallanzani (1765) showed that boiling the meat in a flask and sealing the flask immediately prevented the appearance of these microscopic organisms, thereby disproving Needham's theory.

- Louis Pasteur (1861) demonstrated that, in boiled infusion, bacteria could grow only if the infusion were contaminated with bacteria carried by dust particles in air. His careful and controlled studies proved that bacteria were able to reproduce, and life could not originate by spontaneous generation.

What are their functions?

- Theodor Schwann (1837) and Hermann Helmholtz (1843) proposed that putrefaction and fermentation were connected with the presence of the organisms derived from air.
- Pasteur (1875)
  - Showed that wine fermentation from grapes and souring of wine were caused by microorganisms.
  - Proved that spoilage of meat and milk was associated with the growth of microorganisms.
  - Showed the association of microorganisms with several diseases.
  - Developed vaccines against a few human and animal diseases caused by microorganisms.
- Robert Koch, in Germany 1880-1890, isolated pure cultures of bacteria responsible for anthrax, cholera, and tuberculosis.

- **With time, the importance of microorganisms was recognized, and microbiology was divided into:**
  - **Medical microbiology.**
  - **Soil microbiology.**
  - **Plant pathology.**
  - **Food microbiology.**

Development of food microbiology

- Between 8000 and 1000 B.C., many food preservation methods such as drying, cooking, baking, smoking, salting, sugaring (with honey), low-temperature storage (in ice), storage without air (in pits), fermentation (with fruits, grains, and milk), pickling, and spicing were used, probably mainly to reduce spoilage.

❖ Following the discovery of the ubiquitous existence of microorganisms by Leeuwenhoek (1670) some individuals started associating the possible role of these organisms with food spoilage, food fermentation, and foodborne diseases.

❖ The major developments of ideas on the possible roles of microorganisms in foods and their scientific proofs were initiated by Pasteur in the 1870.

❖ Some of the major developments in the 19th century are listed here:

Food Fermentation

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

Food Spoilage

• Francois Nicolas (1804) developed methods to preserve food 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.

• Peter Durand (1819) developed canning preservation of foods in a steel can.

• L. Pasteur (1870) recommended heating of wine at 62°C for 30 mints to destroy souring bacteria. Later this method was modified and named pasteurization and used to kill mainly vegetative pathogens and many spoilage bacteria.

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

Foodborne Diseases

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

- John Snow (1849) suggested spread of cholera through drinking water contaminated with sewage. In 1854, Filippo Facini named the cholera bacilli as *Vibrio cholera*, which was isolated in pure form by Robert Koch in 1884.
- William Budd (1856) 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.
- Theodor *Escherich* (1885) isolated *Bacterium coli* (later named *Escherichia coli*) from the feces and suggested that some strains were associated with infant diarrhea.
- A. A. Gartner (1888) 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.
- J. Denys (1894) associated pyogenic *Staphylococcus* with death of a person who ate meat prepared from a diseased cow.
- Marie von Ermengem (1895) isolate *Bacillus botulinus* (*Clostridium botulinum*) from contaminate meat and proved that it caused botulism.

❖ In the early 20th century

- The importance of sanitation in the handling of food to reduce contamination by microorganisms was recognized.
- Specific methods were studied to prevent growth as well as to destroy the spoilage and pathogenic bacteria.
- There was also some interest to isolate beneficial bacteria associated with food fermentation.

Characteristics of predominant microorganisms in food

The microbial groups important in foods consist of several species and types of bacteria, yeast, molds, and viruses. Although some algae and protozoa as well as some worms

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 low pH, low water activity (A_w), and high osmotic pressure.
- 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: *Aspergillus, Alternari,Fusarium, Geotrichum, Mucor, Penicillium, Rizopus.*

B. Important yeast 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.
- Some of the most common genera of yeasts are: *Saccharomyces, Pichia, Rhodotorula, Torulopsis, Candida, Zygosaccharomyces.*

C. Important viruses

- Viruses are important in food.
- Some are able to cause enteric disease, and thus, if present in food, can cause foodborne diseases.
- Hepatitis A and Norwalk-like viruses have been implicated in foodborne outbreaks.

- Several other enteric viruses, such as poliovirus, echo virus, and Coxsackie virus, can cause foodborne diseases.
- Some bacteriophages can be very important because they can cause fermentation failure.

D. Important bacterial genera

- In *Bergey's Manual of Determinative Bacteriology* (1993), more than 560 genera are listed in 35 groups. A few that are important in food:

1. ***Gram-Negative Aerobes***

   Campylobacter, Pseudomonas, Xanthomonas, Acetobacter, Gluconobacter, Acinetobacter, Morexella, Alteromonas, Flavobacterium, Alcaligenes, Brucella, Psychrobacter.

2. ***Gram-Negative Facultative Anaerobes***

   Citrobacter, Escherichia, Enterobacter, Edwaredsiella, Erwinia, Hafnia, Klebsiella, Morganella, Proteus, Salmonella, Shigella, Serratia, Yersinia, Vibrio, Aeromonas, Plesiomonas.

3. ***Rickettsias***

   Coxiella (G-ve) very small cell (0.2×0.5µm) grow on host cells.

4. ***Gram-Positive Cocci***

   Micrococcus, Staphylococcus, Streptococcus, Enterococcus, Lactococcus, Leuconostoce, Pediococcus, Sarcinia.

5. ***Gram-Positive, Endospore-Forming Rods***

   Bacillus, Sporolactobacillus, Clostridium.

6. ***Gram-Negative, Endospor-Forming Rods***

   Desulfotomaculum.

7. ***Gram-Positive, Nonsporulating Regular Rods***

   Lactobacillus,Carnobacterium, Brochothrix, Listeria.

8. ***Gram-Positive, Nonsporeforming Irregular Rods***

   Corynebacterium, Brevibacterium, Propionibacterium.

Important bacterial groups in foods

❖ Among the microorganisms found in foods, bacteria constitute major important groups.

- Many different species can be present in food
- Their rapid growth rates
- Ability to utilize food nutrients
- Ability to grow under a wide range of temperatures, aerobiosis, pH, and water activity, as well as to better survive adverse situations, such as survival of spores at high temperature.

❖ Bacteria in foods have been arbitrarily divided into several groups on the basis of similarities in certain characteristics.

A. Lactic acid bacteria

- Produce relatively large quantities of lactic acid from carbohydrates.
- *Lactococcus, leuconostoc, Pediococcus, Lactobacillus,* and *Streptococcus thermophilus*.

B. Acetic acid bacteria

- Produce acetic acid, such as *Acetobacter aceti*.

C. Propionic acid bacteria

- Produce propionic acid (*Propionibacterium freudenreichii*).

D. Butyric acid bacteria

- Produce butyric acid in relatively large amounts.
- Some *Clostridium spp.* such as *Clostridium butyricum*.

E. Proteolytic bacteria

- Can hydrolyze proteins because they produce extracellular proteinases.
- *Micrococcus, Staphylococcus, Bacillus, Clostridium, Pseudomonas, Alteromonas, Flavobacterium, Alcaligenes*, some in *Enterobacteriaceae,* and *Brevibacterium*.

F. Lipolytic bacteria

- Able to hydrolyze triglycerides because they produce extracellular lipases.
- *Micrococcus, Staphylococcus, Pseudomonas, Alteromonas,* and *Flavobacterium*.

G. Saccharolytic bacteria

- Able to hydrolyze complex carbohydrates.
- *Bacillus, Clostridium, Aeromonas, Pseudomonas,* and *Enterobacter*.

H. Thermophilic bacteria

- Able to grow at 50°C and above.
- *Bacillus, Clostridium, Pediococcus, Streptococcus*, and *Lactobacillus*.

I. Psychrotrophic bacteria

- Able to grow at refrigerated temperature (≤5°C).
- *Pseudomonas, Alteromonas, Alcaligenes, Flavobacterium, Serratia, Bacillus, Clostridium, Lactobacillus, Leuconostoc, Carnobacterium, Listeria, Yersinia*, and *Aeromonas*.

J. Thermoduric bacteria

- Able to survive pasteurization temperature treatment.
- *Micrococcus, Enterococcus, Lactobacillus, Pediococcus, Bacillus* (spores), and *Clostridium* (spores).

K. Halotolerant bacteria

- Able to survive high salt concentrations (≥10%).
- *Bacillus, Micrococcus, Staphylococcus, Padiococcus, Vibrio,* and *Corynebacterium*.

L. Aciduric bacteria

- Able to survive at low pH (<4.0).
- *Lactobacillus, Pediococcus, Lactococcus, Enterococcus,* and *Streptococcus*.

M. Osmophlic bacteria

- Can grow at a relatively higher osmotic environment.
- Some species from genera *Leuconostoc, Staphylococcus* and *Lactobacillus*.

N. Gas-Producing bacteria

- Produce gas (CO_2, H_2, H_2S) during metabolism of nutrients.
- Species from genera *Leuconostoc, Lactobacillus, Propionibacterium, Escherichia, Clostridium*, and *Desulfotomaculum*.

O. Slime Producers

- Produce slime because they synthesise polysaccharides.
- *Xanthomonas, Leuconostoc, Alcaligenes, Enterobacter, Lactococcus,* and *Lactobacillus*.

P. Spore Formers

- Have the ability to produce spores.
- *Bacillus*, *Clostridium,* and *Desulfotomaculum.*

Q. Aerobes

- Require oxygen for growth and multiplication.
- *Pseudomonas, Bacillus*, and *Flavobacterium*.

R. Anaerobes

- Cannot grow in the presence of oxygen (*Clostridium*).

S. Facultative Anaerobes

- Able to grow in both the presence and absence of oxygen.
- *Lactobacillus, Pediococcus, Leuconostoc, enteric pathogens*, and some species of *Bacillus, Serratia*, and coliforms.

T. Coliforms

- Species from *Escherichia, Enterobacter, and Klebsiella.*

U. Fecal Coliforms

- Mainly *Escherichia coli* is included in this group.

V. Enteric Pathogens

- Pathogenic *Salmonella, Shigella, Campylobacter, Yersinia, Escherichia, Vibrio,* Listeria, and others that can cause gastrointestinal infection.

Sources of microorganisms in food

Introduction

- The internal tissues of healthy plants (fruits and vegetables) and animals (meat) 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 natural (including internal) sources and from external sources to which a food comes into contact from the time of production until the time of consumption.
- Beside natural microorganisms, a food can be contaminated with different types of microorganisms coming from outside sources such as air, soil, sewage, water, feeds, humans, food ingredients, equipment, packages, and insects.

Predominant microorganisms in different sources

A. Plants (fruits and vegetables)

- 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.
- Pathogens, especially of enteric types, can be present if the soil is contaminated with untreated sewage.
- Diseases of the plants, damage of the surface (before, during, and after harvest), long delay between harvesting and washing, and unfavorable storage and transport conditions after harvesting and before processing can greatly increase microbial numbers as well as predominant types.

- Improper storage conditions following processing can also increase their numbers.

B. 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, hair, and feathers.
- Many, as carriers, can harbor pathogens such as *Salmonella serovars, pathogenic Escherichia coli*, *Campylobacter jejuni, Yersinia enterocolitica,* and *Listeria monocytogenes* without showing symptoms.
- Laying birds have been suspected of asymptomatically carrying *Salmonella* in the ovaries and contaminating the yolk during ovulation.
- 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 and supplying contaminated water and feed (e.g., contaminated with salmonellae) can also change their normal microbial flora.

C. 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
    - 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.
- If the surroundings contain a source of pathogens (e.g., animal and poultry farms or a sewage treatment plant), different types of bacteria, including pathogens and viruses, can be transmitted via the air.

- ❖ Microbial contamination of food from the air can be reduced by controlling dust particles in the air (using filtered air), using positive air pressure, reducing humidity level, and installing UV light.

D. Soil

- ❖ Soil, especially the type used to grow agricultural produce and raise 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 (e.g., *Enterobacter, Pseudomonas, Proteus, Micrococcus, Enterococcus, Bacillus,* and *Clostridium*) 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.
- ❖ Removal of soil (and sediments) by washing and avoiding soil contamination can reduce microorganisms in foods from this source.

E. Sewage

- ❖ Sewage, especially when used as fertilizer in crops, can contaminate food with microorganisms, the most significant of which are different enteropathogenic bacteria and viruses.
- ❖ This can be a major concern with organically grown food and many imported fruits and vegetables, in which untreated sewage and manure might be used as fertilizer.
- ❖ Pathogenic parasites can also get in food 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 kill the pathogens.
- ❖ Also, effective washing of foods following harvesting is important.

F. Water

- ❖ Water is used to produce, process, and, under certain conditions, store foods.
- ❖ It is used for

- irrigation of crops
- drinking by food animals and birds
- raising fishery and marine products
- washing foods
- processing (pasteurization, canning, and cooling of heated foods)
- storage of foods (e.g., fish on ice)
- washing and sanitation of equipment and processing and transportation facilities.

- 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.

G.Humans

- Between production and consumption, foods come in contact with different people handling the foods.
- They include people working in farms and food processing plants, and those handling foods at restaurants.
- 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 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 generalized diseases (e.g., flu) can amplify the situation.

- ❖ In addition to spoilage bacteria, pathogens such as *Sta. aureus*, *Salmonella serovars*, *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.

H. Food ingredients

- ❖ In prepared or fabricated foods, many ingredients or additives are included in different quantities.
- ❖ Many of these ingredients can be the source of both spoilage and pathogenic microorganisms.
- ❖ Various spices generally have very high populations of mold and bacterial spores.
- ❖ The ingredients should be produced under sanitary conditions and given antimicrobial treatments.
- ❖ In addition, setting up acceptable microbial specifications for the ingredients will be important in reducing microorganisms in food from this source.

I. Equipment

- ❖ A wide variety of equipment is used in harvesting, slaughtering, transporting, processing, and storing foods.
- ❖ Many types of microorganisms from air, raw foods, water, and personnel can get into 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.
- *Salmonella, Listeria, Escherichia, Enterococcus, Micrococcus, Pseudomonas, Lactobacillus, Leuconostoc, Clostridium, Bacillus spp*., and yeasts and molds can get in food from equipment.

J. Miscellaneous

- Foods might be contaminated with microorganisms from several other sources, namely packaging and wrapping materials.
- Many types of packaging materials are used in food.
- Because 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.
- Any failure to produce microbiologically acceptable products can reduce the quality of food.

Microbiological standard of food

Introduction

Consumers want the assurance that they are receiving a safe sanitary food supply. Therefore, for centuries, governments throughout the world have regulated the food supply. Of the major regulatory agencies protecting the food supply is:

1. World Health Organization (WHO)
2. Food Agriculture Organization (FAO)
3. Food and Drug Administration (FDA)
4. American Public Health Association (APHA)
5. U.S. Department of Agriculture.

Adulterated and misbranded food

Adulterated and misbranded foods may not be offered for sale. According to the FDA, a food is adulterated if it:

- Is poisonous or harmful to health at detrimental concentrations.
- Contains filth of decomposed.
- Contains a food or coloring agent that is not approved or certified.
- Was prepared or packed under unsanitary conditions, making it contaminated.
- Is derived from a diseased animal.
- Contains any excessive levels of residue.
- Was subject to radiation, other than where permitted.
- Has any valuable constituent omitted.
- Substitutes a specified ingredient with an unspecified ingredient.
- Is damaged or conceals defects.
- Is increased in bulk weight or reduced in its strength, making it appear better than it is.

Bacteriological standard of food

Microbiological standard of meat

Type of meat	**Microbial Test**	**Maximum count in 1gram**
Fresh red meat	T.B.C. *E. coli* *Salmonella*	$1 - 5 \times 10^6$ 10-50 --------
Mincemeat red meat	T.B.C. *E. coli* *Salmonella*	$1 - 5 \times 10^7$ 50-100 --------
Frozen red meat	T.B.C. *E. coli* *Salmonella*	$1 - 5 \times 10^6$ --------- --------
Fresh and Frozen chicken	T.B.C. *E. coli* *Salmonella*	$1-5 \times 10^5$ 10-50
Fresh and Frozen Fish	T.B.C. Coliform *Staph. aureus*	$1-5 \times 10^5$ 200 100
Canned sardine	Thermophiles	100

Microbiological standard of milk and dairy production

Milk grade	Microbial Test	Maximum count in 1gram
(A)Raw milk	T.B.C Coliform	2×10^5 10
(A)Pasteurized milk	T.B.C Coliform	3×10^4 10
(B)Raw milk	T.B.C Coliform	1×10^6 10
(B)Pasteurized milk	T.B.C Coliform	5×10^4 10
Certified milk - Raw	T.B.C Coliform	1×10^4 10
- Pasteurized	T.B.C Coliform	500 1
Milk Powder - Extra quality	T.B.C Coliform	5×10^4 90
- Standard quality	T.B.C Coliform	1×10^5 90

Microbiological standard of butter

Butter	T.B.C	1×10^4
	Mold and Yeast	20
	Psychrophiles	1×10^4
	Coliform	10
	Streptococcus	10
	L.A.B	1×10^3
	P.A.B	50

Microbiological standard of ice cream

Ice Cream	T.B.C	10×10^{4}
	Coliform	10
	Staph. aureus	10
	Salmonella	-------

Microbiological standard of vegetables and fruits

Fresh vegetables	Coliform *Salmonella*	1×10^{6} ------------
Frozen and cooling vegetables	*E. coli* *Salmonella*	10 -----
Dry fruits	*E. coli*	3

Microbiological standard of wheat flour, the spices, and the cherries

Wheat flour	Molds *B.* spores *B. cereus* *Cl. Perfringens*	1×10^{2} 1×10^{2} 1×10^{3} 1×10^{2}
The spices	T.B.C Molds Coliform	1×10^{4} 1×10^{2} 10
The cherries	Molds Coliform	1×10^{2} 30

Common Microbial Spoilage of foods

Type of spoilage	Causing microbe	Description of spoilage
1.Color change	A. *Pseudomonas* spp. *Bacillus subtillus* *Flavobacterium* *Micrococcus* *Serratia* *Lactobacillus* B. Non-pigment yeast Pigment yeast *Rhodotorulla* C. Nearly all food molds	Fluorescent yellow, green pigmentation, Blue (pyocyanin) Black (melanin production) Yellow Rose Red Green White, Colorless Red or Pink = Wide range of soluble or insoluble pigment
2.Sliminess	*Erwinia* *Bacillus* spp. Molds *Pseudomonas viscose* *Leuconostoc mesenteroids*	Light slime (pectin lysis) = = Heavy slime (Dextran formation)
3.Chang in flavor and odor **a.** Destruction of natural flavor compounds of foods: 1. Diacetyl 2. Organic acid	 *Pseudomonas* spp. Many types of Bacteria	 Diacetyl → acetyl methyl carbinol Certain chemical compounds

<u>b</u>. Production of flavor compounds:		
1.Souring	*Lactobacillus* *Acetobacter* *Propionobacterium* *Clostridium butyricum*	Sugar→ Lactic acid Sugar→ Acetic acid Sugar→ Propionic acid Sugar→ Butyric acid
2.Bitterness	*Pseudomonas* spp. *Bacillus*	a. protein hydrolysis b. fatty acid degradation c. Sugar→ Aldonic acid
3.Sulphide odor	*Proteus* *Clostridium* *Deselfovibrio*	protein→ Sulphur A.A. →H_2S Sulphate reduction→ H_2S
4.Rancidity	Lipolytic bacteria & mold	Lipid→ F.A.→ Ketone → Aldehydes Tryptophane → indole
5.Unclean flavor	*E. coli*	Ether formation
6.Ethanol odor	*Pseudomonas* spp.	Production of odor
7.Earthiness	*Streptomyces*	Reaction between free amino acids & free fatty acids
8.Soapiness	*Pseudomonas* spp.	Production of flavor compounds
9.Fruit and vegetable flavor (potato, cabbage)	*Pseudomonas* spp.	

Microbial growth characteristics

Introduction

- ❖ Microorganisms grow or multiply in numbers when exposed to a favorable environment such as food.
- ❖ Their growth is associated with food spoilage, foodborne diseases, and food bioprocessing.

Natural of microbial growth in food

A. Mixed Population

- ❖ Normally, a food harbors a mixed population of microorganisms that can include different species and strains of bacteria, yeasts, and molds.
- ❖ Depending on the environment some of the species or strains can be in optimum or near-optimum growth condition.
  - ➢ Because of rapid rate of growth during storage, they will outnumber the others and become predominant.
  - ➢ This can occur even if they are initially present in low numbers.
  - ➢ This is often the case in foods kept for a long time under a specific condition, such as at refrigerated temperature.
- ❖ Another situation can arise if a food contains, among the mixed population, two species initially present in equal numbers and both growing optimally under the specific intrinsic and extrinsic environments of the food.
  - ➢ After a storage period, the one with shorter generation time becomes predominant.
  - ➢ Many foods most often get spoiled by bacteria than by yeasts and molds because, in general, bacteria have shorter generation time.

B. Sequence of Growth

- ❖ Among the different microbial types normally present in a food, different species can become predominant in sequence during storage.
- ❖ Initially, depending on the environment, one or two types may grow optimally and create an environment in which they can no longer grow rapidly.
- ❖ However, another type in the mixed population can find this changed environment favorable for growth and grow rather rapidly.

- This shift in predominance can occur several times during the storage of a food.

C. Growth in Succession or Diauxic Growth

- Microorganisms that can metabolize two or more nutrients in a food, one preferred over the other and present in limiting concentrations, show growth in stages separated by a short lag phase.
- Initially a bacterial strain grows by utilizing the preferred nutrient and after a short lag of adaptation grows by utilizing the other nutrient.
- During each stage, the growth curve has exponential and stationary phases with the lag phase in-between.
- An example is the growth of certain bacterial strains (such as some lactic acid bacteria and Gram-negative bacteria) in fresh meat.
    - A strain grows initially by utilizing the limiting concentrations of carbohydrate present, followed by utilization of nonprotein nitrogenous (NPN; such as amino acids) substances.

D. Symbiotic Growth

- Symbiosis or helping one another, during growth often occurs in food containing two or more types of microorganisms.
- One type may produce metabolic products that the second type needs for proper growth but cannot produce by itself.
- In turn, the second species produces a nutrient that stimulates the first one to grow better.
- This is found in the production of some fermented foods such as yogurt.
    - Initially, *Streptococcus thermophilus* hydrolyzes milk proteins by its extracellular proteinases and generates amino acids, which are necessary for good growth of *Lactobacillus delbrueckii*. *Lactobacillus*, in turn, produces formate, which stimulates the growth of *Streptococcus* species (both are necessary to produce a desirable product).

E. Synergistic Growth

- This is observed during symbiotic growth of two or more microbial types in a food.
- In synergistic growth, each type is capable of growing independently and producing some metabolites at lower rates.

- However, when the types are allowed to grow in a mixed population, both the growth rate and the level of by-product formation greatly increase.
- The increase is more than the additive of the amounts produced by growing the two separately.
    - For example, both *Str. thermophilus* and *Lab. delbrueckii* subsp. *bulgaricus*, when growing in milk independently, produce 8 to 10 ppm acetaldehyde, the desirable flavor component of yogurt. However, when growing together in milk, 30 ppm or more of acetaldehyde is produced, which is much higher than the additive amounts produced independently by the two species and is necessary for a good-quality yogurt.

F. Antagonistic Growth

- Two or more types of microorganisms present in a food can adversely affect the growth of each other; sometimes one can kill the other.
- This occasionally occurs due to the production of one or more antimicrobial compounds by one or more strains in the mixed population.
    - Some Gram-positive bacteria produce antibacterial proteins that can kill many other types of Gram-positive bacteria.
    - Similarly, some yeasts can produce wall-degrading enzymes and reduce the growth of molds.

Factors influencing microbial growth in food

Introduction

The ability of microorganisms (except viruses) to grow or multiply in a food is determined by the food environment as well as the environment in which the food is stored, designated as the intrinsic and extrinsic environment of food, respectively.

Intrinsic factors or food environment

Intrinsic factors of a food include nutrients, growth factors, and inhibitors (or antimicrobials), water activity, pH, and oxidation–reduction potential.

A. Nutrients and Growth

- Microbial growth is accomplished through the synthesis of cellular components and energy.
- The necessary nutrients for this process are derived from the immediate environment of a microbial cell (food).
- These nutrients include carbohydrates, proteins, lipids, minerals, and vitamins.
- Water is not considered a nutrient, but it is essential as a medium for the biochemical reactions necessary for the synthesis of cell mass and energy.
- Microorganisms normally present in food vary greatly in
- Nutrient requirements (bacteria requiring the most, followed by yeasts and molds).
- Their ability to utilize large and complex carbohydrates (e.g., starch), large proteins (e.g., casein in milk), and lipids.
- Molds are the most capable of doing this (by producing specific extracellular enzymes).

1. Carbohydrates in Foods

- Major carbohydrates present in different foods, either naturally or added as ingredients, can be grouped on the basis of chemical nature as follows:
- Monosaccharides
  - Hexoses: glucose, fructose, mannose, galactose
  - Pentoses: xylose, arabinose, ribose, ribulose, xylulose

- Disaccharides
  - Lactose (galactose + glucose)
  - Sucrose (fructose + glucose)
  - Maltose (glucose + glucose)
- Oligosaccharides
  - Raffinose (glucose + fructose + galactose)
  - Stachyose (glucose + fructose + galactose + galactose)
- Polysaccharides
  - Starch (glucose units)
  - Glycogen (glucose units)
  - Cellulose (glucose units)
  - Inulin (fructose units)
  - Hemicellulose (xylose, galactose, mannose units)
  - Dextrans (α-1, 6 glucose polymer)
  - Pectins
  - Gums and mucilages

- All microorganisms normally found in food metabolize glucose, but their ability to utilize other carbohydrates differs considerably.
- Molds are the most capable of using polysaccharides.
- Food carbohydrates are metabolized by microorganisms principally to supply energy through several metabolic pathways.
- Some of the metabolic products can be used to synthesize cellular components of microorganisms.
- Microorganisms also produce metabolic by-products associated with food spoilage (CO_2).
- Some are also metabolized to produce organic acids, such as lactic, acetic, propionic, and butyric acids, which have an antagonistic effect on the growth and survival of many bacteria.
- Microorganisms can also polymerize some monosaccharides to produce complex carbohydrates such as dextran, capsular materials, and cell wall (or outer membrane and middle membrane in Gram-negative bacteria).

- Some of these carbohydrates from pathogens may cause health hazards (forming complexes with proteins), some may cause food spoilage (such as slime defect).

2. Proteins in Foods

- The major proteinaceous components in foods are simple proteins, conjugated proteins, peptides, and nonprotein nitrogenous (NPN) compounds (amino acids, urea, ammonia, creatinine, trimethylamine).
- Proteins and peptides are polymers of different amino acids without or with other organic (e.g., a carbohydrate) or inorganic (e.g., iron) components and contain 15 to 18% nitrogen.
- Simple food proteins are polymers of amino acids, such as albumins (in egg), globulins (in milk), glutelin (gluten in cereal), prolamins (zein in grains), and albuminoids (collagen in muscle).
- They differ greatly in their solubility, which determines the ability of microorganisms to utilize a specific protein.
- Many microorganisms can hydrolyze albumin, which is soluble in water.
- In contrast, collagens, which are insoluble in water or weak salt and acid solutions, are hydrolyzed only by a few microorganisms.
- Proteins are present in higher quantities in foods of animal origin than in foods of plant origin.
- But plant foods, such as nuts and legumes, are rich in proteins.
- Microorganisms differ greatly in their ability to metabolize food proteins.
- Most transport amino acids and small peptides in the cells; small peptides are then hydrolyzed to amino acids inside the cells, such as in some *Lactococcus* spp.
- Microorganisms also produce extracellular proteinases and peptidases to hydrolyze large proteins and peptides to small peptides and amino acids before they can be transported inside the cells.
- Soluble proteins are more susceptible to this hydrolytic action than are the insoluble proteins.
- Hydrolysis of food proteins can be either undesirable (texture loss in meat) or desirable (flavor in cheese).

- Amino acids inside microbial cells are metabolized via different pathways to synthesize cellular components, energy, and various by-products.
- Many of these byproducts can be undesirable (e.g., NH_3 and H_2S production causes spoilage of food, and toxins and biological amines cause health hazards) or desirable (e.g., some sulfur compounds give cheddar cheese flavor).

3. Lipids in Foods

- Lipids in foods include compounds that can be extracted by organic solvents, some of which are free fatty acids, glycerides, phospholipids, waxes, and sterols.
- Lipids are relatively higher in foods of animal origin than in foods of plant origin, although nuts, oil seeds, coconuts, and olives have high amounts of lipids.
- Lipids are, in general, less preferred substrates for the microbial synthesis of energy and cellular materials.
- Many microorganisms can produce extracellular lipases, which can hydrolyze glycerides to fatty acids and glycerol.
- Some microorganisms also produce extracellular lipid oxidases, which can oxidize unsaturated fatty acids to produce different aldehydes and ketones.
- In general, molds are more capable of producing these enzymes.
- However, certain bacterial groups such as *Pseudomonas, Achromobacter*, and *Alcaligenes* can produce these enzymes.
- Lysis of dead microbial cells in foods causes release of intracellular lipases and oxidases, which then can carry out these reactions.
- In many foods the action of these enzymes is associated with spoilage (such as rancidity), whereas in other foods the enzymes are credited for desirable flavors (such as in mold-ripened cheeses).
- Some beneficial intestinal microorganisms, such as *Lactobacillus acidophilus* strains, can metabolize cholesterol and are believed to be capable of reducing serum cholesterol levels in humans.

4. Minerals and Vitamins in Foods

- Microorganisms need several elements in small amounts, such as phosphorous, calcium, magnesium, iron, sulfur, manganese, and potassium.
- Most foods have these elements in sufficient amounts.
- Many microorganisms can synthesize B vitamins, and foods also contain most B vitamins.

- In general, most foods contain different carbohydrates, proteins, lipids, minerals, and vitamins in sufficient amounts to supply necessary nutrients for the growth of molds, yeasts, and bacteria, especially Gram-negative bacteria normally present in foods.
- Some foods may have limited amounts of one or a few nutrients for rapid growth of some Gram-positive bacteria, especially some fastidious *Lactobacillus* species.
- It is not possible or practical to control microbial growth in a food by restricting nutrients.

B. Growth Factors and Inhibitors in Food

- Foods can also have some factors that either stimulate growth or adversely affect growth of microorganisms.
- The growth factors are naturally present in some foods.
  - An example is the growth factors in tomatoes that stimulate growth of some *Lactobacillus* species.
- Foods also contain many chemicals, either naturally or added, that adversely affect microbial growth.
  - Some of the natural inhibitors are lysozyme in egg, agglutinin in milk, and eugenol in cloves.
  - The inhibitors, depending on their mode of action, can prevent or reduce growth of and kill microorganisms.

C. Water Activity and Growth

Principle:

- In a food system, total water or moisture is present in free and bound forms.

- Bound water is the fraction used to hydrate hydrophilic molecules and to dissolve solutes, and is not available for biological functions; thus, it does not contribute to *Aw*.
- Water activity (*Aw*) is a measure of the availability of water for biological functions and relates to water present in a food in free form.
- The *Aw* of a food can be expressed by the ratio of water vapor pressure of the food (*P*, which is <1) to that of pure water (*Po*, which is 1), i.e., *P*/*Po*.

Aw of Food:

- The *Aw* of food ranges from 0.1 to 0.99.
- The *Aw* values of some food groups are as follows:
  - cereals, sugar, salt, dry milk, 0.10 to 0.20
  - honey, chocolate, dried egg, <0.60
  - jam, jelly, dried fruits, parmesan cheese, nuts, 0.60 to 0.85
  - fermented sausage, dry cured meat, sweetened condensed milk, 0.85 to 0.93
  - evaporated milk, tomato paste, bread, fruit juices, salted fish, sausage, processed cheese, 0.93 to 0.98
  - fresh meat, fish, fruits, vegetables, milk, eggs, 0.98 to 0.99.
- The *Aw* of foods can be reduced by removing water (desorption) and increased by the adsorption of water.
- The *Aw* of a food can be reduced by several means, such as adding solutes, ions, hydrophilic colloids, and freezing and drying.

Aw and Microbial Growth:

- The free water in a food is necessary for microbial growth.
- Each microbial species (or group) has an optimum, maximum, and minimum *Aw* level for growth.
- In general, the minimum *Aw* values for growth of microbial groups are as follows:
  - most molds, 0.8, with xerophilic molds as low as 0.6
  - most yeasts, 0.85, with osmophilic yeasts, 0.6 to 0.7
  - most Gram-positive bacteria, 0.90
  - Gram-negative bacteria, 0.93.

- Some exceptions are growth of *Staphlococcus aureus* at 0.85 and halophilic bacteria at 0.75.

- The *Aw* need for spore-forming bacteria to sporulate and the spores to germinate and the toxin-producing microorganisms to produce toxins is generally higher than the minimum *Aw* for their growth.
- As an example, if minimum *Aw* for growth of a bacterial strain at pH 6.8 is 0.91, then at pH 5.5, it can be 0.95 or more.

D. pH and Growth

Principle:

- pH indicates the hydrogen ion concentrations in a system and is expressed as – log [H+], the negative logarithm of the hydrogen ion or proton concentration.
- It ranges from 0 to 14, with 7.0 being neutral pH.

pH of Food:

- On the basis of pH, foods can be grouped as high-acid foods (pH below 4.6) and low-acid foods (pH 4.6 and above).
- Most fruits, fruit juices, fermented foods (from fruits, vegetables, meat, and milk), and salad dressings are high-acid (low-pH) foods, whereas most vegetables, meat, fish, milk, and soups are low-acid (high-pH) foods.
  - Tomato, however, is a high-acid vegetable (pH 4.1 to 4.4).
- The acid in the foods can either be present naturally (as in fruits), produced during fermentation (as in fermented foods), or added during processing (as in salad dressings).

pH and Microbial Growth:

- The pH of a food has a profound effect on the growth and viability of microbial cells.
- Each species has an optimum and a range of pH for growth.
  - In general, molds and yeasts are able to grow at lower pH than do bacteria, and Gram-negative bacteria are more sensitive to low pH than are Gram-positive bacteria.
- The pH range of growth
  - for molds is 1.5 to 9.0
  - for yeasts, 2.0 to 8.5

- for Gram-positive bacteria, 4.0 to 8.5
- for Gram-negative bacteria, 4.5 to 9.0.

- Individual species differ greatly in lower pH limit for growth; for example, *Pediococcus acidilactici* can grow at pH 3.8 and *Sta. aureus* can grow at pH 4.5, but normally *Salmonella* cannot.
- When the pH in a food is reduced below the lower limit for growth of a microbial species, the cells not only stop growing but also lose viability.

E. Redox Potential, Oxygen, and Growth

Principle:

- The redox or oxidation–reduction (O–R) potential measures the potential difference in a system generated by a coupled reaction in which one substance is oxidized and a second substance is reduced simultaneously.
- The process involves the loss of electrons from a reduced substance (thus it is oxidized) and the gain of electrons by an oxidized substance (thus it is reduced).
- The redox potential, designated as Eh, is measured in electrical units of millivolts (mV).
- In biological systems, the oxidation and reduction of substances are the primary means of generating energy.
- If free oxygen is present in the system, then it can act as an electron acceptor.
- In the absence of free oxygen, oxygen bound to some other compound, such as NO_3 and SO_4, can accept the electron.
- In a system where no oxygen is present, other compounds can accept the electrons.
- Thus, presence of oxygen is not a requirement of O-R reactions.

Redox Potential in Food:

- The redox potential of a food is influenced by its chemical composition, specific processing treatment given, and its storage condition (in relation to air).

- Fresh foods of plant and animal origin are in a reduced state, because of the presence of reducing substances such as ascorbic acid, reducing sugars, and –SH group of proteins.
- Processing, such as heating, can increase or decrease reducing compounds and alters the Eh.
- A food stored in air will have a higher Eh than when it is stored under vacuum or in modified gas (such as CO_2 or N_2).
- Oxygen can be present in a food in the gaseous state or in dissolved form.

Redox Potential and Microbial Growth:

- Growth of microorganisms and their ability to generate energy by the specific metabolic reactions depend on the redox potential of foods.
- The Eh range at which different groups of microorganisms can grow are as follows:
  - aerobes, +500 to +300 mV
  - facultative anaerobes, +300 to +100 mV
  - anaerobes, +100 to –250 mV or lower.
- However, this varies greatly with concentrations of reducing components in a food and the presence of oxygen.
- Molds, yeasts, and *Bacillus, Pseudomonas, Moraxella*, and *Micrococcus* genera are some examples that have aerobic species.
- Some examples of facultative anaerobes are the lactic acid bacteria and those in the family *Enterobacteriaceae*.
- The most important anaerobe in food is *Clostridium*.
- An example of a microaerophile is *Campylobacter* spp.
- The presence or absence of oxygen and the Eh of food determine the growth capability of a particular microbial group in a food and the specific metabolic pathways used during growth to generate energy and metabolic by-products.
- This is important in microbial spoilage of a food (such as putrification of meat by *Clostridium* spp. under anaerobic conditions).

External Factors

- Extrinsic factors important in microbial growth in a food include the environmental conditions in which it is stored.

- These are temperature, relative humidity, and gaseous environment.
- The relative humidity and gaseous condition of storage, respectively, influence the *Aw* and Eh of the food.

A. Temperature and Growth

Principle:

- Microbial growth is accomplished through enzymatic reactions.
- It is well known that within a certain range, with every 10°C rise in temperature, the catalytic rate of an enzyme doubles.
- This relationship changes beyond the growth range.
- Because temperature influences enzyme reactions, it has an important role in microbial growth in food.

Food and Temperature:

- Depending on processing conditions, a food can be exposed to high heat (65°C - 100°C).
- For long-term storage, a food can be kept at 5°C (refrigeration) to –20°C or below (freezing).
- Some relatively stable foods are also kept between 10 and 35°C.
- Some ready-to-eat foods are kept at warm temperature (50° to 60°C) for several hours (in the supermarket).

Microbial Growth and Viability:

- Microorganisms important in foods are divided into three groups on the basis of their temperature of growth:
  - thermophiles (grow at relatively high temperature), with optimum 55°C and range 45 to 70°C.
  - mesophiles (grow at ambient temperature), with optimum at 35°C and range 10 to 45°C.
  - psychrophiles (grow at cold temperature), with optimum at 15°C and range –5 to 20°C.
- Two other terms used in food microbiology are very important with respect to microbial growth at refrigerated temperature and survival of microorganisms to low heat treatment or pasteurization, because both methods are widely used in the storage and processing of foods.

- **Psychrotrophs** are microorganisms that grow at refrigerated temperature (0 to 5°C), irrespective of their optimum range of growth temperature.
- They usually grow rapidly between 10 and 30°C.
- Molds; yeasts; many Gram-negative bacteria from genera *Pseudomonas, Achromobacter, Yersinia, Serratia*, and *Aeromonas*; and Gram- positive bacteria from genera *Leuconostoc, Lactobacillus, Bacillus, Clostridium*, and *Listeria* are included in this group.
- Microorganisms that survive pasteurization temperature are designated as **thermodurics**.
- They include species from genera *Micrococcus, Bacillus, Clostridium, Lactobacillus, Pediococcus*, and *Enterococcus*.
- Bacterial spores are also included in this group.
- When the foods are exposed to temperatures beyond the maximum and minimum temperatures of growth, microbial cells die rapidly at higher temperatures and relatively slowly at lower temperatures.
- Microbial growth and viability are important in reducing food spoilage and enhancing safety against pathogens, as well as in food bioprocessing.

Microbial food spoilage

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.

- **Important factors in microbial food spoilage**
- **Spoilage of Specific Food Groups**
- **Food Spoilage by Microbial Enzymes**

Important factors in microbial food spoilage

- Microbial food spoilage occurs as a consequence of either microbial growth in a food or release of microbial extracellular and intracellular (following cell lysis) enzymes in the food environment.
- Spoilage by microbial growth occurs much faster than spoilage by microbial extra- or intracellular enzymes in the absence of viable microbial cells.

Significance of microorganisms

A. Microbial types

- Foods normally contain many types of molds, yeasts, and bacteria capable of multiplying and causing spoilage (Viruses do not multiply in foods).
- The highest incidence of spoilage, especially rapid spoilage, of processed foods is caused by bacteria (because of shorter generation time), followed by yeasts and molds.

B. Microbial numbers

- To produce detectable changes in a food, microorganisms (mainly bacteria and yeasts) must multiply and attain certain levels, often referred to as the "spoilage detection level."
- Depending on the specific nature of spoilage and microbial types, the spoilage detection level can range from 10^{6-8} cells/g, /ml, or /cm^2.

❖ In the hypothetical example (Figure.1), the population reached the spoilage detection level within 7 d with a high initial load (5×10^5/g) as opposed to 20 d with a low initial load (5×10^2/g) during storage at 12°C. However, when the product with a low initial load was stored at 4°C (to increase the generation time), it took 55 d for the spoilage bacteria to reach the spoilage detection level.

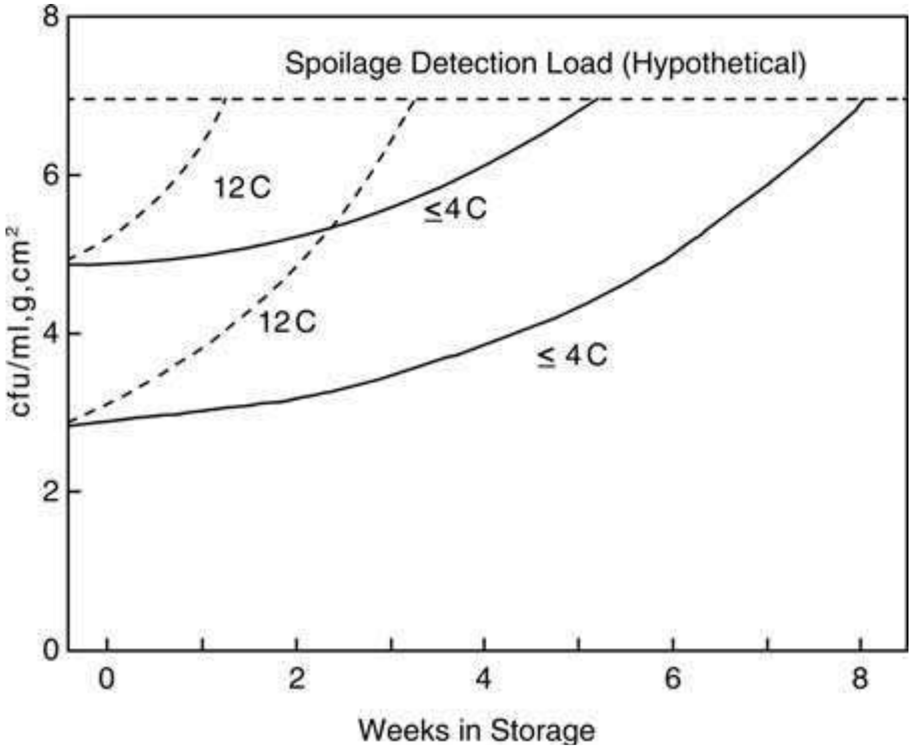

Figure .1 Graphical illustration showing the influence of initial bacterial levels and storage temperatures on the shelf life of a refrigerated product.

C. Predominant microorganisms

❖ Among the different species initially present and capable of growing in a particular food, only those with the shortest generation time under the storage conditions attain the numbers rapidly and cause spoilage.

❖ In a study, a beef sample (pH 6.0) was found to initially contain 10^3 bacterial cells/g, with relative levels of *Pseudomonas spp*. 1%, *Acinetobacter* and *Morexella* 11%, *Brochothrix thermosphacta* 13%, and others (*Micrococcus, Staphylococcus, Enterobacteriaceae*, lactic acid bacteria, etc.) 75%. Following aerobic storage at 2°C for 12 d, the population reached 6×10^7 cells/g, with the relative levels of *Pseudomonas spp*. 99% and all others 1%.

Some important food spoilage bacteria

Bacterial species from only several genera have been implicated more with spoilage of most foods.

A. Psychrotrophic bacteria

- Capable of growing at 5°C and below, but multiply quite rapidly at 10 to 25°C and even at higher temperatures.
- Psychrotrophic bacteria (also many yeasts and molds that are psychrotrophic) can cause spoilage in foods stored on ice and in refrigerators.

1. If the food is stored under aerobic conditions, psychrotrophic aerobes are the predominant spoilage bacteria.
    - They include *Pseudomonas fluorescens*, *Pse. fragi*, *Acinetobacter*, *Moraxella*, and *Flavobacterium* (Some molds and yeasts are included in this group.)
2. In foods stored under anaerobic conditions, anaerobic and facultative anaerobic bacteria predominate.
    - They include *Lactobacillus* spp., *Alcaligenes* spp., *Leuconostoc* spp., and some *Enterococcus* spp., *Enterobacter* spp., *Serratia liquifaciens*, and *Proteus* spp., (and some microaerophilic yeasts).
3. If the food is given low-heat treatment before storage at low temperature, psychrotrophic thermoduric bacteria can cause it to spoil.
    - They include
        - Facultative anaerobes, such as spores of *Bacillus coagulans* and *Bac. megaterium*, some strains of *Lab. viridescens*
        - Anaerobes, such as spores of *Clostridium* spp.

B. Thermophilic bacteria

- Grow between 40 - 90°C, with optimum growth at 55 to 65°C.
- Microorganisms can multiply in warm foods and cause spoilage are:
    1. Spores of some thermophilic *Bacillus* and *Clostridium* spp. can be present in heat-treated foods, which at warm temperature germinate and multiply
    2. Some thermoduric vegetative bacteria surviving low-heat processing (such as pasteurization)
    3. Thermophiles getting in food as post heat contamination

C. Aciduric bacteria

- Can grow relatively rapidly in food at pH 4.6 or below.
- Lactic acid bacteria, yeasts and molds have been associated with such spoilage.

Significance of foods

A. Food types

- Foods differ greatly in their susceptibility to spoilage by microorganisms. This is mainly because of their differences in intrinsic factors (Aw, pH, O–R potential, nutrient content, antimicrobial substances, and protective structures).
- Based on susceptibility of spoilage, foods can be grouped as
  - **Perishable** (spoil quickly, in days)
  - **Semiperishable** (have a relatively long shelf life, few weeks, or months)
  - **Nonperishable** (have a very long shelf life, many months, or years).

B. Food nutrients

- Microorganisms differ greatly in their abilities to metabolize different food nutrients.
- The same nutrient (substrate) can be degraded to produce different end products under aerobic and anaerobic metabolism.
  - Glucose is metabolized (catabolized) by *Micrococcus* spp. aerobically to produce CO_2 and H_2O, and by *Lab, acidophilus* anaerobically to produce mainly lactic acid.
  - *Saccharomyces cerevisiae* metabolizes glucose aerobically to CO_2 and H_2O, but anaerobically to ethanol and CO_2.
- Some microorganisms can also synthesize polymeric compounds as end products, such as dextran production by *Leu. mesenteroides* while metabolizing sucrose.
- Some microorganisms can also secrete extracellular enzymes to break down large molecular nutrients in a food (such as breakdown of starch by amylase produced by some molds).
- Some microorganisms can synthesize pigments while growing in a food (such as *Micrococcus luteus* producing a yellow pigment).

Table .1 lists some of the end products of microbial metabolism of food nutrients that are attributed to food spoilage. The end products vary with the nature of metabolism (e.g., aerobic respiration, anaerobic respiration, or fermentation).

Table.1 Some End Products from Microbial Metabolism of Food Nutrients.

Food Nutrient	End Products
Carbohydrates	CO_2, H_2, H_2O_2, lactate, acetate, formate, succinate, butyrate, isobutyrate, isovalerate, ethanol, propanol, butanol, isobutanol, diacetyl, acetoin, butanediol, dextran, levans
Proteinaceous and NPN compounds	CO_2, H_2, NH_3, H_2S, amines, keto-acids, mercaptans, organic disulfides, putrescine, cadaverine, skatole
Lipids	Fatty acids, glycerol, hydroperoxides, carbonyl compounds (aldehydes, ketones), nitrogenous bases

C. Utilization of food nutrients

- In general, for energy production, microorganisms prefer to use metabolizable monosaccharides, disaccharides, and large carbohydrates first; followed by NPN, small peptides, and large proteinaceous compounds; and finally, lipids.
- However, again metabolic characteristics depend on whether a particular species can use a specific carbohydrate (such as the ability or inability to utilize lactose) and the concentration of it present.
- Also, with any nutrient, small molecules are used first before large molecules (polymers).
- *Pse. fluorescens* growing aerobically in fresh meat with limited amounts of glucose will first metabolize it and then start metabolizing free amino acids and other NPN compounds. If it is allowed to grow for a long time, it will produce extracellular proteinases to break down meat proteins to produce small peptides and amino acids for further metabolism. With time, it may even be able to produce lipases to break down meat lipids and use up some fatty acids.

D. Microbial growth in succession

- Intrinsic and extrinsic factors or environments of a food dictate which, among the mixed microbial species normally present, will multiply rapidly and become predominant to cause spoilage.
- However, as the predominant types grow, they produce metabolites and change the food environment.
- In the changed environment, some other species, initially present but previously unable to compete, may be in a favorable position to grow rapidly and again change the food environment further to enable a third type to grow rapidly.
- Sequential growth of *Lactococcus spp*., aciduric lactose-negative *Bacillus sp*., and Gram-negative rods (such as *Pseudomonas* spp.) in a milk sample can be used as a hypothetical example.
  - -Initially, rapid growth of *Lactococcus spp*. (able to metabolize lactose) under a favorable growth condition will reduce the pH from original 6.5 to 5.0 and reduce the growth rate of many other microbial species present.

    -As the pH drops below 5.0 the generation time of *Lactococcus spp*. becomes longer.

    -However, *Bacillus* sp., because of its aciduric nature, can then starts multiplication, metabolize proteins, and increase the pH.

    -In the high pH, the *Pseudomonas* spp. present initially can then grow by metabolizing NPN and proteinaceous compounds and increase the pH further by producing basic metabolites (amines, NH_3).

Spoilage of Specific Food Groups

Introduction

- ❖ The relative numbers (population level) of a specific type of microorganism initially present (without growth) in a food depend on the intrinsic and extrinsic conditions to which the food is exposed.
- ❖ Growth of some of the microbial species among those initially present enables the microorganisms to reach the spoilage detection level.
  ❖ Microbial types, food types and food environments have important roles in determining the predominant spoilage microflora in a food.

Raw and ready-to-eat meat products

Normal microbiological quality and its significance

- ❖ Normally, carcasses contain an average of $10^{1\text{-}3}$ bacterial cells / in^2.
- ❖ Different enteric pathogens, *Salmonella serovars, Yersinia enterocolitica, Campylobacter jejuni, Escherichia coli, Clostridium perfringens,* and *Staphylococcus aureus,* both from animals or birds and humans, can be present, but normally at a low level.
- ❖ Carcasses of birds, as compared with those of animals, generally have a higher incidence of *Salmonella* contamination coming from fecal matter.
- ❖ Chilled meat has mesophiles, such as *Micrococcus, Enterococcus, Staphylococcus, Bacillus, Clostridium, Lactobacillus, coliforms,* and other Enterobacteriaceae, including enteric pathogens.
- ❖ However, because the meats are stored at low temperature (–1 to 5°C), the psychrotrophs constitute major problems.
  - ➢ The predominant psychrotrophs in raw meats are some *lactobacilli* and *leuconostocs, Brochothrix thermosphacta, Clost. laramie*, some *coliforms, Serratia, Pseudomonas, Alteromonas, Achromobacter, Alcaligenes, Acinetobacter, Morexella, Aeromonas,* and *Proteus*.
  - ➢ Psychrotrophic pathogens include *Listeria monocytogenes* and *Yer. enterocolitica*. The microbial load of fresh meat varies greatly, with bacteria predominating.
- ❖ The pH of the meat (which is low in beef, 5.6, but high in birds, 6.0), high protein content, and low carbohydrate level, along with the environment, determine which types predominate during storage.

- ❖ Heat treatment, especially at an internal temperature of 70°C or higher, kills most microorganisms, except some thermodurics, which include *Micrococcus*, some *Enterococcus*, and maybe some *Lactobacillus* and spores of *Bacillus* and *Clostridium*.

Spoilage of meat

- ❖ Fresh meats from food animals and birds contain a large group of potential spoilage bacteria that include species of *Pseudomonas, Acinetobacter, Moraxella, Shewanella, Alcaligenes, Aeromonas, Escherichia, Enterobacter, Serratia, Proteus, Micrococcus, Enterococcus, Lactobacillus, Leuconostoc, Carnobacterium*, and *Clostridium*, as well as yeasts and molds.
- ❖ Different enteric pathogens can be present, but normally at a low level (*Salmonella serovars, Yersinia enterocolitica, Campylobacter jejuni, Escherichia coli, Clostridium perfringens,* and *Staphylococcus aureus)*
- ❖ Spoilage by strict aerobes in the form of
    - ➢ Off odor is detected at a population of 10^{7-8} cells/cm^2
    - ➢ Slime at 10^{8-9} cells/cm^2.
    - ➢ *Acinetobacter* and *Morexella* metabolize amino acids and produce undesirable odors.
- ❖ In vacuum-packaged, *Lactobacillus curvatus* and *Lab. sake*
    - ➢ Metabolize glucose to produce lactic acid
    - ➢ Metabolize the amino acids leucine and valine to isovaleric and isobutyric acids (cheesy odor). This spoilage is not considered to be highly undesirable.
    - ➢ However, when they metabolize cysteine and produce H_2S gas, the products have undesirable odor and color.

-Read meat

Refrigerated meat microbial spoilage includes:

1. Off odor and slime
    - Off odor is the first symptom of meat spoilage followed by formation of slime layer on the surface.
    - Microorganisms responsible of this spoilage are psychrotrophs e.g., *Pseudomonas* species (80%), *Achromobacter, Flavobacterium, Micrococcus, Serratia* and *Lactobacillus*.

2. Discoloration

	Microorganisms	**color**
Bacteria	*Pseudomonas mephitica*	Green spots
	Pseudomonas pyocyanea	Blue spots
	Pseudomonas geniculata	Dark brown
	Flavobacterium, Micrococcus	Yellow
	Serratia	red
molds	*Cladosporium herbarum*	Black spots
	Thamnidium elegans	White spots
	Penicillum	Green spots
yeasts	*Rhodotorula*	Pink spots

3. Putrefaction and Rancidity
   - Putrefaction occurs under anaerobic conditions
     Proteins → proteinases → NH_3 or H_2S…
   - Rancidity
     Lipids -------→ fatty acids (e.g., butyric acid) + glycerol
   - Putrefaction and rancidity caused mainly by *Pseudomonas* spp. (because of their ability to produce enzymes even in low temperature).

4. Meat souring
   - Sugar (low level) -------→ organic acid
   - Rarely occurs in refrigerated meat (by psychrotrophic lactic acid bacteria).
   - Occurring at room temperature by mesophilic bacteria (e.g., *E. coli* and some lactic acid bacteria).

-Poultry

❖ Not differs from red meat spoilage.

❖ Carcasses of birds generally have a higher incidence of *Salmonella* contamination.

❖ Microbial flora are *Pseudomonas, Flavobacterium, Achromobacter, Alcaligenes, Micrococcus, Microbacterium, Lactobacillus* and also *Proteus,* *Bacillus, Escherichia, Salmonella, Streptococcus, Corynebacterium, Staphylococcus…*

- ❖ Caused 10% of foodborne diseases e.g.
  - ➢ *Salmonella* infection ….1/2
  - ➢ *Clostridium perfringens* poisoning ….1/4
  - ➢ *Staphylococcus* poisoning …. 1/4

-Fish and shellfish

Normal microbiological quality and its significance

- ❖ The microbial population in these products varies greatly with the pollution level and temperature of the water.
- ❖ Bacteria from many groups, as well as viruses, parasites, and protozoa, can be present in the raw materials.
- ❖ Products harvested from marine environments can have halophilic vibrios as well as *Pseudomonas, Alteromonas, Flavobacterium, Enterococcus, Micrococcus, coliforms,* and pathogens such as *Vib. parahaemolyticus, Vib. vulnificus*, and *Clost. botulinum*.
- ❖ Freshwater fish generally have *Pseudomonas, Flavobacterium, Enterococcus, Micrococcus, Bacillus,* and coliforms.
- ❖ Fish and shellfish harvested from water polluted with human and animal waste can contain *Salmonella, Shigella, Clost. perfringens, Vib. cholerae*, and hepatitis A and Norwalk-like viruses.

Following harvest, microorganisms can grow rapidly in fish and crustaceans because of high Aw and high pH of the tissue and availability of large amounts of nonprotein nitrogenous compounds.

Spoilage of fish

- ❖ Fish are susceptible to spoilage through:
  - ➢ Protein hydrolysis by autolytic enzymes (proteinases).
  - ➢ Oxidation of unsaturated fatty acids (high in fatty fish).
  - ➢ Microbial growth (protein putrefaction and lipid rancidity).
- ❖ Microbial spoilage is determined by the microbial types, their level, fish environment, fish types, methods used for harvest, and subsequent handling.

- ➢ Clean water → low number of normal flora e.g., *Pseudomonas, Flavobacterium, Vibrio…*
- ➢ Rivers and lakes → normal flora+ *E. coli, Bacillus, Clostridium, Lactobacillus*, ...
- ➢ Polluted water → *Salmonella, Shigella* (pathogen)

- ❖ 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.
- ❖ However, because of the relatively shorter generation time, spoilage by psychrotrophic *Pseudomonas* spp. predominates under aerobic storage at both refrigerated and slightly higher temperature.

Fresh fish	Spoiled fish
1. Bright surface (light color)	1. Dark color
2. Covered with light mucous layer	2. Mucous increased
3. Bright eyes	3. Eyes losses brightness
4. Pink or red gills	4. Gills color turns to gray
5. Strong skin and meat, with fresh fishy smell	5.Weak skin and meat, with putrefactive smell

Shell egg and liquid egg

Normal microbiological quality and its significance

- ❖ Each shell, depending on the contamination level, can have 10^7 bacteria.
- ❖ Washing helps reduce bacterial level considerably.
- ❖ Eggshells can harbor different types of bacteria, namely *Pseudomonas, Alcaligenes, Proteus, Citrobacter, E. coli, Enterobacter, Enterococcus, Micrococcus*, and *Bacillus*.
- ❖ They can also have *Salmonella* from fecal contamination.

Spoilage of eggs

- ❖ Bacteria, especially motile Gram-negative, can enter through pores of eggshells, particularly if the shells are wet.

- Several antimicrobial factors present in egg albumin, such as lysozyme, conalbumin (binds iron), avidin (binds biotin), or alkaline pH (8.0 to 9.0), can control bacterial 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*
  - 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 and produce different types of fungal rots.

Raw and pasteurized milk

Normal microbiological quality and its significance

- Milk is high in proteins and carbohydrates (lactose), which many microorganisms can utilize for growth.
- Because both raw milk and pasteurized milk contain many types of bacteria as predominant microorganisms, they are refrigerated; yet they have limited shelf life.
- In raw milk, microorganisms come from inside the udder, animal body surfaces, feed, air, water, and equipment used for milking and storage.
  - The predominant types from inside a **healthy udder** are *Micrococcus, Streptococcus*, and *Corynebacterium*.
  - If a cow has **mastitis**, *Streptococcus agalactiae, Sta. aureus, coliforms*, and *Pseudomonas* can be excreted in relatively high numbers.
- During refrigerated storage before pasteurization, only psychrotrophs can grow in raw milk.
  - They include *Pseudomonas, Flavobacterium, Alcaligenes,* and some *coliforms* and *Bacillus spp*.

- They can affect the acceptance quality of raw milk (e.g., by making flavor and texture undesirable).
- Some of them can produce heat-stable enzymes (proteinases and lipases).

❖ Microorganisms present in pasteurized milk are those that survive pasteurization of raw milk (e.g., the thermodurics) and those that enter after heating and before packaging (e.g., post-pasteurization contaminants).

➢ Thermodurics surviving pasteurization include *Micrococcus*, some *Enterococcus* (e.g., *Ent. faecalis*), *Streptococcus*, some *Lactobacillus* (e.g., *Lab. viridescens*), and spores of *Bacillus* and *Clostridium*.

Spoilage of milk and milk products

A. Raw milk

- 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.
- Contaminated microorganisms are
  1. *Mycobacterium bovis* → caused tuberculosis (t. b) in cows. Killed by pasteurization.
  2. *Coxiella burnetti* → caused Q fever.
  3. *Brucella* → Malta fever. (Source animal udder)
  4. *Staph. aureus* → excreted intestinal toxins. (Caused udder inflammation, then transmitted to milk)
- Microbial spoilage of raw milk:
  1. Low acidity without curd (some coccus bacteria).
  2. Acid curd (caused by *Streptococcus* & *Lactobacillus*)
     Strep. & *Lact.* → Lactic acid (↓pH) → casein↓ → whey (contain globulin and lacto albumin) & solid curd without gases.
  3. Sweet curd (caused by *Bacillus subtilis, B. cereus, Pseudomonas putrefaciens, Streptococcus liquefaciens*).
     They are able to exert rennin enzyme which precipitate casein.
     The curd is soft (loos), and the whey produced have rankness (putrefaction smell).

4. Gassy curd (caused by coliforms bacteria and some yeasts which produce acids and gasses)
5. Putrefaction & Rancidity
   a) Putrefaction caused by bacteria able to produce caseinase.
   b) Rancidity caused by bacteria able to produce lipase
      Lipids → fatty acids + rancid test.

   The two reactions need acidic environment.
6. Ropiness (caused by *Pseudomonas viscosa* and *Alcaligenes viscolactic*)
7. Change of test and color

Change of test

Souring	Lactic acid bacteria
Bitter flavor	Hydrolysis of protein & lipid
Carmel flavor	*Streptococcus lactis*
Fishy flavor	*Pseudomonas icthyosmia*
Fruity flavor	*Pseudomonas perolens*
Earthy flavor	*Streptomyces*

Change of color

Pseudomonas pyocyaniae	blue
Pseudomonas fluorescens	Green-yellow
Serratia marcescens & *Micrococcus roseus*	red
Micrococcus luteus & *Flavobacterium*	yellow

B. Cheese

❖ Spoilage caused by psychrotrophic bacteria

Pseudomonas viscosa	Dark color with brown and yellow viscose materials. Stinky smell and bitter test
Pseudomonas fluorescens	Protein and lipid lysis with stinky smell
Pseudomonas fragi	Light color with fruit smell

- Spoilage of refrigerated cheeses generally includes:
  1. Surface slime layer

2. Surface discoloration
3. Off odor and flavor

❖ Spoilage caused by mesophilic bacteria (common in Iraqi cheeses)

➢ *Streptococcus lactis*	Souring and unfavorable flavor
➢ Coli forms	Acid, gas and alcohol formation
➢ *Bacillus* & *Clostridium*	Protein and lipid lysis
➢ *Staphylococcus aureus*	Exerted intestinal poisons
➢ Pathogenic bacteria	e.g., *Salmonella*

❖ Spoilage caused by molds (common in Europe, in solid and semisolid cheeses)
Mold grows in the surface and forms color spots.
e.g., *Cladosporium, Geotrichum, Penicillium, Mucor, Aspergillus*…

C. Butter

❖ Growth of bacteria (*Pseudomonas* spp.), yeasts (*Candida* spp.), and molds (*Geotrichum candidum*) on the surface causes flavor defects (putrid, rancid, or fishy) and surface discoloration.
❖ In unsalted butter, coliforms, *Enterococcus*, and *Pseudomonas* can grow favorably in the water phase (which has nutrients from milk) and produce flavor defects.

Vegetables, fruits, and nuts

Normal microbiological quality and its significance

❖ A leafy vegetable has more microorganisms from the air, whereas a tuber has more from the soil.
- ➢ Generally, vegetables have 10^{3-5} microorganisms/cm^2 or 10^{4-7} /g.
- ➢ Some of the predominant bacterial types are lactic acid bacteria, *Corynebacterium, Enterobacter, Proteus, Pseudomonas, Micrococcus, Enterococcus*, and spore formers.

- They also have different types of molds, such as *Alternaria, Fusarium*, and *Aspergillus*.
- Vegetables can have enteric pathogens, especially if animal and human wastes and polluted water are used for fertilization and irrigation.
- They include *Lis. monocytogenes*, *Salmonella, Shigella, Campylobacter, Clo. botulinum,* and *Clo. perfringens*.
- They can also have pathogenic protozoa and parasites.

❖ Fruits, because of their high carbohydrate content and low pH, favor the growth of different types of molds, yeasts, and lactic acid bacteria.
- In general, microbial populations are 10^{3-6}/g.
- Improperly harvested and processed fruits can have pathogens that survive, grow, and cause foodborne disease.
- Molds, yeasts, and bacteria can cause different types of spoilage.

❖ Microorganisms enter nuts from soil (peanuts) and air (tree nuts).
- Raw nuts can have 10^{3-4} microorganisms/g, with *Bacillus* and *Clostridium* spores, *Leuconostoc, Pseudomonas*, and *Micrococcus* predominating.
- Because of a low Aw, bacteria do not grow in the products.
- However, when used as ingredients, they can cause microbiological problems in the products.
- Molds can grow in nuts and produce mycotoxins (from toxin-producing strains such as aflatoxins by *Aspergillus flavus*).

Spoilage of vegetable and fruits

A. Vegetables

❖ 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.

❖ 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.

- Microorganisms grow more rapidly in damaged or cut vegetables.

B. Fruits

- 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.
- According to the changes in appearance, the mold spoilage is designated as black rot, gray rot, soft rot, brown rot, or others.
- Yeasts from genera *Saccharomyces, Candida, Torulopsis,* and *Hansenula* are associated with fermentation of some fruits, such as apples, strawberries, citrus fruits, and dates.

Cereal, starch, and gums

Normal microbiological quality and its significance

- They are rich in amylose and amylopectin, but can also have simple sugars (e.g., in grains) and protein (e.g., in lentils).
- Unprocessed products (grains) may contain high bacterial levels (aerobic plate count $\approx 10^4$/g, coliform $\approx 10^2$/g, yeasts, and molds $\approx 10^3$/g).
  - They may also contain mycotoxins produced by toxicogenic molds.
- Processed products may also contain a wide variety of yeasts, molds, and bacteria.
- Flours and starches may have higher microbial counts, similar to those of grains.
  - They can contain bacterial spores and psychrotrophs.
  - Some pathogens, such as *Salmonella, Sta. aureus,* and *Clo. perfringens*, have also been isolated.
  - Depending on the product, they can either grow or be the source for spoilage and pathogenic microorganisms as well as mycotoxins.
- Gums also may be the source of yeasts, molds (also mycotoxins), bacterial spores, and lactic acid bacteria.

Spoilage of cereals and their products

A. Grains and seeds

- Normally have 10 to 12% moisture (Aw ≤0.6) and thus inhibits microbial growth.
- During harvesting, processing, and storage, if the Aw increases above 0.6, some molds can grow.
  - *Aspergillus*, *Penicillium*, and *Rhizopus*.

B. Breads

- The Aw of breads is normally low enough (0.75 to 0.9) to prevent growth of bacteria.
- However, some molds (bread molds: *Rhizopus stolonifer*) can grow, especially if moisture is released because of starch crystallization during storage.
- Molds are killed during baking; however, spores can get in from air and equipment following baking.
- A specific type of bread spoilage designated as **ropiness** and characterized by a soft, stringy, brown mass with fruity odor, is caused by the growth of some mucoid variants of *Bac. subtilis*.
- The spores, coming from flour or equipment, survive baking and then germinate and grow inside within 1 to 2 d.

The most important bread spoilage bacteria & molds.

	Molds or bacteria	**Spoilage types**
molds	*Rhizopus nigricans*	White growth with black spots
	Aspergillus niger	Black-brown growth
	Penicillium expansum	Green growth
	Monilia sitophila	Red-pink growth
	Mucor	White hairy (fluffy) growth
bacteria	*Bacillus subtilis*	Ropiness
	Aerobacter cloacae	Souring and gasses
	E. coli	Souring and gasses

Canned food

Normal microbiological quality and its significance

- Canned foods prepared and processed to obtain commercial sterility can have spores of thermophilic spoilage bacteria, namely *Bacillus stearothermophilus*, *Clo. thermosaccharolyticum*, and *Desulfotomaculum nigrificans*.
- In canned products stored at 30°C or below, thermophilic spores do not germinate to cause spoilage.
- However, if the cans are temperature-abused to 40°C or higher, the spores germinate; subsequently, the cells multiply and spoil the products.

Spoilage of canned foods

- Canned foods are heat treated to kill microorganisms present, and the extent of heat treatment is predominantly dependent on the pH of a food.
- Canned food spoilage is both due to

\- Nonmicrobial spoilage (chemical and enzymatic reactions)

- Production of hydrogen, CO_2, browning, corrosion of cans
- liquification, gelation, and discoloration

\- Microbial spoilage

A. Thermophilic spore-formers

- Cause three types of spoilage of low-acid (high-pH) foods, when the cans are temperature abused at 43°C and above.

1. Flat sour spoilage:

- The cans do not swell but the products become acidic because of carbohydrates fermentation.
    - Germination (43°C) and growth (30°C) of facultative anaerobic *Bac. stearothermophilus*.

2. Thermophilic anaerobe (TA) spoilage:

- Growth of anaerobic *Clo. thermosaccharolyticum* (30°C) with the production of large quantities of H_2 and CO_2 gas and swelling of cans and with sour and cheesy odor.

3. Sulfide stinker spoilage:

- Caused by the Gram-negative anaerobic sporeformer *Desulfotomaculum nigrificans* (at 43°C).

- The spoilage is characterized by a flat container but darkened products with the odor of rotten eggs due to H_2S produced.

B. Spoilage due to insufficient heating

- ❖ Germinate and grow of *Clostridium* spores to cause spoilage.
  - Spoilage can be from the breakdown of carbohydrates
    - *Clo. butyricum* and *Clo. pasteurianum*
    - Carbohydrates fermentation produces volatile acids and H_2 and CO_2 gas, causing swelling of cans.
  - Spoilage can be from the breakdown of proteins
    - *Clo. sporogenes* and *Clo. putrefacience* (also proteolytic *Clo. botulinum*)
    - Metabolize proteins and produce H_2S, mercaptans, indole, ammonia, CO_2 and H_2 (causing swelling of cans).
  - *Clo. botulinum* production of toxins
- ❖ Spores of some facultative anaerobic *Bacillus spp*., such as *Bac. subtilis* and *Bac. coagulans*, grow, with the production of acid and gas (CO_2).

C. Spoilage due to container leakage

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

Sugars and confectioneries

Normal microbiological quality and its significance

Sugar can have thermophilic spores of *Bac. stearothermophilus, Bac. coagulans, Clo. thermosaccharolyticum,* and *Des. nigrificans*, as well as mesophilic bacteria (e.g., *Lactobacillus* and *Leuconostoc*), yeasts, and molds.

- ➢ When sugars are used as ingredients in food products, the spores can survive and cause spoilage of products.
- ➢ Pathogens are not present in refined sugar unless contaminated.

- ❖ Confectioneries include a large variety of products (chocolate, cream, jellies...) with a sweet taste (have low Aw (0.84) and some have low pH).
  - ➢ They may contain many types of bacteria, yeasts, and molds.
  - ➢ Although they may harbor *Lactobacillus, Leuconostoc*, spores of *Bacillus* and *Clostridium*, and yeasts and molds, only a few osmotolerant yeasts and molds can grow.

Spoilage of sugars and confectioneries

- Not susceptible to bacterial spoilage (Aw ≤ 0.8).
- Under aerobic conditions, some xerophilic molds can produce visible spoilage.
- Osmophilic yeasts from genera *Candida, Zygosaccharomyces*, *Saccharomyces*, and *Torulopsis* can ferment these products.
    - To prevent growth of yeasts, chemical preservatives are added.

Soft drink, fruit and vegetable drinks, juices, and bottled water

Normal microbiological quality and its significance

- Soft drinks can have different types of microorganisms, but only aciduric microorganisms, such as molds, yeasts, lactic acid bacteria, and acetic acid bacteria, can multiply.
- In fruit juices, molds, yeasts, *Lactobacillus* spp., *Leuconostoc* spp., and acetic acid bacteria can grow.
    - Spoilage of fruit juices by acid-resistant spore forming species from genus *Alicyclobacillus* has currently been recognized.
    - Some pathogens (e.g., acid-tolerant *Salmonella* spp. and *E. coli*) can remain viable for a long time (≥30 d) in the acid products.
- Vegetable juices can have molds, yeasts, and lactic acid bacteria along with *Bac. coagulans, Clo. butyricum,* and *Clo. pasteurianum*.
- Bottled water should not contain more than 10 to 100 bacteria and >10 coliforms/100 ml.
    - The indigenous flora are mainly *Flavobacterium, Alcaligenes*, and *Micrococcus*.

Spoilage of soft drinks, fruits juices and, vegetable juices

- In carbonated beverages, some yeast species from genera *Torulopsis, Candida, Pichia, Hansenula*, and *Saccharomyces* can grow and make the products turbid. Some *Lactobacillus* and *Leuconostoc* species can also grow to cause cloudiness and *Leuconostoc* can cause ropiness (due to production of dextrans) of the products.
- Noncarbonated beverages can be similarly spoiled by yeasts, *Lactobacillus*, and *Leuconostoc* spp. In addition, if there is enough

dissolved oxygen, molds (*Penicillium, Aspergillus, Mucor*, and *Fusarium*) and *Acetobacter* can grow; the latter produces acetic acid to give a vinegar-like flavor.

- Fruit juices are susceptible to spoilage by molds, yeasts, *Lactobacillus, Leuconostoc,* and *Acetobacter* spp. Molds and *Acetobacter* can grow if enough dissolved oxygen is available. Yeasts can cause both oxidation (producing CO_2 and H_2O) and fermentation (producing alcohol and CO_2) of the products. *Leu. mesenteroides* and some strains of *Lab. plantarum* can produce slime due to production of dextran and other exopolysaccharides.
- In fruit drinks, *Lactobacillus* and *Leuconostoc* spp. Can also convert citric and malic acids (additives) to lactic and acetic acids and reduce the sour taste (flat flavor). Because concentrated fruit drinks and fruit preserves have a low Aw (0.9), only osmophilic yeasts can grow; molds also can grow if oxygen is available.

Mayonnaise and salad dressing

Normal microbiological quality and its significance

- Microorganisms are introduced into the products through ingredients, equipment, and air.
- However, except for aciduric microorganisms, most others die, especially when stored for a long time at room temperature.
- Among aciduric microorganisms, molds (*Geotrichum and Aspergillus spp*.), yeasts (*Saccharomyces spp*.), and several species of *Lactobacillus* (*Lab. fructivorans, Lab. brevis*) and some *Bacillus spp.* (*Bac. subtilis, Bac. mesentericus*) have been isolated.
    - Normally, their numbers should not exceed 10/g.
- If pathogens are introduced (e.g., *Salmonella* through eggs), they are expected to be killed rapidly; however, they may survive longer in low-calorie, high-pH products kept at refrigerated temperatures.

Spices and condiments

Normal microbiological quality and its significance

- Some spices, unless given antimicrobial treatments (irradiation), may contain microorganisms as high as 10^{6-7}/g.
- The most important are spores of molds, *Bacillus*, and *Clostridium* spp. Also, *micrococci, enterococci*, yeasts, and several pathogens such as *Salmonella* spp., *Sta. aureus,* and *Bac. cereus* have been found.
- They can also have mycotoxins.
- Although used in small amounts, they can be the source of spoilage and pathogenic microorganisms in food.

Spoilage of mayonnaise, salad dressings, and condiments

- Normally contain some molds, yeasts, spores of *Bacillus* and *Clostridium*, and aciduric bacteria such as *Lactobacillus*.
- The major factors controlling microbial growth are low pH, and relatively low Aw.
- Molds can grow only on the surface exposed to air.
  - Some microaerophilic and facultative anaerobic yeasts and heterolactic *Lactobacillus* spp. can also multiply to produce CO_2.

Pickles

Spoilage of pickles

- In salt stock pickles containing 15% salt, yeasts and halophilic bacteria can grow, especially if the acidity is not sufficient.
- Pickles with low salt (<5%) can have a bloating defect from CO_2 production by yeasts, heterofermentative lactic acid bacteria, and coliforms.
- *Candida, Torulopsis*, and *Saccharomyces* spp. are often the causative yeasts.

The most important pickles spoilage.

Spoilage types	Microorganisms
1. Surface film(white slime layer)	Yeast (*Mycoderma, Candida*) Lactic acid→CO_2 + H_2O
2. Floated pickles	Yeast activity inside cucamber (e.g. *Torulopsis*

	caroliniana)
3. Slimy pickles	*Leuconostoce mesenteroides*
4. Soft pickles (in low salt conc. & low acidity pickles)	Bacteria (e.g. *Aerobacter, Aeromonas, Bacillus*..) and molds(e.g. *Penicillium, Fusarium, Alternaria…)*
5. Black pickles	*Bacillus subtilis*

Food Spoilage by Microbial Enzymes

- ❖ A food is considered spoiled when the changes are detectable and the microbial population has reached to 10^{7-9}/ml, /g, or /cm^2.
- ❖ The changes are brought about by the catalytic actions of a large number of microbial enzymes.
- ❖ A bacterial cell contains many enzymes, many of which are intracellular, and some are extracellular.
  - ➢ Intracellular enzymes are involved in the metabolism of small nutrient molecules of food that are transported inside the cells. Many intracellular enzymes can also act on intracellular large molecules, such as endonucleases, mucopeptidase, lipases, and proteinases.
  - ➢ Extracellular enzymes, after synthesis, either remain bound to the cell surface or are excreted in the food environment. Many can hydrolyze large nutrient molecules of food (e.g., polysaccharides, proteins, and lipids) to small molecules before they are transported into the cells.
- ❖ After microbial cells die normally or are killed by nonthermal treatments so that the intracellular and extracellular enzymes are not inactivated or destroyed, the enzymes can cause food spoilage even in the absence of viable cells or growth of microorganisms.
- ❖ In thermally processed foods, several heat-stable enzymes of the microorganisms retain their activity even after the producer cells are killed.
- ❖ *Pseudomonas, Aeromonas, Flavobacterium, Shewanella, Serratia,* and *Acinetobacter* produce heat-stable enzymes.

- ❖ Many species from these genera are normally present in raw milk, meat, fish, and other food products.
- ❖ During refrigerated storage, psychrotrophs are able to grow and produce heat-stable enzymes in foods.

Spoilage of foods with heat-stable microbial enzymes

Several examples are used here to emphasize the spoilage potential of these enzymes.

A. Pasteurized milk

- ❖ Heat-stable proteinases and lipases of psychrotrophic bacteria are not inactivated by pasteurization and can cause proteolysis of casein and lipolysis of milk lipids to produce flavor defects.

B. Ultrahigh temperature (UHT)-treated milk products

- ❖ Spoilage of UHT-treated milks (heated at 140 to 150ºC for 1-5 s) during storage at 20ºC has been observed in the form of
  - ➢ Bitter flavor, sediments, and gel formation, due to the action of heat-stable proteinases and
  - ➢ Rancid flavor from the action of heat-stable lipases.

C. Cheeses

- ❖ Extracellular proteinases of psychrotrophic bacteria in raw milk reduce cheese yield and increase the levels of nitrogenous compounds in whey.
- ❖ Lipases have also been implicated in the development of off-flavor in cheese.

D. Cream and butter

- ❖ Lipases are responsible for off-flavor development in cream.
- ❖ Butter containing residual heat-stable bacterial lipases undergoes rapid lipid hydrolysis (rancidity) even during storage at –10°C.

E. Milk powder

- ❖ Powdered milk is used as ingredients in a wide variety of foods that can have high amounts of proteins and lipids.
- ❖ These products can develop off-flavor and texture defects from the action of heat-stable proteinases and lipases during storage.

Spoilage of foods by microbial enzymes at low temperature

- ❖ As our likings for the refrigerated foods increase and the foods are stored for a longer time, problems associated with these enzymes will increase.
- ❖ The enzymes could be the exoenzymes which acted on specific food components during long storage.
- ❖ At low temperatures, bacterial cells can die, lyse, and release intracellular enzymes.
  - ➢ Some of these enzymes can be active at low temperature to produce a defect that can be detected during long storage.

Microbial foodborne diseases

☒ Important Facts in Foodborne Diseases

- ☒ **Foodborne Intoxications**
- ☒ **Foodborne Infections**
- ☒ **Foodborne Toxicoinfections**
- ☒ **Parasites**
- ☒ **Indicators of Bacterial Pathogens**

Important Facts in Foodborne Diseases

Foodborne Disease Outbreak

❖ Foodborne disease is an outbreak when two or more people become sick with a similar illness from the consumption of the same food from the same source.

❖ However, in the case of botulism, because of a high fatality rate, even when only one person has the illness, it is considered an outbreak.

Types of Microbial Foodborne Diseases

❖ Foodborne diseases in humans result from the consumption of either food and water contaminated with viable pathogenic bacterial cells (or spores in the case of infant botulism) or food containing toxins produced by toxigenic bacteria and molds.

❖ On the basis of mode of illnesses, these can be arbitrarily divided into three groups:

1. Intoxication or poisoning
2. Infection
3. Toxicoinfection

❖ Many pathogenic bacterial species and viruses have been implicated with foodborne (and waterborne) disease outbreaks; some have occurred at a higher frequency than others (Table.1).

❖ Certain food types or foods prepared under specific conditions and environments have been implicated more frequently with foodborne disease than others. (Table 2).

Table.1 Predominant Bacterial and Viral Pathogens Associated with the Confirmed Foodborne Diseases from 1983 to 1987 in the U.S.

Bacteria and Viruses[a]	Outbreaks		Cases		Deaths	
	No.	%	No.	%	No.	%
Staphylococcus aureus	47	7.6	3,181	6.2	0	0
Clostridium botulinum	74	11.9	140	0.3	10	17.0
Salmonella	342	55.1	31,245	61.1	39	66.0
Shigella spp.	44	7.1	9,971	19.5	2	3.4
Escherichia coli	7	1.1	640	1.3	4	6.8
Campylobacter spp.	28	4.5	727	1.4	1	1.7
Clostridium perfringens	24	3.9	2,743	5.4	2	3.4
Bacillus cereus	16	2.6	261	0.5	0	0
Hepatitis A virus	29	4.7	1,067	2.0	1	1.7
Norwalk-like virus	10	1.5	1,164	2.3	0	0
Total	621	100	51,139	100	59	100

[a]Not included in the table are *Brucella* spp., *Streptococcus* spp., *Vibrio* spp., and several others, which combined were associated with 18 (2.8%) outbreaks. *Lis. monocytogenes* caused 3 outbreaks affecting 259 people, with 70 deaths.

Table.2 Predominant Food Types Associated with Confirmed Foodborne Disease Outbreaks of Bacterial and Viral Origin from 1983 to 1988 in the U.S.

Food Types	No. of Outbreaks	%	Predominant Pathogens (% No. of Outbreaks)
Meat products[a]	91	14.0	*Salmonella* (53%), next *Sta. aureus*
Fish products[b]	20	3.0	*Clo. botulinum* (50%)
Egg products	11	2.0	*Salmonella* (82%)
Dairy products	26	4.0	*Salmonella* (27%)
Salads[c]	33	5.0	*Salmonella, Sta. aureus, Shigella* spp.
Baked food	0	1.0	*Sta. aureus*
Fruits and vegetables	44	7.0	*Clo. botulinum*
Mushrooms	2	0.5	*Clo. botulinum*
Beverages	3	0.5	*Salmonella*
Ethnic foods[d]	19	3.0	*Clo. perfringens, Bac. cereus, Salmonella*
Multiple foods[e]	123	19.0	*Salmonella* (59%)
Unknown foods[f]	254	40.0	*Salmonella* (68%), *Shigella* spp., viruses

[a] Includes beef, ham, pork, sausages, chicken, turkey, and stews.
[b] Includes shellfish and fin fish.
[c] Includes potato, chicken, fish, egg, and other salads.
[d] Includes fried rice, Chinese food, and Mexican food.
[e] More than one food was involved in an outbreak.
[f] Although food was implicated with an outbreak, involvement of a food was not confirmed.

Foodborne Intoxications

- Foodborne intoxication or food poisoning of microbial origin occurs by ingesting a food containing a preformed toxin.

Staphylococcal Intoxication

- Staphylococcal food poisoning caused by toxins of *Staphylococcus aureus*.
- The number of outbreaks and number of cases of staphylococcal gastroenteritis is much higher than several other microbial foodborne disease outbreaks.

- Characteristics

- *Sta. aureus* are Gram-positive cocci, and nonsporulating.
- The cells are killed at 66^{O}C in 12 min, and at 72^{O}C in 15 s.
- *Sta. aureus* are facultative anaerobes but grow rapidly under aerobic conditions.
- They are poor competitors to many other microorganisms found in foods.
- *Sta. aureus* are naturally present in the nose, throat, skin, and hair (feathers) of healthy humans, animals, and birds.
- *Sta. aureus* can be present in infections, such as cuts, abscesses, and facial acne in humans.

- Toxins and Toxin Production

- Enterotoxigenic strains of *Sta. aureus* produce seven different enterotoxins: A, B, C1, C2, C3, D, and E.
- They are heat-stable proteins of molecular weight 26 to 30 kDa and differ in toxicity.

- Disease and Symptoms

- A healthy adult has to consume 30 g or ml of a food containing 100 to 200 ng toxins produced by 10^{6-7} cells/g or /ml.
- The symptoms occur within 2 to 4 h, with a range of 30 min to 8 h, and are directly related to the amounts of toxin ingested and an individual's resistance.
- The disease lasts for 1 to 2 d and is rarely fatal.
  - The primary symptoms are salivation, nausea and vomiting, abdominal cramps, and diarrhea.
  - Some secondary symptoms are sweating, chills, headache, and dehydration.

- Food Association

- Many foods have been implicated in staphylococcal foodborne outbreaks (bakery product,beef,egg, and chicken).

- In general, the bacterium grows in the food and produces toxins without adversely affecting the acceptance quality.

Botulism

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

- Characteristics

- Cells of *Clo. botulinum* are Gram-positive rods, obligate anaerobes, and form single terminal spores.
- Spores are highly heat resistant (killed at 115°C), but cells are killed at moderate heat (pasteurization).
- *Clo. botulinum* strains, on the basis of the type of toxin production, have been divided into six types: A, B, C, D, E, and F.
    - Of these, A, B, E, and F are associated with human foodborne intoxications.
- The strains can grow between 3.3 ^{o}C and 48°C, with the optimum at 30°C to 35°C.
- Spores of *Clo. botulinum* are widely distributed.
- Type A and B spores are more prevalent in soil, sewage, and fecal matters of animals, whereas Type E spores are generally found in marine environments.

- Toxins and Toxin Production

- The toxins of *Clo. botulinum* are neurotoxic proteins.
- In general, toxins associated with food intoxication in humans are extremely potent, and only a small amount of toxin is required to produce the symptoms and cause death.
- The toxins are heat labile and can be destroyed in a contaminated food by high and uniform heat, such as 90^{o}C for 15 min or boiling for 5 min. Radiation at 5 to 7 m.rad can also destroy them.

- Disease and Symptoms

- Highly potent toxins, only a very small amount (1 ng/kg body weight) is necessary for severe symptoms and even death.
- At the initial stage (generally 12 to 36 h, but can be 2 h), some gastrointestinal disorders (e.g., nausea, vomiting, diarrhea, and constipation) may be evident.

- Neurological symptoms develop within a short time, especially if the amount of botulin consumed is high.
- In general, neurological symptoms include blurred or double vision; difficulty in swallowing, breathing, and speaking; dryness of the mouth; and paralysis of different involuntary muscles, which spreads to the diaphragm, lungs, and heart.
- Death usually results from respiratory failure.

- Food Association

- Type E was associated predominantly with fish (fermented, improperly cooked, and smoked fish and fish eggs) and Types A and B were associated with vegetables (e.g., green beans, corn, spinach, pepper, and mushrooms).

Mycotoxicosis

- 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.

- Characteristics

- Toxigenic strains from several species and genera and the toxins they produce include
  - *Asp. flavus, Asp. parasiticus* (both produce aflatoxins),
  - *Asp. nidulans*, *Asp. virsicolor* (sterigmatocystin),
  - *Penicillium viridicatum* (ochratoxin),
  - *Pen. patulum* (patulin),
  - *Pen. roquefortii* (roquefortin),
  - and *Cla. purpurea* (ergotoxin).
- In general, molds grow best in humid and warm environments.
- They are aerobic and thus need air for growth.
- They can grow, though slowly, at very low *Aw* (0.65), low temperature (refrigerated temperature), and low pH (3.5).
- The spores are present in soil, and dust.
- Many foods can have viable spores or mycelia, especially before a heat treatment.

- Toxins and Toxin Production

- ❖ Mycotoxins include a large number of toxins produced by different toxigenic species and strains of molds.
- ❖ Mycotoxins are produced by toxigenic mold strains as secondary metabolites.
- ❖ Toxin production, in general, is directly related with the growth rate of a mold strain.

- Food Association

- ❖ The growth of toxigenic mold strains and the presence of specific mycotoxins have been detected in many foods, such as corn, wheat, barley, rice, beans, peas, peanuts, bread, cheeses, dry sausages, spices.
- ❖ Consumption of mycotoxin-contaminated food can cause mycotoxicosis in humans.
- ❖ Many mycotoxins are resistant to heat used in the normal preparation of foods.

Foodborne Infections

- ❖ Foodborne infection occurs from the consumption of food (and water) contaminated with pathogenic enteric bacteria and viruses.

Salmonellosis by *Salmonella*

❖ *Salmonella typhi* and *Sal. paratyphi* were considered (before the 1940) the major causes of worldwide foodborne and waterborne diseases caused by *Salmonella* in humans.

- Characteristics

❖ *Salmonella* cells are
  - ➢ Gram-negative, nonsporulating, facultative anaerobic, motile rods.
  - ➢ Mesophilic, with optimum growth temperature between 35 and 37^{o}C and killed by pasteurization
  - ➢ sensitive to low pH (4.5)
  - ➢ Survive in frozen and dried states for a long time.
  - ➢ Can multiply in many foods without affecting the acceptance qualities.

❖ Salmonellae are natural inhabitants of the gastrointestinal tracts of many animals.

❖ They have also been isolated from soil, water, and sewage contaminated with fecal matters.

- Toxins

❖ The ability of the pathogens to invade and damage the cells is attributed to the production of a thermostable cytotoxic factor.

- Disease and Symptoms

❖ For foodborne salmonellosis, individual has to consume 10^{5-6} cells

❖ Following ingestion of the pathogen, symptoms appear in 8 to 42 h.

❖ An individual remains in a carrier state for several months following recovery.

❖ The general symptoms are abdominal cramps, diarrhea, nausea, vomiting, chills, fever, and prostration.

❖ It can be fatal, especially to the sick, infants, and the elderly.

- Food Association

❖ Foods of animal origin have been associated with large numbers of outbreaks.

❖ These include beef, chicken, pork, eggs, milk, and products made from them.

Listeriosis by *Listeria Monocytogenes*

- Human listeriosis has been recognized for a long time.
- Individuals with normal health may not develop symptoms or show a very mild enteric form of the disease.
- However, it is highly fatal to newborns, infants, the elderly, and pregnant women.

- Characteristics

- *Lis. monocytogenes* is a Gram-positive, psychrotrophic (grows between 1 and 44^{o}C, with optimum growth at 35 to 37^{o}C), facultative anaerobic, nonsporulating, small rod.
- The cells are relatively resistant to freezing, drying, high salt, and pH 5.0 and above.
- They are sensitive to pasteurization temperature (71.7^{o}C for 15 s or 62.8^{o}C for 30 min).
- *Lis. monocytogenes* is isolated from many environmental samples, such as soil, sewage, water, and dead vegetation.
- It is isolated from the intestinal contents of animals and birds.
- A large proportion of uncooked meat, milk, egg, seafoods, and fish, as well as leafy vegetables and tubers (potatoes and radishes), contains *Lis. monocytogenes*.
- Many heat-processed foods, such as pasteurized milk and dairy products, and ready-to-eat meat contain the organism.

- Toxin

- The virulence factors of *Lis. monocytogenes* is a specific type of hemolysin, listeriolysin O.
- The toxin causes death of the cells.

- Disease and Symptoms

- Most often, symptoms appear 1 to 7 d following ingestion and include mild flu-like symptoms with slight fever, abdominal cramps, and diarrhea.
- The symptoms subside in a few days, but the individual sheds *Lis. monocytogenes* in the feces for some time.

Pathogenic *Escherichia coli*

- ❖ Gram-negative, motile, nonsporulating, rod-shaped, facultative anaerobic bacterium, a normal inhabitant of the intestinal tract of humans and warm-blooded animals and birds.
- ❖ Pathogenic strains of *Esc. coli* are subdivided into four groups.

1. ***Enteropathogenic* Esc. coli *(EPEC)*** - infant diarrhea.
2. ***Enterotoxigenic* Esc. coli *(ETEC)*** -diarrhea in travelers, as well as in infants. The pathogens produce an invasive factor and enterotoxins to cause the disease.
3. ***Enteroinvasive* Esc. coli *(EIEC)*** - These strains are known to cause dysentery similar to that that caused in shigellosis.
4. ***Enterohemorrhagic* Esc. coli *(EHEC)*** - cause severe bloody diarrhea (hemorrhagic colitis) humans.

Gastroenteritis Due to EIEC

- ***Toxins***: The pathogens produce several polypeptides (invasive factors).

- ***Disease and Symptoms***: Following ingestion of the pathogen (10^6 cells) and incubation period, symptoms appear as abdominal cramps, profuse diarrhea, headache, chills, and fever. Symptoms can last for 7 to 12 d, but a person can remain a carrier and shed the pathogens in feces for a long time.

Gastroenteritis due to EHEC

- ***Toxins***: *Esc. coli* O157:H7 produces a verotoxin , or Shiga toxin. More than one toxin can be involved in the disease and the symptoms related to it.

- ***Disease and Symptoms***: *Esc. coli* O157:H7 causes hemorrhagic colitis, hemolytic uraemic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP). Symptoms occur 3 to 9 d after ingestion and generally last for 4 d. The colitis symptoms include a sudden onset of abdominal cramps, watery diarrhea (which in 35 to 75% of cases turns to bloody diarrhea), and vomiting.

Shigellosis (Bacillary Dysentery) by *Shigella* spp.

- ❖ The genus *Shigella* contains four species: *Shigella dysenteriae, Shi, flexneri, Shi. boydii* and *Shi. sonnei*.
- ❖ In general, food service establishments have been implicated in more outbreaks, and poor personal hygiene has been the major cause.

- Characteristics

- ❖ Gram-negative, facultative anaerobic rods.

- The strains grow between 7 and 46^{o}C, with an optimum at 37^{o}C.
- The cells survive for days under different physical and chemical stresses, such as refrigeration, freezing, 5% NaCl, and pH 4.5.
- They are killed by pasteurization.
- The intestine of humans and some primates is the only habitat known (carry and shed it without showing any symptoms).

- Toxins

- The toxin is designated as Shiga toxin (ST).
- The invasive trait is expressed at 37^{o}C but not at 30^{o}C.

- Disease and Symptoms

- The infective dose is very low, 10^{1-3} cells in adults.
- Following ingestion of a contaminated food, the symptoms occur in 12 h to 7 d.
- Symptoms last for 5 to 6 d (2 to 3 weeks in severe cases).
- The symptoms include abdominal pain, diarrhea often mixed with blood, mucus and pus, fever, chills, and headache.

Campylobacteriosis by *Campylobacter spp.*

- *Campylobacter jejuni* and *Cam. coli* are considered the most common causative agents of human diarrheal disease in many countries worldwide.
- The foods implicated most often in campylobacteriosis were raw milk and improperly cooked chicken.

- Characteristics

- *Campylobacter jejuni* is a Gram-negative, nonsporulating, rod-shaped bacterium.
- The strains are microaerophilic .
- Growth temperature ranges between 32 and 45^{o}C, with optimum 42^{o}C.
- They do not generally grow well in many foods. However, they survive well under refrigeration and for months in the frozen state.
  - *Cam. jejuni* is an enteric organism.
  - It has been isolated in high frequency from feces of animals and birds.

- Toxins

- *Cam. jejuni* has a thermolabile enterotoxin that is responsible for enteric disease symptoms.
- In addition, the strains produce an invasive factor.

- Disease and Symptoms

- The infective dose is considerably low, only 500 cells.
- Following ingestion, symptoms of the disease occur in 2-5 d.
- Symptoms generally last for 2 to 3 d.
- The main symptoms are enteric and include abdominal cramps, profuse diarrhea, nausea, and vomiting.
- Other symptoms include fever, headache, and chills. In some cases, bloody diarrhea has been reported.

Yersiniosis by *Yersinia enterocolitica*

- *Yer. enterocolitica* is a psychrotroph and can grow at $0^{o}C$.

- Characteristics

- *Yer. enterocolitica* cells are Gram-negative short rods, nonsporeforming, and facultative anaerobic.
- The strains grow between 0 and $44^{o}C$, with an optimum growth at 25 to $29^{o}C$.
- Cells are sensitive to pasteurization.

- *Yer. enterocolitica* is a normal inhabitant of intestines of animals and birds.

- Toxins

- Both the pathogenic and nonpathogenic strains produce a heat-stable toxin.
- Pathogenic strains also carry an invasive factor.
- Only after colonization is the heat-stable toxin capable of causing the disease.

- Disease and Symptoms

- High dose (10^7 cells) is required for the disease.
- Young children are more susceptible to foodborne yersiniosis.
- Symptoms are severe abdominal pain, diarrhea, nausea, vomiting, and fever.
- Symptoms generally appear 24 to 30 h following consumption of a contaminated food and last 2 to 3 d.

Gastroenteritis by *Vibrio* spp.

- In the genus *Vibrio*, four species have been implicated in foodborne illnesses:
  - *Vibrio cholerae* (included in the toxicoinfection)
  - *Vib. mimicus* = = =
  - *Vib. Parahaemolyticus*
  - *Vib. vulnificus*.

A. *Vibrio parahaemolyticus* Gastroenteritis

- The high incidence is directly related to the consumption of raw seafoods (in Japan).
- Gram-negative, nonsporulating, curved rods.
- Growth temperature ranges between 5 to 42^{o}C, with optimum 30 to 37^{o}C.
- The cells are extremely sensitive to drying, heating (pasteurization), and refrigeration and freezing.
- Halophilic bacteria, distributed in coastal waters worldwide.

-Toxin and Toxin Production:

- The foodborne pathogenic strains can cause hemolysis because of the presence of a heat-stable hemolysin.
- If the toxin forms in a food, heating does not destroy it.

- Disease and Symptoms:

- Individual has to consume 10^{5-7} cells to develop symptoms.
- The cells are sensitive to low stomach pH.
- Symptoms appear 10 to 24 h following ingestion of live cells and last for 2 to 3 d.
- Symptoms include nausea, vomiting, abdominal cramps, diarrhea, headache, fever, and chills. The disease is not normally fatal.

B. *Vibrio vulnificus* Septicemia

- Highly lethal pathogen (ability to invade the bloodstream).
- Following consumption of contaminated seafood (raw oysters) the cells penetrate the intestinal wall and produce primary septicemia in 20 to 40 h.
- Symptoms are chills, fever, and prostration, with occasional vomiting and diarrhea.

Enteric Viruses

- ❖ Hepatitis A and Norwalk-like viruses are more predominant.
- ❖ They are of enteric origin and are excreted in very high numbers in human feces.
- ❖ Pasteurization can effectively kill the enteric viruses.

- Disease and Symptom:

- ❖ Can cause infection at a considerably low dose level.
- ❖ Following ingestion of hepatitis, A viruses, symptoms occur after 4 weeks, with a range of 2 to 7 weeks.
  - ➢ The general symptoms are fever, nausea, vomiting, abdominal discomfort, and inflammation of liver, which may follow with jaundice.
  - ➢ Symptoms may last for 1 to 2 weeks or longer.
- ❖ NLV cause gastroenteritis, characterized by vomiting and diarrhea.
  - ➢ Symptoms appear 12 to 24 h after ingestion and last for 1 to 2 d.
- ❖ The viruses are excreted in the feces of infected persons.

Other Foodborne Infections

A. Brucellosis

- ❖ Human brucellosis is caused by *Brucella* spp.
- ❖ They are Gram-negative, non-spore forming, aerobic small rods pathogenic to animals and humans.
- ❖ The organisms are located in the udders and uterus thus can be excreted in milk.
- ❖ Pasteurization of milk and milk products kills *Brucella* cells.
- ❖ Symptoms of brucellosis in humans include undulant fever, profuse sweats, body aches, aching joints, chills, and weakness.
- ❖ Symptoms appear in 3 to 21 d following consumption of a contaminated food.

B. Streptococcal Infection

- ❖ Pathogen, isolated from lactating animals with mastitis.
- ❖ It is a Gram-positive coccus and has been associated with human pharyngitis with symptoms of sore throat, fever, chills, and weakness. Some strains can cause scarlet fever.

- ❖ Consumption of contaminated raw milk causes the infection.

C. Q Fever

- ❖ Q fever in humans is caused by a rickettsia, *Coxiella burnetii*.
- ❖ Animals carry this organism without any symptom. People handling animals, raw milk, and meat can be infected by the rickettsia and develop symptoms of Q fever.
- ❖ Symptoms include fever, anorexia, muscular pain, and headache.
- ❖ Symptoms appear 2 to 4 weeks after infection.
    - ❖ Foodborne infection occurs from the consumption of raw or improperly pasteurized milk and milk products.

Foodborne Toxicoinfections

The pathogenesis and disease symptoms of several pathogens associated with foodborne and waterborne gastroenteritis are somewhat different from classical food poisoning or foodborne infection caused by the pathogens. (Although the differences are not always very clear).

Clostridium Perfringens Gastroenteritis

- The outbreaks generally occur with some foods that were prepared in advance by heating and then kept warm for several hours before serving (cafeterias, restaurants, and schools).

- Characteristics

- The cells are Gram-positive rods, and spore formers. anaerobic but can tolerate some air (oxygen).
- The vegetative cells are sensitive to low-heat treatment (pasteurization), but the spores are extremely heat resistant.
- The temperatures of growth of vegetative cells and germination of spores and outgrowth range between 10 and 52^{o}C. The optimum growth occurs at 45^{o}C.
- Spores and vegetative cells are found in soil; dust; intestinal contents of animals, birds, and humans; and sewage.

- Toxins and Toxin Production

- The enterotoxin associated with the foodborne disease is a heat-labile protein.
  - It is an intracellular protein produced by the cells during sporulation in the intestine and released.
  - Unlike toxins of food poisoning microorganisms, the enterotoxin is produced in the digestive tract.

- Disease and Symptoms

- The enterotoxin causes only gastroenteritis.
- The symptoms appear 8 to 24 h following ingestion of a large number of viable cells ($\geq 5\times10^5$/g) through a food.
- The main symptoms are diarrhea and abdominal pain. Nausea, vomiting, and fever also can occur but are less common.
- Symptoms generally disappear within 24 h.

- Food Association

- Raw meat from animals and birds is most commonly contaminated with the spores and cells from the digestive tract content, whereas vegetables and spices commonly get them from soil and dust.

Bacillus cereus Gastroenteritis

- Characteristics

- The cells are Gram-positive rods, which form endospores in the middle of the cells.
- Cells are sensitive to pasteurization.
- Spores can survive high heat treatment used in many cooking procedures.
- *Bac. cereus* is aerobic but can also grow under some degree of anaerobic environment.
- The cells can multiply in a temperature range of 4 to 50^{o}C, with the optimum 35 - 40^{o}C.
  - Spores and cells of *Bac. cereus* are common in soil and dust and can be readily isolated in small numbers from many foods, which include both raw and finished products.
  - Intestinal tracts of 10% of healthy adult humans have *Bac. cereus* under normal conditions.

- Toxins and Toxin Production

- The strains produce at least two types (emetic and enteric) of enterotoxins, each probably associated with specific types of symptoms.
- Only when the cells are lysed are the toxins released.
- They occur in the intestinal tract but can also occur in foods.
- Thus, the cases can also be regarded as food poisoning, as in staphylococcal food poisoning.

- Disease and Symptoms

- In general, a large number of cells (10^{6-8}/g) need to be ingested to produce gastroenteritis.
- The two types of enterotoxins produce two types of symptoms.
  - The enterotoxin associated with the diarrheal form is a heat-labile protein
    - Symptoms occur 6 to 12 h
    - Symptoms include abdominal pain, profuse watery diarrhea, and perhaps nausea, but no vomiting or fever
    - Recovery is usually within 24 h.
    - Symptoms similar to those produced by *Clo. perfringens*.

- The enterotoxin associated with the emetic form is a heat-stable protein.
    - The symptoms occur 1 to 5 h
    - Symptoms are nausea and vomiting; abdominal pain and diarrhea may also be present.
    - Symptoms last for 24 h.
    - Symptoms are similar to those of staphylococcal gastroenteritis.

- Food Association

- In the diarrheal outbreaks, a variety of foods, including vegetables, salads, meats, pudding, sauces, and soups, has been implicated, mostly because of their improper cooling.
- However, in the emetic form, outbreaks mostly involve rice and sometimes other starchy foods.

Cholera

- Caused by *Vib. cholerae*

- Characteristics

- Gram-negative, curved rod.
- It is sensitive to heat and is killed by the temperature used for cooking.
- The optimum temperature of growth is between 30 and 35^{o}C.
- Cholera is a human disease.
- The disease results from the ingestion of infective doses of *Vib. cholerae* cells through food and water contaminated with feces of humans suffering from the disease.
- Marine environments (Seafoods) may serve as long-term reservoirs.
- Contaminated water can also be the source of the disease.

- Toxins and Toxin Production

- The toxin is a heat labile.
- Following ingestion of *Vib. cholerae* cells in sufficient numbers, the cells colonize the small intestine and multiply rapidly and produce toxins.

- When the cells die and lyse, the toxins are released into the intestine.

- Disease and Symptoms

- *Vib. cholerae* is not contagious.
- The infective dose for cholera is 10^6 viable cells per person.
- The incubation period ranges from 1 to 5 d.
- The symptoms include the sudden onset of profuse watery diarrhea and vomiting. Loss of fluid results in dehydration.

- Food Association

- Food can serve as a source of *Vib. cholerae* if it is contaminated directly with human feces from the patient or previously contaminated water.
- Consumption of raw seafood and partially cooked (not enough to kill *Vib. cholerae* cells) were mainly involved.

Escherichia Coli Gastroenteritis

- Enteropathogenic (EPEC) and enterotoxigenic (ETEC) *Esc. coli* types produce diarrheal diseases when ingested in large numbers through contaminated foods and water.
- The symptoms are more like those in cholera.

- Characteristics

- Gram-negative small, curved rods, nonsporulating.
- The strains are facultative anaerobes.
- Growth occurs between 10 and 50°C, optimum at 30 - 37°C.
- The cells are sensitive to low-heat treatment, such as pasteurization.
- The carriers can shed the organisms in feces and can contaminate food and water directly or indirectly.
- Many animals harbor it and contaminate soil, water, and food.

- Toxins and Toxin Production

- ETEC subgroup produce (either LT or ST, or both)
  - Heat labile (LT)
  - Heat stable (ST)
  - Factors that enable the cells to colonize, multiply, and initiate infection.

- EPEC subgroups produce
  - LT toxin
  - Several others produce toxins different from LT and ST
- The toxins are generally detected in a growing culture within 24 h at 35°C.

- Disease and Symptoms

- EPEC strains associated with infant diarrhea
- ETEC strains are the cause of traveler's diarrhea, a nonfatal diarrheal disease.
- Ingestion of a high level (10^{6-9} cells) of viable cells of the organisms by adults is necessary for the symptoms to occur within 24 to 72 h.
- Symptoms include mild to severe diarrhea that lasts for 24 to 30 h.

Parasites

- Included in this group are several intestinal and tissue helminths (roundworms, flatworms, and tapeworms) and protozoa that are known to cause human illness and where a food association has either been confirmed or suspected.

A. Trichinosis by *Trichinella spiralis*

- *R*oundworm.
- A person gets infected by consuming raw or insufficiently cooked meat of infected animals (pigs).
- The symptoms of trichinosis appear in 2 to 28 d following ingestion.
  - Initial symptoms are nausea, vomiting, and diarrhea, followed by fever, swelling of eyes, muscular pain, and respiratory difficulties.

B. Anisakiasis by *Anisakis simplex*

- Nematode
- Human infection results from the consumption of raw infected fish.
- Symptoms appear after a few days and generally include irritation of throat and digestive tract.

C. Taeniasis by *Taenia* spp.

- Tapeworm, disease caused by
  - *Taenia solium* from pork.
  - *Tae. saginata* from beef.
- The illness results from the ingestion of raw or improperly cooked meat contaminated with larvae of the tapeworms.
- Symptoms appear within a few weeks and are digestive disorders.
- If an organ is infected by the larvae, especially a vital organ, the consequences can be severe.

D. Toxoplasmosis by *Toxoplasma gondii*

- 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 gondii*.
- In many people, it does not cause any problems.
- However, in individuals with low resistance, the symptoms are generally flu-like, with fever and headache.

E. Giardiasis by *Giardia lamblia*

- Intestinal protozoan.
- Contaminated raw vegetables and poor personal hygiene are considered the major causes of the disease.
- The main symptoms are acute or chronic diarrhea and abdominal pain.
- The oocysts and cysts are excreted in the feces of the affected individuals.

F. Cryptosporidiosis by *Cryptosporidium parvum*

- Intestinal protozoan causes cryptosporidiosis in humans through the consumption of contaminated food and water with the oocysts.
- The major symptoms of infection in humans are watery diarrhea, fever, muscle aches, dehydration.

G. Cyclosporiasis by *Cyclospora cayetanensis*

- Coccidian intestinal protozoan.
- Consumption of food and water contaminated with the oocysts and subsequent infection results in the disease.
- The main symptoms are watery diarrhea, cramps, nausea, vomiting, fever, and fatigue.

Indicators of Bacterial Pathogens

- ❖ All pathogenic microorganisms implicated in foodborne diseases are considered enteric pathogens, except *Staphylococcus aureus, Bacillus cereus, Clostridium botulinum* (except in the case of infant botulism), *Clo. perfringens,* and toxicogenic molds.
- ❖ A food contaminated with fecal materials may theoretically contain one or more of these pathogens and can thus be potentially hazardous to consumers.

Coliform Group

A. Coliforms

1. Organisms and Sources

- ❖ The term *coliform* does not have taxonomic value; rather, it represents a group of species from several genera, namely, *Escherichia, Enterobacter, Klebsiella, Citrobacter*, and probably *Aeromonas* and *Serratia*.
- ❖ The main reason for grouping them together is their many common characteristics.
- ❖ They are all Gram-negative, non-spore forming rods.
- ❖ Some species can grow at higher temperature (44.5^{o}C), whereas others can grow at 4 to 5^{o}C.
- ❖ All are sensitive to low-heat treatments and are killed by pasteurization.
- ❖ They can be present in feces of humans and warm-blooded animals and birds.

2. Occurrence and Significance in Food

- ❖ Coliforms are expected to be present in many raw foods and food ingredients of animal and plant origin.
- ❖ In some plant foods, they are present in very high numbers because of contamination from soil.
- ❖ Because they can grow in foods, some even at refrigerated temperature, a low initial number can reach a high-level during storage.

- In heat-processed products, their presence is considered post heat-treatment contamination from improper sanitation.

B. Fecal Coliforms

1. Organisms and Sources

- Fecal coliform bacteria also constitute a group of bacteria and include those coliforms whose specificity as fecal contaminants is much higher than that of coliforms.
- This group includes mostly *Esc. coli*, along with some *Klebsiella* and *Enterobacter* spp.
- Non fecal coliforms are eliminated by using a high incubation temperature (44.5 or 45.0 ^{O}C) for 24 h in selective broths containing lactose.
- Lactose fermentation, with the production of gas, is considered a presumptive positive test.

2. Occurrence and Significance in Food

- Some fecal coliforms are present in raw foods of animal origin.
- They can be present in plant foods from contaminated soil and water.
- High numbers can be due to either gross contamination or growth from a low initial level, probably because of improper storage temperature.
- Their presence in heat-processed (pasteurized) foods is probably because of improper sanitation after heat treatment.
- A food can be accepted or rejected based on the numbers present.
- This group is extensively used as an indicator in foods of marine origin and in water and wastewater.

C. *Escherichia coli*

1. Organisms and Sources

- *Esc. coli* strains conform to the general characteristics described for coliform groups.
- Initially, *Esc. coli* types were used as indicators of fecal contamination and possible presence of enteric pathoge, with the considerations that they are nonpathogenic.
- However, it is now known that some variants and strains of *Esc. coli* are pathogenic (e.g., *Esc. coli* O157:H7).

2. Occurrence and Significance in Food

- *Esc. coli* is present in the lower intestinal tract of humans and warm-blooded animals and birds.
- Its presence in raw foods is considered an indication of direct or indirect fecal contamination.
- In heat-processed foods, its presence is viewed with great concern.
- Its value as an indicator of fecal contamination and the possible presence of enteric pathogens is much greater than that of coliform and fecal coliform groups.

Enterobacteriaceae Group

- The methods recommended to detect coliforms and fecal coliforms and *Esc. Coli* are based on the ability of these bacterial species to ferment lactose to produce gas and acid.
- In contrast, some enteric pathogens do not ferment lactose, such as most *Salmonella*.
- Thus, instead of only enumerating coliforms or fecal coliforms in a food, enumeration of all the genera and species in the Enterobacteriaceae family is advocated.
  - Because this family includes not only coliforms but also many genera and species that are enteric pathogens, enumeration of the whole group can be a better indicator of the level of sanitation, possible fecal contamination, and possible presence of enteric pathogens (Table-3).

Enterococcus Group

A. Characteristics and Habitat

- The genus *Enterococcus* is includes many species that were previously grouped as fecal streptococci and other streptococci.
- They are Gram-positive, non-spore forming, nonmotile cocci or coccobacilli, and facultative anaerobes.
- They can grow between 10 and 45^{O}C, and some species can grow at 50^{O}C. Some can survive pasteurization temperature.
- They are found in the intestinal tracts of humans and warm- and cold-blooded animals, birds, and insects.
- Some can be species specific whereas others can be present in humans, warm-blooded animals, and birds.

- ❖ Among the currently recognized species, several are found in the intestine of humans and food animals and birds, including *Enterococcus faecalis, Ent. faecium, Ent. durans, Ent. gallinarum, Ent. avium*, and *Ent. hirae*.
- ❖ They probably do not multiply in water but can survive longer than many coliforms.
- ❖ They can grow in most foods.

Table-3 Genera, Habitat, and Association to Foodborne Illnesses of the *Enterobacteriaceae*

Genera[a]	Main Habitat	Associated with Foodborne Illness[b]
Escherichia	Lower intestine of humans and warm-blooded animals and birds	Only the pathogenic strains
Shigella	Intestine of humans and primates	All strains
Salmonella	Intestine of humans, animals, birds, and insects	All are considered pathogenic
Citrobacter	Intestine of humans, animals, birds; also soil, water, and and sewage	Can be opportunistic
Klebsiella	Intestine of humans, animals, birds; also soil, water, and grain	Can be opportunistic
Enterobacter	Intestine of humans, animals, birds; widely distributed in nature, mostly plants	Can be opportunistic
Erwinia	Mostly in plants	No association
Serratia	Soil, water, plants, and rodents	Can be opportunistic
Hafnia	Intestine of humans, animals, birds; also soil, water, and sewage	No association
Edwardsiella	Cold-blooded animals and water	No association
Proteus	Intestine of humans, animals, birds; also soil and polluted water	Can be opportunistic
Providencia	Intestine of humans and animals	Can be opportunistic
Morganella	Intestine of humans, animals, and reptiles	Can be opportunistic
Yersinia	Intestine of humans and animals; also environment	Some species or strains are pathogenic
Obesumbacterium	Brewery contaminant	No association
Xenorhabdus	Nematodes	No association
Kluyvera	Soil, sewage, and water	Can be opportunistic
Rahnella	Fresh water	No association
Tatumella	Human respiratory tract	No association

[a] Some are of nonfecal origin.
[b] The pathogenic species and strains in the indicated genera are confirmed with foodborne and waterborne illnesses. Some species and strains in some genera, indicated as opportunistic, are suspected of being foodborne pathogens.

B. Occurrence and Significance in Food

- ❖ *Enterococcus* can get in different foods through fecal contamination or through water, vegetation, or equipment and processing environments, and may not be of fecal origin.

- In this respect, its value as an indicator of fecal contamination and possible presence of enteric pathogens in food is questionable.
- Currently, their presence in high numbers, especially in heat-processed (pasteurized) foods, can be used to indicate their possible presence in high numbers in raw materials and improper sanitation of the processing equipment and environment.

Control of microorganisms in food

With respect to spoilage and pathogenic microorganisms, the objective is to minimize their numbers or completely eliminate them from food.

Several methods, individually or in combinations, are used to achieve these goals by

- **Control of access (Cleaning and Sanitation)**
- **Control by physical removal**
- **Control by Heat**
- **Control by Low Temperature**
- **Control by Reduced Aw**
- **Control by Low pH and Organic Acids**
- **Control by Modified Atmosphere (or Reducing O–R Potential)**
- **Control by Antimicrobial Preservatives**
- **Control by Irradiation**

Control of access (Cleaning and Sanitation)

- Spoilage and pathogenic microorganisms enter in food from different sources.
- One of the major objectives to produce a safe food with desirable shelf life is to minimize the access of microorganisms in food from various sources.
- This can be achieved by
  - Proper plant design
  - Training personnel
  - Designing equipment that can be sanitized effectively

- Establishing an efficient cleaning and sanitation procedure.
  - Many cleaning and sanitizing chemicals are available commercially
- (Chlorine-Based sanitizers, Iodophores, Quaternary Ammonium compounds, H_2O_2).

Control by physical removal

1. **Centrifugation** is used in some; liquid foods, such as milk, fruit juices, and syrups, to remove suspended undesirable particles.
2. **Filtration** is used in some liquid foods, such as soft drinks, fruit juices, and water, to remove undesirable solids and microorganisms.
3. **Trimming:** fruits and vegetables showing damage and spoilage are generally trimmed.

- In this manner, areas heavily contaminated with microorganisms are removed (e.g., trimming the outside leaves in cabbage, remove visible mold growth from hard cheeses).

4. **Washing** foods, equipment and work areas under cleaning and sanitation.
   - Fruits and vegetables are washed regularly to reduce temperature and remove soil.
   - Shell eggs are washed to remove fecal materials and dirt.
   - Carcasses of food animals (beef and lamb) are washed to remove hair, soil particles, and microorganisms.

Control by Heat

- The desirable effect of heat on the taste of foods of animal and plant origin was probably accidentally discovered, following a natural forest fire, by our ancestors long before civilization.

- ❖ They also possibly recognized that heated foods did not spoil as rapidly as raw foods or cause health hazards.
- ❖ Since then, heat has been used to roast, boil, bake, and concentrate foods to improve taste and to enhance shelf life (and probably safety).

Objectives

- ❖ The main objective (microbiological) of heating food is to destroy vegetative cells and spores of microorganisms that include molds, yeasts, bacteria, and viruses (including bacteriophages).
- ❖ Heating of foods also helps destroy undesirable enzymes (microbial and food) that would otherwise adversely affect the acceptance quality of food.
  - ❖ 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.

Influencing Factors

A. Nature of Food

- ❖ Composition (amount of carbohydrates, proteins, lipids, and solutes)
  - ▪ Provide protection to microorganisms against heat.
- ❖ *Aw* (moisture): Microorganisms in liquid food are more susceptible to heat destruction than in a solid food.
- ❖ pH: Microorganisms are more susceptible to heat damage in a food that has higher *Aw* or lower pH.
  - ❖ Antimicrobial content (natural or added).
  - ▪ In the presence of antimicrobials, not inactivated by heat, microorganisms are destroyed more rapidly.

B. Nature of Microorganisms

Factors that influence microbial sensitivity to heat include

- ❖ Inherent resistance of species and strains
  - ➢ Cells of molds, yeasts, and many bacteria (except thermoduric and thermophilic), as well as viruses, are destroyed within 10 min at 65°C.

- Most thermoduric and thermophilic bacterial cells important in foods are destroyed in 5 to 10 min at 75 to 80°C.
- Yeast and most mold spores are destroyed at 65 to 70°C in a few minutes
- Many bacterial spores are destroyed at 100°C in 30 min
- All spores are destroyed at 121°C in 15 min (sterilization temperature and time).

❖ Stage of growth
- Cells at the exponential stage of growth are more susceptible to heat than resting cells (stationary phase).

❖ previous exposure to heat

❖ Initial load.
- The higher the initial microbial load in a food, the longer the time it takes at a given temperature to reduce the population to a predetermined level.

C. Nature of Process

❖ Liquid food is heated more rapidly than a solid food, and a container with high conduction (metal) is better.

❖ A product can have a cold point at the center (in a solid food in a can) or near the end (in a liquid food in a can), which may not attain the desired temperature within the given time.

❖ Finally, it needs to be emphasized that heating a food at a given temperature for a specific time means that every particle of that food should be heated to the specified temperature(71.6°C) and stay at that temperature for the specified time (15 sec; milk pasteurization).

❖ This time is also called the holding time.

Mathematical Expressions

Expressions are helpful to design a heat-treatment method for a food.

A. Decimal Reduction Time (D Value)

The D value is the time in minutes during which the number of a specific microbial (cells or spores) population exposed to a specific temperature is reduced by 90%. It is expressed as DT = t min, where T is the temperature (°C) and t is the time (min). It can be determined by using the expression:

$$D_T = \frac{t}{\log_{10} x - \log_{10} y}$$

Where x and y represent, respectively, microbial numbers before and after exposing at temperature T for t min. It also can be determined by plotting $\log_{10}$ survivors against time of exposure (min) for a specific temperature (Figure 3). The 12*D* concept is used in heat processing of high-pH foods to destroy the most heat-resistant spores of the pathogenic bacteria *Clostridium botulinum*.

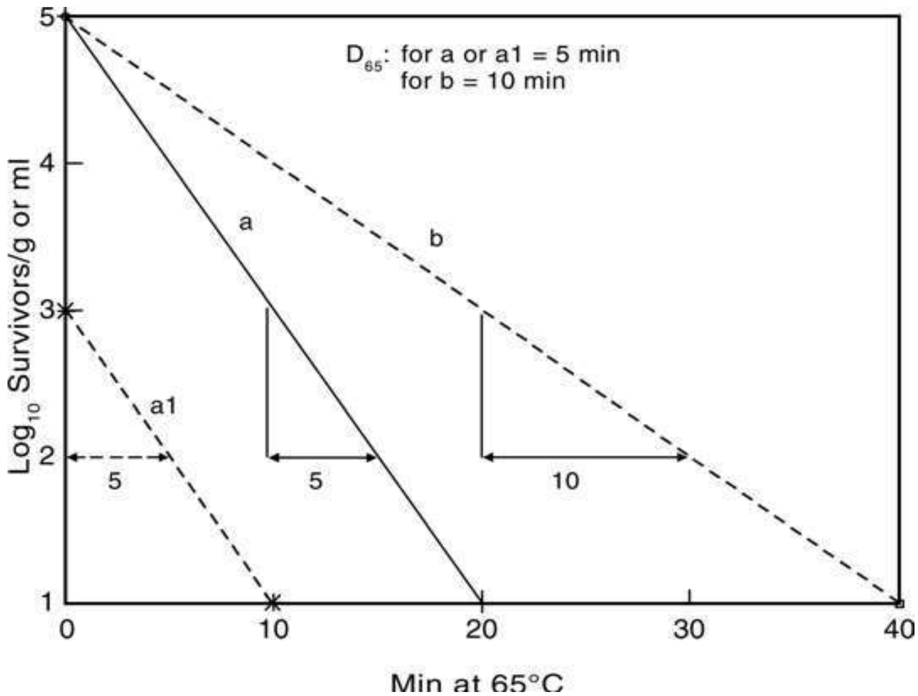

Figure.3 Graphical presentation of decimal reduction time (*D*). The graph also illustrates the number of *D* required with high and low populations of bacteria with the same heat sensitivity (a and a1) to obtain a desired survivor level (say 101/g or /ml) and different *D* values for two bacterial species with different heat sensitivities at 65°C (a and b).

B. Thermal Death Time (TDT), and Z Value,

TDT is the time in log that is necessary to completely destroy a specific number of microbial cells or spores in a population at a specific temperature. It indicates the relative sensitivity of a microorganism to different temperatures. A TDT curve can be constructed either by plotting log time of complete destruction against temperature or by plotting log *D* values against temperature (Figure 4).

The slope of the curve is the Z value, which indicates the temperature required to change the D value (or TDT) to transverse by 1 log. A value of Z = 10 in °C implies that if the D value of bacterial spores at 100°C is 50 min, at 110°C it will be 5 min, and at 120°C it will be 0.5 min.

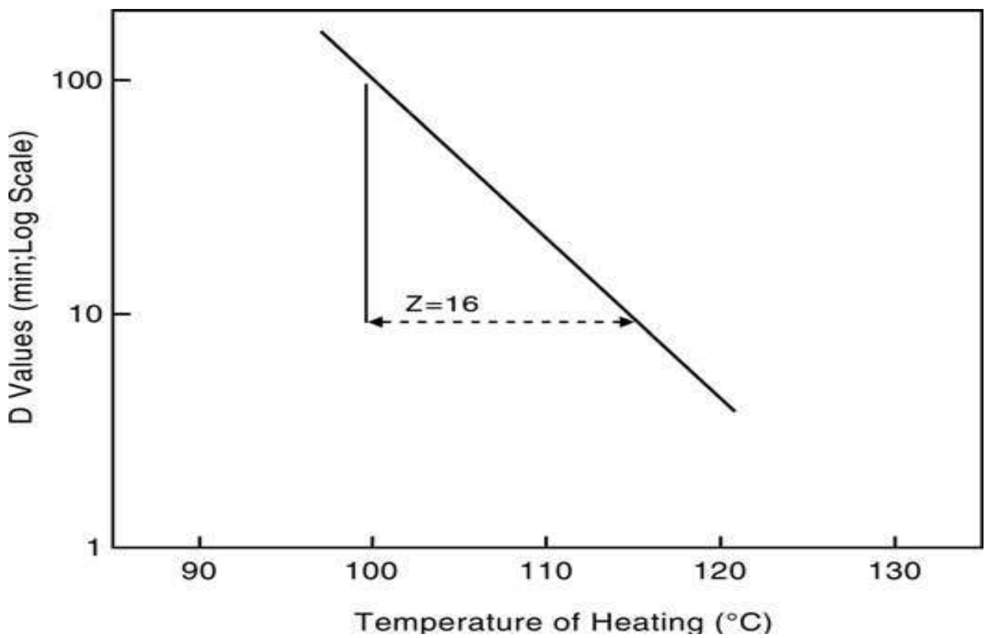

Figure 4 Hypothetical thermal death time curve. *D*, decimal reduction time; *Z*, ℃ required for the thermal death time curve to transverse over a log10 cycle.

Methods

1. **Low-heat processing pasteurization**: The temperature low-heat processing or pasteurization is below 100°C.
2. **High heat processed foods**: The process involves heating foods at or above 100°C for a desired period of time.
3. **Microwave heating**: 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.

Control by Low Temperature

Natural freezing has been used to preserve food during the very early stages of civilization, 10,000 to 12,000 B.C.

Objective

- 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, but spores are not killed at low temperature.
- Low-temperature storage, especially freezing, is also lethal to microbial cells.

Influencing Factors

A. Nature of Process

- Refrigerated foods have limited shelf life, and, with time, microorganisms grow and spoil the products.
  - Different types of bacteria, molds, and yeasts can grow.
  - Spores of some spoilage *Bacillus* and *Clostridium* spp. can germinate.
- In frozen foods, microorganisms (only cells, not spores) slowly die. However, even after long storage, some survive in frozen foods.
  - As the temperature drops further, to –20°C, and water in a food freezes completely, more cells will have sublethal and lethal injury.
- The rate of cooling of a food is also very important for effective control of the growth of pathogenic and spoilage microorganisms.
  - A slow rate of cooling of foods has been implicated as a major cause of foodborne diseases.
- During thawing of a frozen food (such as an uncooked chicken), rapid thawing is desirable in order to control microbial growth, especially growth of pathogens.

B. Nature of Food

- A food with higher solid content (especially high proteins, carbohydrates, and lipids, but low ions), pH closer to 7.0, higher *Aw*, and the absence of microbial inhibitors facilitate growth and survival of microorganisms at refrigeration temperature and cause less death at frozen temperature.

C. Nature of Microorganisms

- Although some microorganisms can grow at as low as –10°C, many mesophilic and thermophilic bacterial cells can be sublethally injured and may die with time at low temperatures above freezing.
- In general, Gram-negative, or rod-shaped bacteria are more susceptible to the damaging effect of freezing than Gram-positive or spherical-shaped bacteria.
- Also, cells from the early exponential phase of growth are more susceptible to freezing than those from the early stationary phase.
- Species and strains of microorganisms also differ greatly in sensitivity and resistance to freezing damage.
- Germination and outgrowth of spores of some *Clostridium* spp. can occur at as low as 2°C and maybe at a slightly higher temperature for some *Bacillus* spp. spores.
- Spores do not lose their viability in frozen foods.

Methods

A. Ice Chilling

- 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.

B. Refrigeration

- 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.

C. Freezing

- The minimum temperature used in home freezers 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 and not for only freezing a food to that low temperature.

Control by Reduced Aw

- The ability of dried seeds, grains, tubers, 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.
- In more recent years, new technologies have helped produce foods with low *Aw* by freeze-drying, freeze concentration, and osmotic-concentration methods.

Objectives

- The main objectives of reducing *Aw* 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.

Influencing Factors

A. Nature of Process

- Solutes differ in their ability to reduce *Aw*.
  - The amounts (% w/w) of NaCl, sucrose, glucose, and inverted sugar required to reduce *Aw* at 25°C of pure water to 0.99 are 1.74, 15.45, 8.9, and 4.11 g; and to 0.92, they are 11.9, 54.34, 43.72, and 32.87 g, respectively.
- These solutes do not freely enter the microbial cells and thus have a greater inhibitory effect on microbial cells as compared with solutes that enter freely in cells (e.g., glycerol), which are required in higher amounts for similar inhibition.

B. Nature of Foods

- In a homogeneous food, *Aw* will remain unchanged provided other factors do not change.
- However, a heterogenous food with ingredients or items of different *Aw* will generate a gradient.
  - This can lead to microbial growth in an item preserved by reduced *Aw* alone and stored with an item of high *Aw* containing a preservative.
- The minimum *Aw* for growth of microorganisms in a food can be higher than that in a broth.
  - Thus, *Sta. aureus* has a minimal *Aw* of growth of 0.86 in a broth, but it does not grow in shrimp at an *Aw* of 0.89.

- As the incubation temperature is moved in either direction from optimum without changing the *Aw*, the microorganisms require a longer time to grow.
  - In a broth of *Aw* of 0.975, a *Clo. botulinum* E strain grew in 6 d at 30°C, in 19 d at 15°C, and in 42 d at 7.2°C.
- Reduced *Aw* and low pH interact favorably in inhibiting microbial growth.
  - A *Clo. botulinum* B strain grew at an *Aw* of 0.99 up to pH 5.3 and at an *Aw* of 0.97 up to pH 6.0, but at an *Aw* of 0.95, it failed to grow even at pH 7.0.

C. Nature of Microorganisms

- Microorganisms differ greatly in their minimal *Aw* requirement for growth, sporulation, and germination (Table 9).
  - In general, molds and yeasts can grow at lower *Aw* values than bacteria.
  - Among pathogenic and spoilage bacteria, Gram-negatives require a slightly higher *Aw* than Gram-positives for growth.

Methods

A. Natural Dehydration

- Natural dehydration is a low-cost method in which water is removed by the heat of the sun.
- The process is slow, spoilage and pathogenic bacteria as well as yeasts and molds 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.
- Depending on the temperature and time of exposure, some microbial cells can die during drying.

C. Freeze-Drying

- Freeze-drying is a relatively costly process.
- The water molecules are removed from the food by sublimation (from solid state to vapor state).

- ❖ Microbial cells are exposed to two stresses — freezing and drying — that reduce some viability.
- ❖ Spores are not affected by the process.

D. Smoking

- ❖ Many meat and fish products are exposed to low heat and smoke for cooking.
- ❖ The heating process removes water from the products, thereby lowering their *Aw*.
- ❖ 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.

Table 9. Minimum A_w for microbial growth at optimum growth temperature.

Microorganism	A_w
Bacteria	
Bacillus cereus	0.95
Bacillus stearothermophilus	0.93
Clostridium botulinum Type A	0.95
Clostridium botulinum Type B	0.94
Clostridium botulinum Type E	0.97
Clostridium perfringens	0.95
Escherichia coli	0.95
Salmonella spp.	0.95
Vibrio parahaemolyticus	0.94
Staphylococcus aureus	0.86
Pseudomonas fluorescens	0.97
Lactobacillus viridescens	0.94
Yeast	
Saccharomyces cerevisiae	9.90
Saccharomyces rouxii	0.62
Debaryomyces hansenii	0.83
Molds	
Rhizopus nigricans	0.93
Penicillium chrysogenum	0.79
Penicillium patulum	0.81
Aspergillus flavus	0.78
Aspergillus niger	0.77
Alternaria citri	0.84

E. Intermediate Moisture Foods

- ❖ Intermediate moisture foods (IMF) that have *Aw* values of 0.70 to 0.90 (with moisture contents of 10 to 40%).
  - ➢ Some of the IMFs include salami, dry sausages, dried fruits, jams, jellies, and honey.

- The low *Aw* value and relatively high moisture is obtained by adding water-binding solutes and hydrophilic colloids.
- Microorganisms can survive in the products, but because of low *Aw*, bacteria cannot grow.
- However, yeasts and molds can grow in some.

Control by Low pH and Organic Acids

- It was observed that over a restricted pH range, many microorganisms present in food can grow, but at lower pH ranges many of them die.
- Once this was recognized, many organic acids were used as food additives.
    - In addition to their effectiveness as food preservatives, they are also used to improve acceptance qualities of foods.
- Organic acids in foods can be:
    - Present naturally such as citric acid in citrus fruits.
    - Produced in different fermented foods such as acetic, lactic, and propionic acids.
    - Added to foods and beverages to reduce the pH.

Influencing Factors

A. Nature of Acids

- The antimicrobial effectiveness of four acids follows the order acetic > propionic > lactic > citric.
- Solubility of the acids in water is important for the desirable effect.
    - Acetate, propionate, lactate, and citrate are very soluble in water, whereas benzoate, sorbate, and paraben are poorly soluble in water, and thus, at the same concentration, have different effectiveness.
- Organic acids also differ in their lipophilic properties, which in turn regulate their ease in entering the cells.
    - Acetic and propionic acids are more lipophilic than lactic acid and have more antimicrobial effectiveness than lactic acid.

B. Nature of Foods

- The normal pH of foods varies greatly from a very acid range (3.0; citrus juice) to an alkaline range (pH 9.0; egg albumen).
- An acid is more inhibitory in a food at a lower pH than in one at a higher pH.
- The buffering action of the food components also reduces the effectiveness of low pH.
- Nutrients can also facilitate repair of sublethal acid injury of microorganisms.

C. Nature of Microorganisms

- Microorganisms important in food vary greatly in the lower limit of pH that allows growth (Table 10).
- In general, Gram-negative bacteria are more sensitive to low pH than are Gram-positive bacteria, and yeasts and molds are the least sensitive.

Acids Used

A. Acetic Acid: more effective against bacteria than yeasts and molds.

B. Propionic Acid: effective against molds and bacteria but almost ineffective against yeasts.

C. Lactic Acid

D. Citric Acid.

E. Sorbic Acid: more effective against molds and yeasts than against bacteria.

F. Benzoic Acid: more effective against yeasts and molds than bacteria.

G. Parabens (Esters of *p*-Hydroxybenzoic Acid) effective at high pH and against bacteria, yeasts, and molds.

Table. 10 Minimum pH at which Growth Occurs

Microorganism	Minimum Growth pH
Gram-Negative Bacteria	
Escherichia coli	4.4
Pseudomonas spp.	5.6
Salmonella	4.5
Vibrio spp.	4.8
Serratia spp.	4.4
Gram-Positive Bacteria	
Bacillus cereus	4.9
Bacillus stearothermophilus	5.2
Clostridium botulinum	4.6
Clostridium perfringens	5.0
Enterococcus faecalis	4.4
Lactobacillus spp.	3.8
Staphylococcus aureus	4.0
Listeria monocytogenes	4.6
Yeasts	
Candida spp.	1.5 to 2.3
Saccharomyces spp.	2.1 to 2.4
Hansenula spp.	2.1
Rhodotorula spp.	1.5
Molds	
Aspergillus spp.	1.6
Penicillium spp.	1.6 to 1.9
Fusarium spp.	1.8

Control by Modified Atmosphere (or Reducing O–R Potential)

❖ The use of modified atmosphere to reduce O–R potential of the environment has been a very widely used method to control growth mainly of aerobic microorganisms in food.

❖ Three terminologies are used to alter the atmosphere in foods in order to preserve and increase their acceptance quality.

1. *Controlled Atmosphere Packaging (CAP).*
2. *Modified Atmosphere Packaging (MAP).*
3. *Vacuum Packaging.*

Objectives

❖ The objectives of MAP are to control or reduce the growth of undesirable microorganisms in food.

❖ The technique also helps retard enzymatic and respiratory activities of fresh foods.

- The growth of aerobes (molds, yeasts, and aerobic bacteria) is prevented in products that are either vacuum packaged or flushed with 100% CO_2, 100% N_2, or a mixture of CO_2 and N_2.
- However, under these conditions, anaerobic and facultative anaerobic bacteria can grow unless other techniques are used to control their growth.

Methods

A. Vacuum Packaging: is predominantly used in many fresh and ready to-eat meat products.

B. Gas Flushing: is used to increase the shelf life of many foods. The gases usually used are a mixture of CO_2 and N_2, with some O_2 for packaging red meats.

Control 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 designated as antimicrobial inhibitors or preservatives.

Objectives

- 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.

Influencing Factors

- compound should have the desired antimicrobial property
    - Compound that kills instead of controlling growth is preferred.
    - Compound effective against many types of microorganisms important in foods is preferred.
    - Compound effective not only against vegetative cells but also against spores is preferred.

- Most compounds do not meet all these requirements.

- Compound should not affect the normal quality of a food (texture, flavor, or color).
- It should not interact with food constituents and become inactive.
- It should have a high antimicrobial property at the pH, *Aw*, Eh, and storage temperature of the food.
- It should be stable during the storage life of the food.
- It should be economical and readily available.
- It should be effective in small concentrations.
- It should be safe for human consumption.

Examples of Antimicrobial Preservatives

Added antimicrobial compounds have to be approved by regulatory agencies. Table.11 lists some antimicrobial preservatives used in foods.

Table. 11 Some Antimicrobial Chemical Preservatives Used in Foods

Acetaldehyde	Dehydroacetate	Lactic acid	Propylene glycol
Acetic acid	Diacetate	Lauric acid	Propylene oxide
Ascorbic acid	Diacetyl	Lysozyme	Propyl gallate
Bacteriocins	Diethyl dicarbonate	Malic acid	Smoke
Benomyl	Diphenyl	Methyl bromide	Sodium chloride
Benzoic acid	Ethyl alcohol	Monolaurin	Sorbic acid
Betapropiolactum	Ethyl formate	Natamycin	Spices
BHA, BHT, and TBHQ	EDTA	Nitrite and nitrate	Succinic acid
Boric acid	Ethylene oxide	Parabens	Sucrose
Caprylic acid	H_2O_2	Peracetate	Sulfites and SO_2
Chitosan		Polyphosphates	Tetracyclines
Citric acid		Propionic acid	Thiabendazole
CO_2 and CO			Tylosin

[a]Not all are permitted in the U. S.

Control by Irradiation

- 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, β-rays, ɣ-rays, and cosmic rays).

- β-rays have very little penetration power.
- X-rays have good penetration power.
- ɣ-rays (photons) have high penetration power (40 cm thick) and may be considered effective and economical for use in foods.
  - The ability of x-rays, β-rays, and ɣ-rays to kill microorganisms was recognized soon after their discovery in the late 19th and early 20th centuries.
  - Irradiated fresh fruits, vegetables, meat, and fish have been approved in many countries.
  - Irradiated foods will have a special logo (Figure 5).

Objectives

- Irradiation 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.
- Irradiation cannot destroy toxins or undesirable enzymes in a food.
- Irradiation is a cold sterilization process, and thus irradiated foods do not show some of the damaging effects of heat on food quality.
- Irradiation can cause oxidation of lipids and denaturation of food proteins, especially when used at higher doses.

Influencing Factors

A. Nature of Process

- Among the several methods available (x-rays, β-rays, ɣ-rays), ɣ-rays have a higher potential for effective and economical use in food preservation.
- The antimicrobial efficiency of ionizing radiation increases as the dose is increased.
- The antimicrobial efficiency decreases in the absence of oxygen (because of reduced oxidizing reactions) and at low *Aw* (because of reduced free-radical formation with less water).

- ❖ Freezing also reduces the efficiency because of reduced availability of reactive water molecules.

B. Nature of Foods

- ❖ Foods can be exposed to ɣ-radiation in packages, cans baskets, and bags (because of its penetration capability).
- ❖ Frozen, dry, or anaerobically packaged foods need higher doses of treatment.

C. Nature of Microorganisms

- ❖ Because of size differences, molds are more sensitive than yeasts, which are more sensitive than bacterial cells; bacterial cells are more sensitive than viruses.
- ❖ Gram-negative are more sensitive than Gram-positive bacteria, and rods are more sensitive than cocci.
- ❖ Some strains designated as radiation resistant such as *Salmonella, Escherichia coli, Enterococcus faecalis,* and *Staphylococcus aureus.*
- ❖ Spores are quite resistant to irradiation, probably because their water content is very low (e.g., spores of *Clostridium botulinum* Type A and *Bacillus pumilus*).

UV Radiation: Because of low penetration power, it has been used to inactivate microorganisms on the surface of foods as well as in air and on walls, shelves, and equipment in the food handling and processing area.

Figure 5: Logo for irradiated foods. The Codex Alimentarius, an international committee on food safety, has developed this logo to put in green color on the package of irradiated foods. Irradiated foods from the U.S. are labeled with this logo, along with the words.