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Ministry of Higher Education and Scientific Research

University of 8 May 1945-Guelma

Faculty of Natural and Life Sciences, Earth and the Universe

Department of Biology

Course support for the Master 2 Applied Biochemistry

Food Microbiology

SHV : 67.5 hours, WHV : 3 hours
Credit : 6, Coefficient : 3

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FORE-WORD

This course is designed to provide students with an understanding of the principles and applications of microbiology in the food sector. Food microbiology is an essential discipline that explores the role of microorganisms in the processing, preservation, and safety of food.

Microorganisms are ubiquitous in our environment and play a crucial role in the food chain, either as beneficial fermentation agents or as potential pathogens. This course will highlight the complex interactions between microorganisms and food, the factors influencing these interactions, and the methods for microbiological control and analysis of foods. We will also explore the risks associated with foodborne intoxications and infections.

This course aims to provide a comprehensive understanding of microorganisms involved in food processing and spoilage, understand the factors influencing microbial growth in food and to evaluate health risks related to foodborne microorganisms and associated management measures.

The course is divided into six chapters: Introduction to Food Microbial Flora: Origin and Role, Classification and Characterization of Major Microorganisms in Food Products, Extrinsic and Intrinsic Factors of Foods Affecting their Microbiology, Food Biodeterioration and Control Methods, Bacterial Food Poisoning and Infections, Bacteriological Analysis of Foods.

The course is not based on any specific reference. Rather, it draws its origins from several textbooks and authoritative sources in the field. These sources collectively contribute to the comprehensive understanding and practical application of food microbiology concepts presented throughout the course.

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Microorganisms are living entities of microscopic size and include bacteria, viruses, yeasts and molds (fungi), algae, and protozoa. For a long time, bacteria have been classified as procaryotes (cells without definite nuclei), and the fungi, algae, and protozoa as eucaryotes (cells with nuclei); viruses do not have regular cell structures and are classified separately.

Microorganisms are present everywhere on Earth, including humans, animals, plants and other living creatures, soil, water, and atmosphere. The interactions between microorganisms, plants, and animals are natural and constant. Since the human food supply consists of plants and animals or products derived from them, it is understandable that our food supply can contain microorganisms in interaction with the food.

1. Food ?

These are complex substances, most often natural, having undergone technological and/or culinary treatment or not, preserved with or without special treatment. Foods are consumed in order to satisfy the body is nutritional and energy needs, but because of their organoleptic, emotional and sociological qualities.

2. Food Microbiology ?

Before the 1970s, food microbiology was regarded as an applied science mainly involved in the microbiological quality control of food. Thus, modern-day food microbiology needs to include a great deal of basic science to understand and effectively solve the microbiological problems associated with food. The discipline includes not only microbiological aspects of food spoilage and foodborne diseases and their effective control and bioprocessing of foods but also basic information of microbial ecology, physiology, metabolism, and genetics.

3. Origin of Food Microbial Flora

Microorganisms are omnipresent in the environment and can be found in soil, water, air, plants, animals, and humans. The microbial flora of food originates from multiple sources during the various stages of food production, processing, and handling. Understanding these sources is crucial for managing microbial contamination and ensuring food safety. The source of food-contaminating microorganisms can be classified into two categories: Endogenous and Exogenous.

3.1. Endogenous origin (primary sources)

Microorganisms exist in the raw material or food before any handling. They come from the animal or plant organism from which the food is produced.

3.1.1. Commensal flora of the organism

In food, commensal microorganisms can influence the overall microbial ecology, impacting both food safety and quality. Most contaminants are of intestinal origin. They can be anaerobic bacteria, aero-anerobic and micro-aerophilic.

3.1.2. Food prepared from a sick organism

This type of contamination is less frequent due to veterinary controls, while plant products do not constitute a great danger for the consumer because phytopathogenic microorganisms are almost harmless to humans and animals.

3.2. Exogenous origin (secondary sources)

The accidental introduction of microorganisms during the processing of the raw material.

3.2.1. 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.

3.2.2. 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) and storage of foods (e.g., fish on ice), washing and sanitation of equipment, and processing and transportation facilities.
- ❖ The water is free from any pollution of animal or human origin; the microorganisms present in the water come from aquatic plants and the soil. The flora is diverse, including Gram-negative bacilli like *Pseudomonas, Aeromonas, Acinetobacter*, and *Alcaligenes*, as well as Gram-positive cocci like Micrococci.
- ❖ Water can collect pollutants of human or animal origin, notably intestinal microorganisms: Enterobacteria, Enterococci, *Clostridium*. Among these, Fecal-origin flora can cause various infections.

3.2.3. Air

- Microorganisms are present in dust and moisture droplets in the air. They do not grow in dust, but are transient and variable, depending on the environment. Their level is controlled by the degree of humidity, size and level of dust particles, temperature and air velocity, and resistance of microorganisms to drying. Generally, dry air with low dust content and higher temperature has a low microbial level.
- Spores of *Bacillus* spp., *Clostridium* spp., and molds, and cells of some Gram positive bacteria (e.g., *Micrococcus* spp. and *Sarcina* spp.), as well as yeasts, can be predominantly present in air. 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 (including bacteriophages), can be transmitted via the air.

3.2.4. Skin flora

- Microorganisms are found on the keratinized layers of the skin.
- Two species are constant regardless of the skin location: *Staphylococcus epidermidis* and *Propionibacterium acnes*.
- Other species are common, such as yeasts and molds.
- Some species are only occasionally present or in specific sites, such as *Staphylococcus aureus* in the nasal passages.

3.2.5. Processing Equipment

Equipment and surfaces in food processing facilities can harbor microorganisms if not properly cleaned and sanitized. Cross-contamination from equipment is a common issue. Contact between food and dirty surfaces increases their microbial load.

4. Role of Microbial Flora in Food

The microbial flora present in food can have both beneficial and detrimental effects. Understanding these roles is essential for managing food quality and safety.

4.1. Beneficial Roles

4.1.1. Fermentation

Certain microorganisms are intentionally added to food to carry out fermentation processes. Fermentation enhances the flavor, texture, and nutritional value of foods. Common examples include lactic acid bacteria in yogurt and sauerkraut, and yeast in bread and beer production.

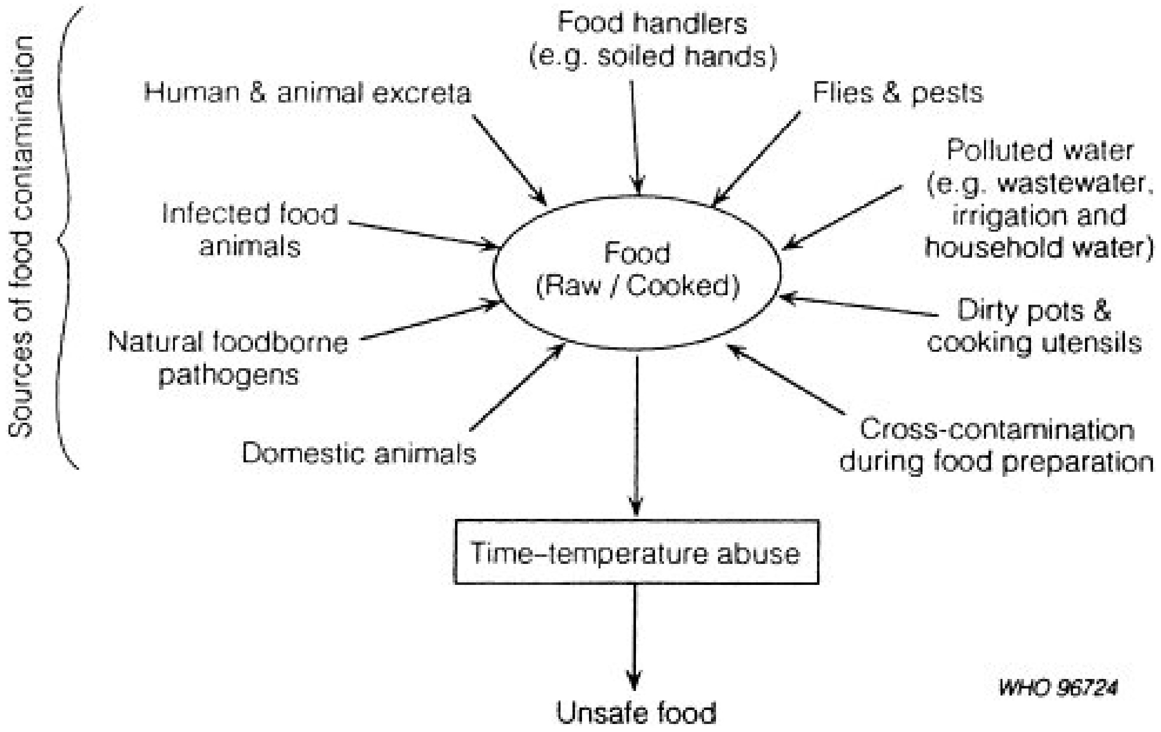

Figure 1. Origin of contaminants in food.

a. Lactic fermentation

- ❖ It is involved in the production of yogurts, fermented milks, sausages, certain cheeses.
- ❖ It is homolactic when, under the action of homofermentative bacteria, lactic acid is in the majority. Among the homofermentative bacteria bacteria of the genera Lactococcus, Lactobacillus and Streptococcus.
- ❖ It is heterolactic when under the action of heterofermentative bacteria we obtain lactic acid and other products, ethanol, ethanoic acid, carbon dioxide. Among heterofermentative bacteria, the genera Leuconostoc and certain Lactobacillus.

b. Alcoholic fermentation

The ethanol in all alcoholic beverages comes from the fermentation of glucose under the effect of zymase, an enzyme produced by yeasts (brewer's yeast: *Saccharomyces cerevisiae*, *Candida utilis*).

c. Acetic fermentation

- ❖ It is to Louis Pasteur (1808-1873) that we discovered the biochemical nature of the vinegar formation process. From 1865, based on Pasteur's research, the industrial production of vinegar boomed. He showed that the ferment is a living being which he calls *Mycoderma aceti* (*Acetobacter aceti*) (vinegar flower).

- Acetic fermentation is a type of fermentation in which ethanol from alcoholic fermentation is oxidized to acetic acid (aerobic).

d. Propionic fermentation

- Fermentation at the end of which glucose is transformed into propionic acid and ethanoic acid, which are responsible for the flavor of cooked cheeses, and carbon dioxide is responsible for the opening of these cheeses.
- The bacteria that produce this type of fermentation are propionic bacteria (genus Propionibacterium).

e. Butyric fermentation

- Fermentation during which glucose is transformed into acetic and butyric acids, carbon dioxide and hydrogen.
- Butyric acid is responsible for the putrid odor and spicy taste of some cooked cheeses. This fermentation takes place under the effect of the bacteria *Clostridium butyricum*.

4.1.2. Probiotics

Some microorganisms, known as probiotics, are beneficial to human health. These microorganisms, often found in fermented foods, can improve gut health and boost the immune system.

4.2. Detrimental Roles

4.2.1. Food Spoilage

Many microorganisms are responsible for the spoilage of food, leading to economic losses and food waste. Spoilage microorganisms cause changes in food color, texture, odor, and taste, making it unfit for consumption. Common spoilage bacteria include *Pseudomonas, Lactobacillus,* and *Clostridium* species.

4.2.2. Foodborne Pathogens

Pathogenic microorganisms can cause foodborne illnesses, posing significant public health risks. Common foodborne pathogens include *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, and Norovirus. These pathogens can lead to symptoms ranging from mild gastroenteritis to severe, life-threatening conditions

The microbial groups significant in foods include various species and types of bacteria, yeasts, molds, and viruses. These microorganisms are important due to their roles in causing foodborne diseases and spoilage, as well as in producing food and food ingredients.

While many bacterial species, some molds, and viruses can cause foodborne diseases, yeasts do not. Most bacteria, molds, and yeasts can grow in foods and potentially cause spoilage, unlike viruses, which cannot grow in foods. Several species of bacteria, molds, and yeasts are considered safe or food-grade and are used to produce fermented foods and ingredients.

Among these groups, bacteria are the largest and most significant due to their ubiquitous presence and rapid growth rate, even in conditions where yeasts and molds cannot grow. Consequently, bacteria are the most important microorganisms in food spoilage and foodborne diseases.

1. Classification of Important Microorganisms in Food

1.1. Bacteria

- ❖ Bacteria are unicellular organisms, typically about 0.5–1.0 x 2.0–10 µm in size. They come in three morphological forms: spherical (cocci), rod-shaped (bacilli), and curved (comma-shaped). Bacteria can form associations such as clusters, chains (two or more cells), or tetrads, and they can be either motile or nonmotile. Their cytoplasmic materials are enclosed within a rigid cell wall and a membrane beneath it. Nutrients in molecular and ionic forms are transported from the environment through the membrane via specific mechanisms, which also contains energy-generating components and forms intrusions called mesosomes in the cytoplasm. Bacterial cytoplasm does not contain membrane-bound organelles, and ribosomes are dispersed within it. The genetic materials, including structural and plasmid DNA, are circular and not enclosed in a nuclear membrane, nor do they contain histones.
- ❖ Based on Gram-stain behavior, bacteria are classified as Gram-negative or Gram-positive. Gram-negative bacteria have a complex cell wall with an outer membrane and a middle membrane, consisting of lipopolysaccharides (LPS), lipoproteins (LP), and phospholipids arranged in a bilayer, with the hydrophobic parts inside and the hydrophilic parts outside. Gram-positive bacteria, on the other hand, have a thick cell wall made of several layers of mucopeptide, providing a thick, rigid structure, and two types of teichoic acids. They also have antigenic properties, useful for serological identification of Gram-positive bacteria.

1.1.1. Gram-positive Cocci

a. *Staphylococcus*

- *Staphylococcus* are gram-positive cocci rounded into regular clusters or diplococci, spherical cells (0.5 to 1 mm); occur singly, in pairs, or clusters; nonmotile; mesophiles; facultative anaerobes; grow in 10% NaCl, devoid of spores and capsules. They are catalase positive and oxidase negative, fermenting glucose without gas.
- *Staphylococcus aureus* strains are frequently implicated in food poisoning.
- *Staphylococcus carnosus* is used for fermentation of certain sausages.

b. *Streptococcus*

- Spherical or ovoid (1 mm); occur in pairs or chains; nonmotile; facultative anaerobes; mesophiles, devoid of spores and rarely encapsulated, catalase negative, mesophilic.
- *Streptococcus pyogenes* is pathogenic and has been implicated in foodborne illnesses.
- *Streptococcus thermophilus* is used in lactic fermentation.

c. *Micrococcus*

These are spherical cells ranging from 0.2 to 2 µm in size. They occur in pairs, tetrads, or clusters and are aerobes and nonmotile. Some species produce yellow colonies. They are mesophiles and resistant to low heat. They are commonly found on mammalian skin and can cause spoilage. An example species is *Micrococcus luteus*.

d. *Enterococcus*

Spheroid cells (1 µm) that occur in pairs or chains. They are nonmotile, facultative anaerobes, and some strains can survive low heat (pasteurization). They are mesophiles and typically found in the intestinal contents of humans, animals, and birds, as well as in the environment. They can establish on equipment surfaces and are used as indicators of sanitation. They are important in food spoilage, *Enterococcus faecalis*.

e. *Lactococcus*

Ovoid, elongated cells (0.5 to 1.0 µm) that occur in pairs or short chains. They are nonmotile, facultative anaerobes, and mesophiles, but can grow at 10°C. They produce lactic acid and are used in the production of many bioprocessed foods, especially fermented dairy foods. Species include *Lactococcus lactis* subsp. *lactis* and subsp. *cremoris*. They are present in raw milk and plants, and several strains produce bacteriocins, some of which have a relatively wide host range against Gram-positive bacteria, making them potential food biopreservatives.

f. *Leuconostoc*

Spherical or lenticular cells that occur in pairs or chains. They are nonmotile, facultative anaerobes, and heterolactic fermenters. They are mesophiles, but some species and strains can grow at or below 3°C. Some are used in food fermentation. Psychrotrophic strains are associated with spoilage (gas formation) of vacuum-packaged refrigerated foods. They are found in plants, meat, and milk. Species include *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Leuconostoc lactis*, *Leuconostoc carnosum*.

g. *Pediococcus*

Spherical cells (1 µm) that form tetrads, mostly present in pairs. They are nonmotile, facultative anaerobes, and homolactic fermenters. They are mesophiles, but some can grow at 50°C and survive pasteurization. Some species and strains are used in food fermentation, while others can cause spoilage of alcoholic beverages. They are found in vegetative materials and some food products. Species include *Pediococcus acidilactici* and *Pediococcus pentosaceus*. Several strains produce bacteriocins with a wide spectrum against Gram-positive bacteria, making them potential food biopreservatives.

h. *Sarcina*

Large, spherical cells (1 to 2 µm) that occur in packets of eight or more. They are nonmotile, produce acid and gas from carbohydrates, and are facultative anaerobes. They are present in soil, plant products, and animal feces and can be involved in the spoilage of foods of plant origin. Species include *Sarcina maxima*.

1.1.2. Gram-positive Bacilli

a. *Bacillus*

- ❖ Rod-shaped, straight cells vary widely in size (small, medium, or large; 0.5–1 x 2–10 µm) and shape (thick or thin). They can exist singly or in chains, be motile or nonmotile, mesophilic or psychrotrophic, and aerobic or facultatively anaerobic. All form spherical or oval endospores, which are highly heat-resistant, with one spore per cell.
- ❖ These bacteria include many species significant in foods due to their ability to cause foodborne disease (e.g., *Bacillus cereus*) and food spoilage, particularly in canned products (e.g., *Bacillus coagulans*, *Bacillus stearothermophilus*). Some species and strains produce enzymes used in food bioprocessing (e.g., *Bacillus subtilis*).
- ❖ They are commonly found in soil, dust, and plant products, especially spices. Many species and strains can produce extracellular enzymes that hydrolyze carbohydrates, proteins, and lipids.

b. *Clostridium*

- Rod-shaped cells that vary widely in size and shape; motile or nonmotile. They are anaerobes, with some species being extremely sensitive to oxygen, and can be mesophilic or psychrotrophic. They form endospores, which are oval or spherical and usually located at one end of the cell, although some species sporulate poorly. These spores are heat resistant.
- These bacteria are found in soil, marine sediments, sewage, decaying vegetation, and animal and plant products.
- Some species are pathogens significant in food safety, such as *Clostridium botulinum* and *Clostridium perfringens*, while others are important in food spoilage, including *Clostridium tyrobutyricum*, *Clostridium saccharolyticum*, and *Clostridium laramie*. Additionally, some species are used as sources of enzymes for hydrolyzing carbohydrates and proteins in food processing.

c. *Lactobacillus*

- Rod-shaped cells that vary widely in shape and size; some are very long while others are coccobacilli. They appear singly or in small and large chains. These bacteria are facultative anaerobes, with most species being nonmotile. They are mesophiles, though some are psychrotrophs. Lactobacilli can be either homo- or heterolactic fermenters and are found in plant sources, milk, meat, and feces.
- Many species are used in food bioprocessing, such as *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus helveticus*, and *Lactobacillus plantarum*. Some are also used as probiotics, including *Lactobacillus acidophilus*, *Lactobacillus reuteri*, and *Lactobacillus casei* subsp. *casei*. Some species, like *Lactobacillus sake* and *Lactobacillus curvatus,* can grow at low temperatures in refrigerated products.

d. *Corynebacterium*

Slightly curved rods with some cells staining unevenly. They are facultative anaerobes, nonmotile, and mesophilic. Corynebacteria are found in the environment, plants, and animals. Some species cause food spoilage, while *Corynebacterium glutamicum* is used to produce glutamic acid.

e. Propionibacterium

Pleomorphic rods (0.5 × 2 µm) that can be coccoid, bifid, or branched. They are present singly or in short chains, often forming V and Y configurations or clumps with a Chinesecharacter-like arrangement. These bacteria are nonmotile, anaerobic, and mesophilic. Dairy propionibacteria, such as *Propionibacterium freudenreichii* in Swiss cheese, are used in food fermentation. They produce proline and propionic acid and are found in raw milk, Swiss cheese, and silage.

f. *Bifidobacterium*

Rods of various shapes, present singly or in chains, and arranged in V or star-like shapes. These nonmotile, mesophilic, anaerobic bacteria metabolize carbohydrates to lactate and acetate. They are found in the colons of humans, animals, and birds. Some species, like *Bifidobacterium bifidum*, *Bifidobacterium infantis*, and *Bifidobacterium adolescentis*, are used as probiotics.

g. *Brevibacterium*

Cells that can change from rod to coccoid shape; they are aerobes, nonmotile, and mesophilic. *Brevibacterium linens* and *Brevibacterium casei** are involved in developing the aroma in several cheese varieties (surface-ripened) due to sulfur compound production (such as methanethiol). In other protein-rich products, they can cause spoilage (in fish). They are found in different cheeses and raw milk.

h. *Carnobacterium*

Similar in many characteristics to lactobacilli cells, these bacteria are found in meat and fish. They are facultative anaerobes, heterofermentative, and nonmotile, and can grow in foods, especially in meat products stored at refrigerated temperatures. Some strains produce bacteriocins. Species include *Carnobacterium piscicola*.

i. *Brochothrix*

Similar in many characteristics to lactobacilli, these facultative anaerobes are homofermentative and nonmotile. They are found in meat and can grow in refrigerated vacuum-packaged meat and meat products. Species include *Brochothrix thermosphacta*.

j. *Listeria*

Short rods (0.5 × 1 µm) that occur singly or in short chains. They are motile, facultative anaerobes that can grow at 1°C, but their cells are killed by pasteurization. The species are widely distributed in the environment and have been isolated from different types of foods. Some strains of *Listeria monocytogenes* are important foodborne pathogens.

1.1.3. Gram-negative Bacilli

1.1.3.1. Gram-Negative Aerobes

a. *Pseudomonas*

- Pseudomonadaceae family currently includes 5 genera: Pseudomonas, Comamonas, Frateuria, Xanthomonas and Zoogloea. 265 species were listed, but the 1974 edition of the Manual Bergey included 29 species, 13 of which were of medical interest. In the 3rd edition of this Systematic Bacteriology manual, more than 40 species have been listed.
- Gram-negative bacilli, aerobic, mobile by ciliature (monotrichous – perititrichous), rarely immobile and non-sporulating.
- Oxidase +, the optimal temperature is 30°C.
- They are ubiquitous and widespread in nature, characterized by their resistance to antibiotics and antiseptics.
- *Pseudomonas aeruginosa* is the most well-known and widespread species within the Pseudomonas genus. It is considered the most pathogenic and serves as the type species for the genus and nosocomial infections. It can also be associated with the deterioration of certain plants.
- The two most common and characteristic pigments are pyocyanin (blue pigment) and pyoverdine (fluorescent yellow-green pigment). They are soluble in culture media and can color them.
- Pseudomonas species are widely found in the environment and include *Pseudomonas fluorescens*, *Pse. aeruginosa*, and *Pse. putida*. They are important spoilage bacteria that can metabolize a wide variety of carbohydrates, proteins, and lipids in foods.

b. *Campylobacter*

Two species, *Campylobacter jejuni* and *Campylobacter coli*, are foodborne pathogens. They are small (0.2 × 1 mm), microaerophilic, helical, and motile. Found in the intestinal tracts of humans, animals, and birds. Mesophiles.

c. *Xanthomonas*

Most characteristics of this group are similar to those of Pseudomonas. They are plant pathogens that can cause spoilage of fruits and vegetables. Strains of *Xanthomonas campestris* are used to produce xanthan gum, a food stabilizer.

d. *Acetobacter*

These bacteria are ellipsoid to rod-shaped (0.6 × 4 mm), occurring singly or in short chains, and can be motile or nonmotile. They are aerobes that oxidize ethanol to acetic acid and are mesophiles. They cause souring of alcoholic beverages and fruit juices and are used to produce vinegar. They can also spoil some fruits. An important species is *Acetobacter aceti*.

e. *Gluconobacter*

This group shares many characteristics with Acetobacter. *Gluconobacter oxydans* causes spoilage of pineapples, apples, and pears.

f. *Acinetobacter*

Rod-shaped (1 × 2 mm), occurring in pairs or small chains, with twitching motility due to polar fimbriae. They are strictly aerobic and grow between 20 and 35°C. Found in soil, water, and sewage. An important species is *Acinetobacter calcoaceticus*.

g. *Moraxella*

Very short rods that frequently approach a coccoid shape (1 × 1.5 mm), occurring singly, in pairs, or in short chains. They may be capsulated and exhibit twitching motility. Optimum growth is at 30 to 35°C. Found in the mucous membranes of animals and humans. An important species is *Moraxella lacunata*.

h. *Alteromonas*

Most species are of marine origin and may be present in foods of marine origin. They require 100 mM NaCl for optimum growth. *Alteromonas putrefaciens* (recently reclassified as *Shewanella putrefaciens*) has many characteristics similar to *Pseudomonas* and is important in fish and meat spoilage. They are psychrotrophs.

i. *Flavobacterium*

Rods with parallel sides (0.5 × 3 mm), nonmotile, and with colored colonies. Some species are psychrotrophs. They cause spoilage of milk, meat, and other protein foods (*Flavobacterium aquatile*).

j. *Alcaligenes*

Rods or coccobacilli (0.5 × 1 mm), motile, and found in water, soil, or fecal material. They are mesophiles that cause spoilage of protein-rich foods (*Alcaligenes faecalis*).

k. *Brucella*

Coccobacilli (0.5 × 1.0 mm), mostly single, and nonmotile. Different species cause diseases in animals, including cattle, pigs, and sheep. They are also human pathogens and have been implicated in foodborne brucellosis. *Brucella abortus* causes abortion in cows.

l. *Psychrobacter*

This genus, created in 1986, contains one specie: *Psychrobacter immobilis*. They are coccobacilli (1 × 1.5 mm), nonmotile, and can grow at 5°C or below, with optimum growth at 20°C, and unable to grow at 35°C. Found in fish, meat, and poultry products.

1.1.3.2. Gram-Negative Facultative Anaerobes

a. *Escherichia*

Escherichia are straight rods (1 × 4 mm), capable of being motile or nonmotile, and they are mesophiles. They reside in the intestinal tracts of humans, warm-blooded animals, and birds. While many strains are harmless, some can cause foodborne diseases in humans and animals. They are used as indicators of sanitation in the coliform and fecal coliform groups. *Escherichia coli* is a significant species within this genus.

b. *Citrobacter*

These are straight rods (1 × 4 mm), typically found singly or in pairs, and they are usually motile. Citrobacter species are mesophiles inhabiting the intestines of humans, animals, birds, and the environment. They are categorized in the coliform group, serving as indicators of sanitation. Notably, *Citrobacter freundii* is an important species.

c. *Enterobacter*

Enterobacter species are straight rods (1 × 2 mm), motile, and mesophiles. They are commonly found in the intestines of humans, animals, birds, and the environment. Included in the coliform group, they serve as indicators of sanitation. Notable species include *Enterobacter aerogenes*.

d. *Erwinia*

Erwinia species are small rods (1 × 2 mm) found in pairs or short chains. They are motile and facultative anaerobes, with optimal growth occurring at 30°C. Many Erwinia strains are plant pathogens causing spoilage of plant products. *Erwinia amylovora* is a notable species within this genus.

e. *Hafnia*

Hafnia are small rods (1 × 2 mm), motile, and mesophiles. They are found in the intestines of humans, animals, birds, and the environment, often associated with food spoilage. *Hafnia alve*i is a significant species.

f. *Klebsiella*

Klebsiella species are medium-sized rods (1 × 4 mm), occurring singly or in pairs. They are motile and capsulated mesophiles found in the intestines of humans, animals, birds, soil, water, and grains. Included in the coliform group, they are used as indicators of sanitation. *Klebsiella pneumoniae* is a key species.

g. *Morganella*

Morganella are small rods (0.5 × 1 mm), motile, and mesophiles. They inhabit the intestines of humans and animals, potentially pathogenic but not directly implicated in foodborne diseases. Notable species include *Morganella morganii*.

h. *Proteus*

Proteus are straight, small rods (0.5 × 1.5 mm), highly motile, and capable of forming swarms on agar media. Some species can grow at low temperatures and are found in the intestines of humans, animals, and the environment. Many Proteus strains are involved in food spoilage, with *Proteus vulgaris* being a notable species.

i. *Salmonella*

Salmonella are medium rods (1 × 4 mm), usually motile, and mesophiles. There are numerous serovars, all considered human pathogens. They reside in the intestines of humans, animals, birds, and insects, causing major foodborne diseases. *Salmonella enterica* ssp. *enterica* is a significant species.

j. *Shigella*

Shigella are medium rods that are nonmotile and mesophiles. They are found in the intestines of humans and primates and are associated with foodborne diseases. *Shigella dysenteriae* is a notable species.

k. *Serratia*

Serratia are small rods (0.5 × 1.5 mm), motile, and can produce colonies in white, pink, or red hues. Some species can grow at refrigerated temperatures and are found in environmental settings, causing food spoilage. *Serratia liquefaciens* is a significant species.

l. *Yersinia*

Yersinia are small rods (0.5 × 1 mm), motile or nonmotile, capable of growing at low temperatures such as 1°C. They are present in the intestines of animals, with *Yersinia enterocolitica* having been involved in foodborne disease outbreaks.

m. *Vibrio*

Vibrio are curved rods (0.5 × 1.0 mm), motile, and mesophiles. They inhabit freshwater and marine environments, with some species requiring NaCl for growth. Several Vibrio species are pathogens involved in foodborne diseases *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, while others cause food spoilage *Vibrio alginolyticus*.

n. *Aeromonas*

Aeromonas are small rods (0.5 × 1.0 mm), occurring singly or in pairs, motile, and psychrotrophs. They are found in water environments, with *Aeromonas hydrophila* being suspected as a potential foodborne pathogen.

1.2. Fungi

1.2.1. Overview and classification

- ❖ Both yeasts and molds are eukaryotic organisms, but they differ in cellular structure and organization. Eukaryotic cells are generally larger in size, ranging from 20 to 100 micrometers, compared to prokaryotic cells which are typically 1 to 10 micrometers in size. Eukaryotic cells possess rigid cell walls composed of carbohydrates and a thin plasma membrane containing sterols. The cytoplasm is dynamic with streaming movement and contains membrane-bound organelles such as mitochondria and vacuoles. Ribosomes can be attached to the endoplasmic reticulum. The DNA is organized into linear chromosomes enclosed within a nuclear membrane. Eukaryotic cell division occurs by mitosis for asexual reproduction and by meiosis for sexual reproduction when applicable.
- ❖ Yeasts are unicellular eukaryotes. The cell has a very variable size depending on the species: from 1 to 10 µm wide and 2-3 to 20-50 µm long. They are spherical, ovoid and cylindrical in shape. However, there are characteristic cell shapes: bottle shape and triangular shape. Under certain culture conditions, yeasts can give mycelial (multicellular) forms. The cell is limited by a rigid wall representing almost 20% of its dry weight with a cytoplasmic membrane and a very small nucleus. They are haploid or diploid; the optimal growth temperature is between 20 and 28°C with a maximum between 35 and 47°C. Yeast grows well in an acidic environment and can adapt to more extreme or alkaline pH levels.

Table 1. Important Bacteria in foods.

Section[a](Group[b])	Description	Family	Genera
2	Gram-negative, aerobic/microaerophilic, motile, helical/vibrioid	Not indicated	*Campylobacter, Arcobacter, Helicobacter*[c]
4	Gram-negative, aerobic, rods and cocci	*Pseudomonadaceae*	*Pseudomonas, Xanthomonas*
		Acetobacteraceae	*Acetobacter, Gluconobacter*
		Nisseriaceae	*Acinetobacter, Morexella*
		Not indicated	*Alteromonas, Flavobacterium, Alcaligenes, Brucella, Psychrobacter*
5	Gram-negative, facultative anaerobic, rods	*Enterobacteriaceae*	*Citrobacter, Escherichia, Enterobacter, Edwardsiella, Erwinia, Hafnia, Klebsiella, Morganella, Proteus, Salmonella, Shigella, Serratia, Yersinia*
		Vibrionaceae	*Vibrio, Aeromonas, Plesiomonas*
9	Rickettsias	*Rickettsiaceae*	*Coxiella*
12 (17)	Gram-positive, cocci	*Micrococcaceae*	*Micrococcus, Staphylococcus*
		Not indicated	*Streptococcus, Enterococcus, Lactococcus, Leuconostoc, Pediococcus, Sarcina*
13 (18)	Gram-positive, endospore-forming rods and cocci	Not indicated	*Bacillus, Sporolactobacillus, Clostridium,* (*Desulfotomaculum*[d])
14 (19)	Gram-positive, nonsporing, regular rods	Not indicated	*Lactobacillus, Carnobacterium, Brochothrix, Listeria*
15 (20)	Gram-positive, nonsporing, irregular rods	Not indicated	*Corynebacterium, Brevibacterium, Propionibacterium, Bifidobacterium*

[a] Sections in *Bergey's Manual of Systematic Bacteriology*.
[b] Groups in *Bergey's Manual of Determinative Bacteriology*. Only those sections (or groups) containing bacteria important in food are listed in this table.
[c] Are included in this group and contain pathogenic species that can be foodborne.
[d] *Disulfotomaculum* cells stain Gram-negative.

❖ Molds are nonmotile, heterotrophic filamentous multicellular fungi: some live in symbiosis with plants, others are parasites of plants or animals, and still others are saprophytes. They are non-photosynthetic. Their body or thallus is made up of two parts: the mycelium (a set of several filaments called hyphae) and the spores. They are acidophilic (pH between 3 and 7), mesophilic (20 to 30°C) and others are psychrotrophic (<15°C). Molds often have significant lytic properties (cellulolytic, pectinolytic, amylolytic, proteolytic, lipolytic) which make them dangerous but also sometimes useful degrading agents (ripening of cheeses, production of enzymes).

- ❖ The classification of yeasts is naturally an integral part of that of fungi. It is based, at least initially, on morphological characters. Yeasts belong to three classes: Ascomycetes, Basidiomycetes, and Deuteromycetes (Fungi imperfecti).
- ❖ The classification of molds is based on purely morphological characters and divided into four classes: Zygomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes (Fungi imperfecti).

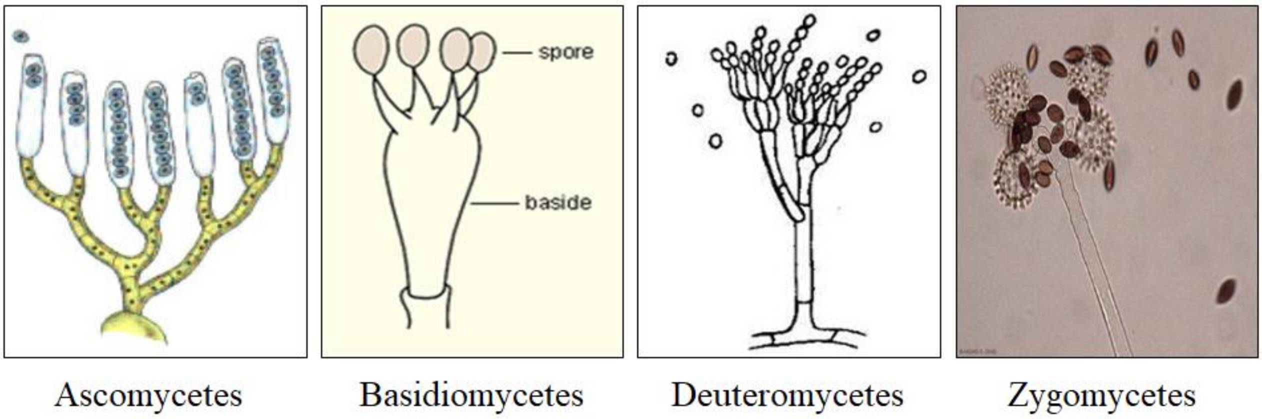

Figure 2. Fungi morphology.

1.2.2. Yeasts

Yeasts play a significant role in the food industry due to their dual capacity to induce spoilage and contribute to bioprocessing. Many yeasts are utilized for producing food additives, with several notable genera highlighted below:

a. *Saccharomyces*

These yeasts exhibit diverse cell shapes (round, oval, elongated) and represent the most pivotal genus. Variants of *Saccharomyces cerevisiae* are crucial in baking for leavening bread and in alcoholic fermentation. They are also known to spoil food by producing alcohol and CO2.

b. *Pichia*

Cells of Pichia are oval to cylindrical and are implicated in spoilage of beer, wine, and brine, forming pellicles. Some species are employed in oriental food fermentation, exemplified by *Pichia membranaefaciens*.

c. *Rhodotorula*

These yeasts are notable for their pigment production, which can discolor foods like meat, fish, and sauerkraut. *Rhodotorula glutinis* is a representative species.

d. *Torulopsis*

Cells of Torulopsis are spherical to oval and contribute to spoilage of milk due to their ability to ferment lactose (e.g., *Torulopsis versatilis*). They also affect fruit juice concentrates and acidified foods.

e. *Candida*

Many Candida species spoil foods rich in acid, salt, and sugar, forming pellicles on liquid surfaces. Some strains, such as *Candida lipolyticum*, are known to cause rancidity in butter and dairy products.

f. *Zygosaccharomyces*

These yeasts spoil high-acid foods such as sauces, ketchups, pickles, and mayonnaise, particularly those with lower acidity and reduced salt and sugar content (e.g., *Zygosaccharomyces bailii*).

1.2.3. Molds

Molds play a crucial role in food due to their ability to thrive in environments where many bacteria cannot survive, such as low pH, low water activity (Aw), and high osmotic pressure. They are diverse and widespread in food, functioning as significant agents of spoilage. Additionally, certain strains are capable of producing mycotoxins, contributing to foodborne illnesses. Molds also play a pivotal role in food bioprocessing, aiding in the production of additives and enzymes.

a. *Aspergillus*

Widely distributed and diverse in food importance, features septate hyphae and produces black asexual spores on conidia. Many are xerophilic, thriving in low water activity, and can spoil grains and various foods like jams, cured ham, nuts, and fruits. Certain species produce harmful mycotoxins, such as aflatoxin from *Aspergillus flavus*. Several strains are also utilized in food processing and the production of additives; for instance, *Aspergillus oryzae* aids in sake production by hydrolyzing starch with alpha-amylase, while *Aspergillus niger* processes citric acid and produces enzymes like beta-galactosidase.

b. *Penicillium*

Also widely distributed, features septate hyphae and produces blue-green conidiophores resembling brush heads. Some species like *Penicillium roquefortii* and *Penicillium camembertii* are integral to cheese production. However, many Penicillium species cause fungal rot in fruits, vegetables, grains, breads, and meats, and some produce mycotoxins like Ochratoxin A.

c. *Fusarium*

Forming cottony growth and sickle-shaped conidia, is associated with rot in citrus fruits, potatoes, and grains. A key species is *Fusarium solani*.

d. *Mucor*

Widely distributed with nonseptate hyphae producing sporangiophores, forms cottony colonies and is involved in food fermentation and enzyme production. *Mucor rouxii* causes vegetable spoilage.

e. *Geotrichum*

With septate hyphae and rectangular arthrospores, forms creamy, yeast-like colonies and commonly grows on dairy products. Notably, *Geotrichum candidum* is recognized as a dairy mold.

f. *Rhizopus*

With aseptate hyphae forming sporangia on sporangiophores, causes spoilage in numerous fruits and vegetables. *Rhizopus stolonifer* is a well-known black bread mold.

g. *Alternaria*

Characterized by septate hyphae and dark-spored conidia, causes tomato rot and imparts rancid flavors in dairy. Some species produce mycotoxins, notably *Alternaria tenuis*.

1.3. Viruses

Viruses are important in food for three reasons. Firstly, some viruses can cause enteric diseases, meaning that their presence in food can lead to foodborne illnesses. Several pathogenic viruses are known to cause foodborne illnesses in humans. However, due to challenges in their detection in food, the full extent of their involvement in foodborne diseases remains uncertain. Viruses, classified as noncellular entities, play a crucial role in food microbiology, particularly enteric viruses and bacteriophages that are widely found in nature.

1.3.1. Enteric viruses

- ❖ Hepatitis A and Norwalk-like viruses have been implicated in foodborne outbreaks. Both belong to the group of single-stranded RNA viruses. Hepatitis A virus is a small, naked, polyhedral enteric virus approximately 30 nm in diameter. Its RNA strand is encapsulated within a capsid.
- ❖ Several other enteric viruses, including poliovirus, echovirus, and Coxsackie virus, can cause foodborne diseases. In countries with lower sanitation standards, these viruses can contaminate food and lead to outbreaks of illness.

1.3.2. Bacteriophages

- ❖ They consist of nucleic acids (either DNA or RNA) encapsulated by proteins forming a head and tail structure.
- ❖ Bacteriophages attach to the surface of host bacterial cells and inject their nucleic acid into the cell. Inside the host cell, numerous phages are produced and released upon cell lysis.
- ❖ Some bacterial viruses (bacteriophages) are used to identify certain pathogens, such as *Salmonella* spp. and *Staphylococcus aureus* strains, based on the sensitivity of these bacteria to various bacteriophages at specific dilutions. Additionally, bacteriophages are employed to transfer genetic traits between bacterial species or strains through a process called transduction, which is commonly used in *Escherichia coli* and *Lactococcus lactis*.
- ❖ Some bacteriophages are significant because they can cause fermentation failure. Many lactic acid bacteria, used as starter cultures in food fermentation, are susceptible to various bacteriophages. These phages can infect and destroy the starter-culture bacteria, leading to product failure. Bacteriophages have been identified for many species within the genera Lactococcus, Streptococcus, Leuconostoc, and Lactobacillus.

The growth and multiplication of microorganisms (excluding viruses) in food are influenced by the food's intrinsic environment and the extrinsic conditions in which it is stored. Because these factors are interrelated, their effects on microbial growth cannot be studied independently.

1. Extrinsic Factors

Extrinsic factors that are crucial for microbial growth in food include the storage conditions: temperature, relative humidity, the gaseous environment and Antimicrobials produced during food manufacturing.

1.1. Temperature

Microbial growth relies on enzymatic reactions. It is widely recognized that within a specific temperature range, each 10 °C increase doubles the catalytic rate of enzymes, while a decrease of 10 °C halves the enzymatic reaction rate. This relationship holds true within the optimal growth temperature range for microorganisms. Temperature profoundly affects enzyme activities, thereby playing a critical role in microbial growth in food.

Microorganisms important in foods are classified into three groups based on their temperature requirements for growth. Each group has an optimal temperature and a specific range within which they can grow effectively:

- **Mesophiles (meso = median):** grow at ambient temperature, with optimum at 35 °C and range 10 to 45 °C;
- **Psychrophiles (psychro = cold):** grow at cold temperature, with optimum at 15 °C and range –5 to 20 °C.
- **Thermophiles (thermo = hot):** grow at relatively high temperature with optimum at 55 °C and range 45 to 70 °C.

These divisions are not defined and often overlap with each other. 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:

- **Psychrotrophs:** microorganisms that grow at refrigerated temperatures (0 to 5 °C) do so regardless of their optimal growth temperature range. They typically exhibit rapid growth between 10 and 30 °C. This group includes molds, yeasts, many Gram-negative bacteria such as those from the genera *Pseudomonas, Achromobacter, Yersinia, Serratia*, and

Aeromonas, as well as Gram-positive bacteria from genera like *Leuconostoc*, *Lactobacillus, Bacillus, Clostridium,* and *Listeria*.

- **Thermotrophs:** microorganisms that can survive pasteurization temperatures are classified as thermoduric. This group includes species from genera such as *Micrococcus, Bacillus, Clostridium, Lactobacillus, Pediococcus,* and *Enterococcus*. Bacterial spores are also part of this category. Thermoduric microorganisms have varying temperature requirements; many can grow at refrigerated temperatures as well as at higher thermophilic temperatures.

1.2. Relative humidity

- Relative humidity is a measure of the amount of water vapor or moisture present in the air relative to the maximum amount of moisture that the air could hold at a given temperature without condensation. It is expressed as a percentage (%) and provides information about the degree of moisture saturation in the air.
- Relative humidity influences both the water activity of the food and the growth of microorganisms on the surface of the food.

1.3. Gaseous environment

For aerobic germs, the presence of O2 promotes their multiplication. An increase in the CO2 content (up to 10%) and a reduction in the oxygen content allow better preservation of food.

1.4. Antimicrobials produced during food manufacturing

These are substances that are either bacteriostatic or bactericidal. The addition of antimicrobial compounds to food products (additives) or the use of various antimicrobial agents in the food production environment (disinfection agents, cleaning agents, etc.) is regulated.

2. Intrinsic Factors

Intrinsic factors of food, such as pH, redox potential, water activity, nutrient content, and antimicrobial agents naturally present in food, each have their own influence on microbial growth when considered individually. However, in a real food system, these factors are present simultaneously and interact with each other. This simultaneous presence and interaction can either enhance or inhibit microbial growth, depending on the specific conditions and combinations present in the food.

2.1. pH

- pH is a measure of the hydrogen ion concentration in a system and is defined as –log (H+) concentration. It spans a scale from 0 to 14, where 7.0 represents a neutral pH. The actual concentration of H+ ions can vary in a system depending on the specific acid present. Different acids contribute varying amounts of H+ ions, influencing the pH of the solution accordingly.
- The pH of the food will promote proliferation of microorganisms if it is close to the optimum pH for growth.
- When the pH of a food drops below the lower limit for a microbial species to grow, the cells not only cease their growth but also experience a loss of viability. The speed at which this loss occurs depends on how much the pH is reduced.
- The pH ranges for growth vary among different types of microorganisms: Molds is 1.5 to 9.0, Yeasts is 2.0 to 8.5, Gram-positive bacteria is 4.0 to 8.5, Gram-negative bacteria is 4.5 to 9.0.
- *E. coli* multiplies between pH 4.4 and pH 9.
- *Lactobacillus* requires a relatively low pH close to 6; these microorganisms are called acidophiles.
- *Vibrio* reproduces at an optimal pH of 9 and are referred to as alkalophiles.

2.2. Redox Potential

- The redox potential is designated as Eh.
- In biological systems, the oxidation and reduction of substances are fundamental processes for energy generation. When free oxygen is available in the system, it serves as the primary electron acceptor. In environments lacking free oxygen, other compounds like NO_3 and SO_4, which have bound oxygen, can function as alternative electron acceptors.
- The redox potential of a food is determined by its chemical composition, the specific processing treatments it undergoes, and its storage conditions, particularly in relation to exposure to air.
- Microorganisms are categorized into aerobes, anaerobes, facultative anaerobes, or microaerophiles based on their ability to grow in different levels of oxygen availability.
- **Strict aerobes:** require free oxygen for their energy generation process because free oxygen serves as the final electron acceptor in aerobic respiration.

- **Strict anaerobes:** Anaerobes cannot grow in the presence of even small amounts of free oxygen because they lack the enzyme superoxide dismutase, which is necessary to neutralize toxic oxygen free radicals.
- **Facultative anaerobes:** have the capability to generate energy using free oxygen when it is available. Alternatively, they can utilize bound oxygen found in compounds such as NO_3 or SO_4 as final electron acceptors through anaerobic respiration. In the absence of oxygen, they can switch to fermentation processes where other compounds accept electrons (or hydrogen) to generate energy anaerobically.
- **Microaerophiles:** grow better in the presence of less oxygen.

2.3. Water Activity

- Water activity (A_w) is a measure of the availability of water for biological functions and relates to water present in a food in free form. In a food system, water exists in both free and bound forms. Bound water is used to hydrate hydrophilic molecules and dissolve solutes but is not accessible for biological functions, thus it does not contribute to A_w.
- A_w is represented as the ratio of the vapor pressure of water in the food (P) to that of pure water (P_o) denoted as Po/P. This ratio ranges from > 0 to < 1, typically between 0 and 1, indicating the availability of water for microbial growth and chemical reactions in food.
- The water activity (A_w) of food varies across different groups:
  - Cereals, crackers, sugar, salt, dry milk: A_w ranges from 0.10 to 0.20,
  - Noodles, honey, chocolate, dried egg: A_w is less than 0.60,
  - Jam, jelly, dried fruits, parmesan cheese, nuts: A_w ranges from 0.60 to 0.85,
  - Fermented sausage, dry cured meat, sweetened condensed milk, maple syrup: A_w ranges from 0.85 to 0.93,
  - Evaporated milk, tomato paste, bread, fruit juices, salted fish, sausage, processed cheese: A_w ranges from 0.93 to 0.98,
  - Fresh meat, fish, fruits, vegetables, milk, eggs: A_w ranges from 0.98 to 0.99.
- Different microbial species or groups have specific requirements for water activity (A_w) levels to support their growth. Generally, the minimum A_w values for growth of various microbial groups are as follows:
  - **Most molds:** around 0.8, with xerophilic molds able to grow at levels as low as 0.6,
  - **Most yeasts:** around 0.85, though osmophilic yeasts can grow at lower levels, typically between 0.6 to 0.7,

- **Most Gram-positive bacteria:** around 0.90,
- **Gram-negative bacteria:** around 0.93.

Table 2. A_w of different types of foods and microorganisms

Microorganisms	Foods
Acinetobacter (0.99)	Meats (0.99)
Clostridium botulinum (0.97)	Raisins (0.986)
Pseudomonas fluorescens (0.957)	Apples (0.98)
E. coli (0.95)	Jam (0.75-0.80)
Salmonella spp. (0.95)	Cereals (< 0.70)
Staphylococcus aureus (0.86)	Chocolate (< 0.60)
Saccharomyces cerevisiae (0.90-0.94)	
Mucor (0.80-0.90)	
Aspergillus flavus (0.78)	

2.4. Nutrient content

❖ Microbial growth occurs as microorganisms synthesize cellular components and generate energy.

❖ These nutrients encompass carbohydrates, proteins, lipids, minerals, and vitamins. While water is not classified as a nutrient, it plays a crucial role as a medium for the biochemical reactions required for synthesizing cellular mass and generating energy.

❖ All foods contain these five major nutrient groupseither naturally or through addition, though the quantity of each nutrient varies significantly depending on the type of food. Generally, meat is abundant in protein, lipids, minerals, and vitamins but lacks carbohydrates. On the other hand, Plant-based foods are typically rich in carbohydrates but may have lower levels of proteins, minerals, and certain vitamins. Some foods, such as milk and many prepared foods, provide ample quantities of all five nutrient groups necessary for microbial growth.

❖ Pathogenic microorganisms are for the most part heterotrophic chemo-organotrophic and must therefore find their energy in the components of the food.

2.4.1. Carbohydrates

❖ Carbohydrates found in various foods, whether naturally occurring or added as ingredients, can be classified based on their chemical nature.

- Lactose is exclusively found in milk, making it possible to appear in foods derived from or containing milk and its derivatives. Glycogen is primarily found in animal tissues, particularly in the liver. Pentoses, many oligosaccharides, and polysaccharides are naturally found in plant-based foods.
- All microorganisms typically present in food metabolize glucose, although their capacity to utilize other carbohydrates varies significantly.

Table 3. Carbohydrates composition found in foods

Types	Composition
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 (composed of glucose units)
	- Glycogen (composed of glucose units)
	- Cellulose (composed of glucose units)
	- Inulin (composed of fructose units)
	- Hemicellulose (composed of xylose, galactose, mannose units)
	- Dextrans (α-1,6 glucose polymer)
	- Pectins
	- Gums and mucilages

2.4.2. Proteins

- The primary protein components in foods include simple proteins, conjugated proteins, peptides, and non-protein nitrogenous (NPN) compounds such as amino acids, urea, ammonia, creatinine, and trimethylamine. Proteins are present in higher quantities in foods of animal origin than in foods of plant origin.
- Microorganisms vary widely in their capacity to metabolize dietary proteins. Many transport amino acids and small peptides into their cells, where small peptides are subsequently broken down into amino acids. For instance, certain *Lactococcus* spp. perform this intracellular hydrolysis.

- Additionally, microorganisms secrete extracellular proteinases and peptidases to break down large proteins and peptides into smaller peptides and amino acids outside the cells. Soluble proteins are more vulnerable to this hydrolytic process compared to insoluble proteins.

2.4.3. Lipids

- Lipids are generally less substrates for the microbial synthesis of energy and cellular materials.
- Many microorganisms can produce extracellular lipases that hydrolyse glycerides to fatty acids and glycerol. Fatty acids can be transported into cells and used for energy synthesis, while glycerol can be metabolised separately.
- Some microorganisms also produce extracellular lipid oxidases that can oxidise unsaturated fatty acids to various aldehydes and and ketones.
- In general, molds are more able to to produce these enzymes. However, certain groups of bacteria, such as *Pseudomonas*, *Achromobacter* and *Alcaligenes* can produce these enzymes.
- Some beneficial intestinal microorganisms, such as *Lactobacillus acidophilus* strains, can metabolize cholesterol and are thought to be able to reduce serum cholesterol levels in humans.

2.4.4. Minerals and Vitamins

- Microorganisms need several elements in small amounts, such as phosphorus, calcium, magnesium, iron, sulphur, manganese and potassium. Most foods contain these elements in sufficient quantities. Many microorganisms can synthesise B vitamins, and foods also contain most B vitamins.
- In general, most foods contain sufficient amounts of various carbohydrates, proteins, lipids, minerals and vitamins to provide the nutrients necessary for the growth of molds, yeasts and bacteria, especially the gram-negative bacteria normally found in foods.
- Some foods may have limited amounts of one or a few nutrients for the rapid growth of some Gram-positive bacteria, especially some fastidious *Lactobacillus* species. If their growth is desired, some carbohydrates, essential amino acids and B vitamins may be added to a food. It is not possible or practical to control microbial growth in a diet by restricting nutrients.

2.5. Antimicrobial agents naturally present in food

- ❖ Antimicrobial substances already exist in foods that are of plant or animal origin.
- ❖ Fresh milk contains lactenins, anti-coliform factors with time-limited activity.
- ❖ Egg contains lysozyme which is active on Gram-positive germs.
- ❖ Cranberries contain benzoic acid, which is active against yeasts and molds;
- ❖ Compounds such as thymol (thyme), eugenol (clove) and cinnamic aldehyde (cinnamon) have antimicrobial activity.

Food produced under proper sanitary conditions contains microorganisms at levels much lower than that spoilage detection. Over time, the growth of certain microbial species among the initial population allows the microorganisms to reach spoilage detection levels. Many factors influence, which species will multiply rapidly to become the dominant spoilage microorganisms. In addition to the types of microorganisms, the types of food and their environments (both intrinsic and extrinsic factors) play crucial roles in determining the dominant spoilage microflora in food.

1. Food spoilage ?

The physicochemical characteristics of the food determine the growth of microorganisms. The proliferation of these microorganisms in a food product results in changes in the organoleptic qualities.

2. Causes of spoilage

Spoilage can result from one or more of the following causes:

- Growth and activity of microorganisms (or occasionally higher forms). This often involves a succession of different organisms;
- Insects;
- Action of the food's own plant or animal enzymes;
- Purely chemical reactions that are not catalyzed by the enzymes of the tissues or microorganisms;
- Physical changes, such as those caused by freezing, burning, drying, pressure, etc.

3. Classification of foods by ease of spoilage

Based on their susceptibility to spoilage, foods can be categorized into three groups:

- **Stable or Nonperishable Foods:** these foods do not spoil unless handled and include products such as sugar, flour, and dry beans;
- **Semiperishable Foods:** when properly handled and stored, these foods remain unspoiled for a considerable time. Examples include potatoes, certain apple varieties, waxed rutabagas, and nutmeats;
- **Perishable Foods:** this group consists of most essential daily foods that spoil quickly unless special preservation methods are used. Examples include meats, fish, poultry, most fruits and vegetables, eggs, and milk.

4. Carbohydrate Spoilage

- **Polymers (starch, cellulose)**; we witness a hydrolysis which causes a modification of the texture;
- **Monomers (glucose, fructose, etc.)**; we witness fermentation which causes a change in taste and aroma.

4.1. Carbohydrates Degradation

- Food carbohydrates encompass a wide range of chemical compounds, including monosaccharides (tetroses, pentoses, and hexoses), disaccharides, oligosaccharides, and polysaccharides.
- Although carbohydrates are the preferred source of energy, microorganisms vary significantly in their ability to degrade specific carbohydrates. Carbohydrates that are utilized at the cellular level, such as monosaccharides, disaccharides, and trisaccharides, can be transported into the cell and hydrolyzed into monosaccharide units before further breakdown. Polysaccharides, on the other hand, are first broken down into mono- and disaccharides by extracellular microbial enzymes (e.g., α-amylase) secreted into the environment before they can be transported and metabolized.

4.1.1. Degradation of Polysaccharides

Molds, certain *Bacillus* spp. and *Clostridium* spp., along with several other bacterial species, can degrade starch, glycogen, cellulose, pectin, and other polysaccharides using extracellular enzymes. The resulting mono- and disaccharides are then transported into the cell for metabolism. The microbial breakdown of these polysaccharides, particularly pectins and cellulose in fruits and vegetables, can alter the texture and diminish the quality and acceptability of these products.

4.1.2. Degradation of Disaccharides

Disaccharides in foods, whether naturally present (such as lactose and sucrose) or produced through microbial degradation (such as maltose from starch), are hydrolyzed into monosaccharides within the cell by specific enzymes. Lactose is broken down by lactase into galactose and glucose, sucrose by sucrase into glucose and fructose, and maltose by maltase into glucose. Many microbial species are unable to metabolize one or more of these disaccharides.

4.1.3. Degradation of Monosaccharides

- ❖ Monosaccharides undergo degradation (catabolism) by aerobic, anaerobic, and facultative anaerobic microorganisms through various pathways that produce a wide array of intermediate and end products. The specific metabolic pathways depend on the type and quantity of monosaccharides, the microbial species involved, and the redox potential of the system.
- ❖ Although all important food microorganisms can metabolize glucose, they exhibit significant variability in their ability to utilize fructose, galactose, tetroses, and pentoses.
- ❖ Fermentable monosaccharides are processed through five major pathways, with many microbial species possessing more than one pathway. These pathways include the Embden–Meyerhof–Parnas (EMP) pathway, the Hexose Monophosphate Shunt (HMS) or pathway, the Entner–Doudoroff (ED) pathway, and two phosphoketolase (PK) pathways (pentose phosphoketolase and hexose phosphoketolase). Microorganisms through diverse pathways encompassing fermentation, anaerobic respiration, and aerobic respiration further metabolize pyruvic acid, a key intermediate produced in these pathways.

Table 4. End products of carbohydrate metabolism by certain microorganisms.

Microbial Type	Fermentation Pattern	Major End Products
Yeasts	Alcohol	Ethanol, CO_2
Lactic acid bacteria	Homofermentative	Lactate
	Heterofermentative	Lactate, acetate, ethanol, CO_2, diacetyl, acetoin
Bifidobacteria	Bifidus (hexose ketolate)	Lactate, acetate
Propionibacteria	Propionic acid	Propionate, acetate, CO_2
Enterobacteriaceae	Mixed acid	Lactate, acetate, formate, CO_2, H_2, succinate
Bacillus, Pseudomonas	Butanediol	Lactate, acetate, formate, 2,3-butanediol, CO_2, H_2
Clostridium	Butyric acid	Butyrate, acetate, H_2, CO_2, butanol, ethanol, acetone, isopropanol

5. Protein Compound Spoilage

- ❖ Protein compounds found in foods encompass various types, including simple proteins such as albumin, globulin, zein, keratin, and collagen, as well as conjugated proteins like myoglobin, hemoglobin, and casein. Additionally, peptides containing two or more amino acids are also present.
- ❖ Generally, microorganisms have the capability to transport amino acids and small peptides into their cells. Proteins and larger peptides present in food undergo hydrolysis into amino

acids and small peptides due to the action of microbial extracellular proteinases and peptidases. Various genera such as *Alcaligenes, Bacillus, Clostridium, Enterococcus, Enterobacter, Flavobacterium, Klebsiella, Lactococcus, Micrococcus, Pseudomonas*, and *Serratia* are known for their production of these extracellular enzymes.

- **Polymers (proteins)**; we witness a hydrolysis which causes a modification of the texture;
- **Amino acids**; we witness decarboxylation, deamination, etc. which causes a modification of taste and odor and formation of toxic molecules.

5.1. Aerobic Respiration (Decay)

- Many aerobic and facultative anaerobic bacteria possess the ability to oxidize amino acids and utilize them as their primary source of carbon, nitrogen, and energy. L-amino acids typically undergo either oxidative deamination or transamination processes to produce respective keto acids, which are subsequently channeled into various metabolic pathways.
- Several amino acids can be oxidized through distinct pathways by numerous bacterial species. Examples include:
  - Conversion of L-threonine to acetaldehyde and glycine;
  - Conversion of L-tryptophan to anthranilic acid;
  - Conversion of L-lysine to glutaric acid;
  - Conversion of L-valine to ketoisovalerate;
  - Conversion of L-leucine to ketoisocaproate;
  - Conversion of L-arginine to citrulline;
  - Conversion of L-histidine to urocanic acid.

5.2. Fermentation (Putrefaction)

- Anaerobic and facultative anaerobic bacteria degrade L-amino acids either individually or in pairs through specific metabolic pathways. When metabolizing single amino acids, they employ processes such as deamination (producing the carbon skeleton and ammonia), decarboxylation (producing amines and carbon dioxide), and hydrolysis (producing the carbon skeleton, carbon dioxide, ammonia, and hydrogen). The resulting carbon skeletons (such as fatty acids, α-keto acids, and unsaturated acids) are utilized to generate energy and other metabolic products.
- Pairwise metabolism of amino acids involves simultaneous oxidation-reduction reactions where one amino acid acts as a hydrogen donor (oxidized) and the other as a hydrogen acceptor (reduced). For instance, alanine, leucine, and valine can be oxidized, while

glycine, proline, and arginine can be reduced in reactions like the Stickland reaction. The products of these reactions include fatty acids, ammonia, and carbon dioxide.

- ❖ The microbial degradation of amino acids yields a wide variety of products depending on the type of microorganism, amino acid substrate, and the redox potential of the environment. These products include keto acids, fatty acids, hydrogen gas, carbon dioxide, ammonia, hydrogen sulfide, amines, and sulfur-containing compounds. Some of these metabolites, such as indole and skatole from tryptophan, putrescine and cadaverine from lysine and arginine, histamine from histidine, tyramine from tyrosine, and sulfur compounds from cysteine and methionine, are associated with food spoilage (causing foul odors) and health risks.
- ❖ In food, the breakdown of amino acids by microbial enzymes (both extracellular and intracellular) influences flavor and texture, playing roles in cheese ripening and yogurt production. For instance, *Lactobacillus acidophilus* converts threonine to acetaldehyde, contributing to the desirable flavor of acidophilus yogurt. Indole production from tryptophan is used to differentiate *Escherichia coli* from other coliforms. The metabolic profiles of amino acids are also used for species identification of bacteria and are important in understanding foodborne pathogen behavior.
- ❖ Additionally, some foodborne pathogens are capable of synthesizing proteins, including toxins like thermostable toxins of *Staphylococcus aureus*, thermolabile toxins of *Clostridium botulinum*, and toxins associated with foodborne infections (such as Shiga toxin). On the other hand, beneficial microbial species can synthesize essential amino acids (e.g., L-lysine), antibacterial peptides (e.g., nisin and pediocin), and enzymes (e.g., amylases and proteinases) in significant quantities, which are used for beneficial purposes in food processing.

Table 5. Products from the microbial decomposition of amino acids.

Chemical process	*Products*
Oxidative deamination	Keto acid + NH_3
Hydrolytic deamination	Hydroxy acid + NH_3
Reductive deamination	Saturated fatty acid + NH_3
Desaturation deamination (at a and b positions)	Unsaturated fatty acid + NH_3
Mutual O-R between pairs of amino acids	Keto acid + fatty acid + NH_3
Decarboxylation	Amine + GO_2
Hydrolytic deamination + decarboxylation	Primary alcohol + NH_3 + CO_2
Reductive deamination + decarboxylation	Hydrocarbon + NH_3 + CO_2
Oxidative deamination + decarboxylation	Fatty acid + NH_3 + CO_2

6. Lipid Compound Spoilage

- ❖ The primary lipids found in food include mono-, di-, and triglycerides; free saturated and unsaturated fatty acids; phospholipids; sterols; and waxes, with glycerides being the most abundant.
- ❖ Microorganisms generally exhibit a low preference for metabolizing lipids due to their hydrophobic nature, especially when present in large quantities. However, in emulsions, microorganisms at the oil-water interface can metabolize lipids.
- ❖ Glycerides are hydrolyzed by extracellular lipases, releasing glycerol and fatty acids. These fatty acids are then transported into microbial cells and metabolized through β-oxidation, generating acetyl-CoA units for further utilization. In conditions where fatty acids are produced rapidly, they can accumulate in the food. Unsaturated fatty acids may undergo oxidation by microbial oxidases, first producing hydroperoxides and then carbonyl compounds like aldehydes and ketones.
- ❖ Several microorganisms known for their production of lipases (hydrolytic enzymes) include genera such as *Alcaligenes, Enterobacter, Flavobacterium, Micrococcus, Pseudomonas, Serratia, Staphylococcus*, as well as molds like *Aspergillus, Geotrichum*, and *Penicillium*. Oxidative enzymes are primarily produced by molds and play roles in both food spoilage and contributing to desirable flavors in mold-ripened cheeses.

7. Physical and Chemical Control Methods

7.1. Physical Control Methods

Microorganisms in solid and liquid foods can be physically removed using various methods. These methods generally partially reduce the microbial load, thereby enhancing the effectiveness of subsequent antimicrobial steps. They are commonly applied to raw foods before further processing.

7.1.1. Centrifugation

- ❖ Centrifugation is employed in certain liquid foods like milk, fruit juices, and syrups to eliminate suspended undesirable particles such as dust, leukocytes, and food particles. This process involves subjecting the food to high centrifugal forces, typically in thin layers.
- ❖ During centrifugation, heavier particles within the liquid food, such as dust, leukocytes, and food particles, move outward and are separated from the lighter liquid mass. While the primary aim of centrifugation is not the removal of microorganisms, certain microbial entities like spores, large bacterial rods, bacterial clamps, chains, yeasts, and molds can be inadvertently removed due to their heavier mass.

- ❖ High centrifugal forces can lead to the removal of up to 90% of the microbial population. After centrifugation, the food typically contains fewer thermoduric microorganisms, particularly bacterial spores that might otherwise survive mild heat treatments like milk pasteurization. This process helps to reduce the microbial load in the pasteurized product, contributing to its microbiological safety and stability.

7.1.2. Filtration

- ❖ Filtration is a widely used method in various liquid foods such as soft drinks, fruit juices, beer, wine, and water. Its primary purposes include removing undesirable solids and microorganisms while imparting a sparkling clear appearance to the final product. Unlike methods involving heat, filtration minimizes or avoids heating altogether, preserving the natural flavors of the products and heat-sensitive nutrients like vitamin C in citrus juices, thus maintaining their natural characteristics.
- ❖ In the context of producing concentrated juices with enhanced flavor and higher vitamin content, filtration plays a crucial role as a processing step. There are several types of filtration systems available for different applications. Typically, coarse filters are initially used to eliminate larger components, followed by ultrafiltration. Ultrafiltration, which utilizes filters with pore sizes ranging from 0.45 to 0.7 micrometers, is highly effective in removing yeasts, molds, and most bacterial cells and spores from liquid products.
- ❖ Additionally, air filtration is employed in certain food-processing operations, such as the spray drying of milk. This method helps in removing dust particles from the air used for drying, thereby reducing the microbial content in food products exposed to air during processing.

7.1.3. Irradiation

7.1.3.1. Electromagnetic Radiation

They are characterized by their wavelength. Their action on microorganisms is of dual interest: food preservation (sterilization) and understanding their mode of action on bacteria can be extrapolated to their effect on human cells. The wavelengths of electromagnetic radiation are as follows:

- **Infrared (> 800 nm), visible (400 to 800 nm), Ultraviolet (≤ 400 nm):** These are less effective because of their longer wavelengths. UV rays are the most commonly used despite their low efficiency. The most active region of their emission spectrum is between 260 and 270 nm. Their penetration is halted by the smallest suspended matter particles.

- **X-rays and γ–rays** release more energy and penetrate deeper into matter. These radiations primarily affect DNA.

Industrially, the following distinctions are made:

- **Radappertization:** irradiation dose between 20 and 50 kGy (kilo Gray);

- **Radicidation:** dose sufficient to eliminate pathogenic bacteria and spoilage flora except spore-forming bacteria. Doses used are below 10 kGy;

- **Radurization:** doses generally between 1 and 5 kGy. This treatment can be applied to packaged goods.

7.1.4. Washing

❖ Fruits and vegetables undergo regular washing to achieve several objectives: reducing temperature to slow down metabolic activity and microbial growth, and removing soil and microorganisms, especially those present in soil.

❖ Similarly, shell eggs are washed to eliminate fecal matter and dirt. Food animal carcasses, such as those from beef, pork, and lamb, are washed to remove hair, soil particles, and microorganisms.

❖ Various methods are employed during carcass washing, including hot water, steam, ozonated water, and water containing disinfectants like chlorine, acetic acid, propionic acid, lactic acid, tripolyphosphates, or bacteriocins such as nisin and pediocin from lactic acid bacteria. These methods have been studied for their effectiveness in reducing microorganisms, especially enteric pathogens like *Salmonella* spp., *Campylobacter jejuni*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*.

7.1.5. Control by Heat

a. Low-Heat Processing or Pasteurization

❖ The temperature used for low-heat processing or pasteurization is **below 100 ºC**. The primary objectives of pasteurization are to eliminate all vegetative cells of pathogens and a significant portion (~90%) of associated microorganisms (yeasts, molds, bacteria, and viruses) that can cause spoilage. In certain foods, pasteurization also inactivates natural enzymes (e.g., phosphatase in milk).

❖ The temperature and duration of pasteurization are carefully selected to achieve microbiological safety while minimizing thermal damage to the food, which can affect sensory qualities (like the cooked flavor in milk) or cause processing challenges (such as coagulation in liquid egg).

- ❖ Pasteurization of milk has a long history and is typically conducted using two methods: heating at 62.8 ºC for 30 minutes or at 71.7 ºC for 15 seconds, known as Low Temperature Long Time (LTLT) and High Temperature Short Time (HTST) methods, respectively.. After pasteurization, milk is promptly cooled to 4 ºC and stored at that temperature until consumption.
  - ❖ Various low-heat treatments are applied to process different foods, including: Ice cream mix: heated to 82.3 °C for 25 seconds or 71.2°C for 30 minutes; Liquid whole egg: treated at 60°C for 3.5 minutes to target *Salmonella* destruction; Fruit juices: typically processed at 60-70°C for 15 minutes or 80-85°C for 1 minute; Wine: Subjected to heating at 82-85°C for 1 minute; Pickles in acid (pH 3.7): Heated to 74°C for 15 minutes; Vinegar: Processed at temperatures ranging from 65.6 to 71.1°C for 1 minute or at 60°C for 30 minutes; Crab meat: reated at 70°C for 10 minutes.

b. High-Heat-Processed Foods

- ❖ The process involves heating foods at or above **100 ºC** for a specified duration, chosen based on product characteristics and the specific microorganisms targeted for destruction. Most products undergo a treatment to achieve commercial sterility, which eradicates microorganisms capable of growing under normal storage conditions.
- ❖ For low-acid or high-pH (pH > 4.6) products, a 12D treatment is employed to eliminate *Clostridium botulinum* Type A and B spores, known for their resilience. However, such products may still harbor viable spores of thermophilic spoilage bacteria (e.g., *Bacillus stearothermophilus, Bacillus coagulans, Clostridium thermosaccharolyticum*, and *Desulfotomaculum nigrificans*), which remain dormant as long as the products are stored at or below 30ºC.
- ❖ For high-acid or low-pH (pH ≤ 4.6) products like tomato products, fruit products, and acidified foods, a lower heat treatment is sufficient. Since *Clostridium botulinum* spores cannot germinate or grow in such acidic conditions, their presence poses minimal health risks. The spore-forming bacteria that can potentially germinate and grow in low-pH products (e.g., *Bacillus coagulans*) and acid-tolerant non-spore-forming bacteria (e.g., *Lactobacillus* and *Leuconostoc* spp.), yeasts, and molds are relatively heat-sensitive. These products are typically heated to approximately 100ºC for a specified duration.

- Commercial sterility can also be achieved through ultrahigh temperature (UHT) processing, where foods are heated at very high temperatures for a brief period. For instance, milk heated to approximately 150°C for 2 to 3 seconds can be stored at room temperature (≤ 30°C) with a shelf life typically extending up to 3 months. However, if the raw milk contains microbial heat-stable proteinases or lipases, it can still spoil despite the high-temperature treatment.

7.1.6. Control by Low Temperature

a. Ice Chilling

- Foods are stored on ice where the surface temperature typically ranges from 0 to 1°C. This method is used for storing a variety of perishable items including fresh fish, seafood, meats, cut fruits, bagged vegetable salads, various types of ready-to-eat salads prepared onsite, high pH and low-calorie salad dressings, sous vide products, and certain ethnic foods like tofu. This approach is becoming increasingly popular.
- However, challenges such as temperature fluctuations (due to container size or melting ice), varying storage durations (from fresh to several days), and potential cross-contamination (raw seafood and ready-to-eat items in open containers within the same display case) can lead to microbiological issues, particularly from foodborne pathogens.

b. Refrigeration

- It consists of lowering the temperature of a food to values slightly above its freezing point. The purpose of refrigeration is to inhibit the growth of mesophilic germs, including most pathogenic microorganisms. Therefore, it is effective only at temperatures between 0 and +4 °C.
- Previously, 7°C was considered adequate for domestic refrigeration units. However, advancements in technology have made it cost-effective to maintain temperatures of 4 to 5°C in domestic refrigerators. For perishable products, a temperature of 4.4°C or lower is considered ideal. In commercial food processing, temperatures as low as 1°C may be used for refrigerating perishable foods such as fresh meat and fish.
- Refrigeration is widely used to preserve raw and processed foods of both plant and animal origin, as well as many prepared and ready-to-eat foods. The popularity of refrigerated foods is increasing rapidly among consumers. Many of these foods are expected to have a shelf life of 60 days or more.

c. Freezing

- Freezing involves storing foods at temperatures below the freezing point, typically -18°C. It is used for long-term preservation of foods (from 4 to 24 months).
- The minimum temperature maintained in household freezers is typically -20°C, at which most of the water in food remains frozen. Dry ice (-78°C) and liquid nitrogen (-196°C) are also used for rapid freezing, not merely to reach such low temperatures but to achieve instant freezing.
- After freezing, foods are generally kept at temperatures around -20 to -30°C. Depending on the type of food, they can be stored at these refrigerated temperatures for several months or even over a year. Frozen storage preserves various items such as raw produce (vegetables, fruits), meat, fish, processed foods, and cooked dishes (ready-to-eat after thawing and reheating).
- At -20°C, microorganisms do not grow in frozen foods; instead, their cells typically die during freezing. However, surviving microorganisms can multiply if the food thaws accidentally or slowly, creating conditions favorable for spoilage and pathogenic microorganisms. Spores may also germinate and grow under suitable post-thawing temperatures and times. Enzymes released by dead microbial cells can degrade the quality of the food, affecting its acceptability.

7.1.7. Control by Drying

a. Natural Dehydration

It is a cost-effective method where water is removed from foods using solar heat. It is commonly employed for drying grains, as well as fruits like raisins, vegetables, fish, meat, milk, and curd, particularly in warmer climates. This process is slow, and depending on the conditions, it can allow for the growth of spoilage and pathogenic bacteria, yeasts, and molds, including toxigenic types.

b. Mechanical Drying

It is a controlled process that achieves drying rapidly, ranging from a few seconds to a few hours. Various methods are utilized, such as tunnel drying (where food moves through a tunnel against hot air), roller drying (liquid spread thinly on a heated drum), and spray drying (liquid sprayed into hot air, drying instantly). Foods dried mechanically include vegetables, fruits, fruit juices, milk, coffee, tea, and meat jerky.

c. Freeze-Drying

Compared to natural and mechanical drying, freeze-drying minimally affects the quality of food but is more costly. It is applicable to both solid and liquid foods. Initially, the food is frozen rapidly at low temperatures, then placed in a high vacuum to remove water by sublimation (from solid to vapor state), preserving its shape and size. Freeze-dried products include vegetables, fruits, fruit juices, coffee, tea, meat, and fish, often as specialty items.

d. Foam-Drying

Foam drying involves whipping a product to create a stable foam that enhances surface area for drying by warm air. Liquid products like egg whites, fruit purees, and tomato paste are dried using this method. Foam drying itself has limited lethality against microbial cells and spores, but prior concentration methods, product pH, and low water activity (A_w) can cause lethal and reversible damage to microbial cells.

e. Smoking

Meat and fish products undergo smoking, where they are exposed to low heat and smoke simultaneously, removing water and lowering water activity (A_w). This process is used for producing many low-heat-processed meats like dry and semidry sausages, as well as smoked fishes. Heat from smoking kills many microorganisms, while low A_w and antimicrobial substances in smoke control growth of survivors.

7.1.8. Control by Modified Atmosphere

a. Vacuum Packaging

❖ A food product packaged under vacuum initially evolves aerobically. Indeed, the vacuum is never deep enough to eliminate oxygen. A partial pressure of oxygen equivalent to 1% of atmospheric pressure is sufficient to saturate respiratory enzymes.

❖ Moreover, oxygen penetrates through the packaging film at a rate that varies depending on the film's permeability to this gas. Tissue and the microorganisms residing within consume oxygen and produce CO_2.

❖ Three possibilities of evolution can be considered:

- **The food consumes more oxygen than it receives and releases more CO_2 than exits the packaging:** the atmosphere becomes anaerobic with a high partial pressure of CO_2.
- **The food consumes as much oxygen as it receives:** the partial pressure of oxygen does not change and remains low. However, the film's permeability to

CO_2 is generally less than that to oxygen. Therefore, the atmosphere becomes enriched in CO_2 and nitrogen.

- **The food consumes less oxygen than it receives:** the atmosphere tends towards a composition similar to air but richer in CO_2.

b. Gas Flushing

Most often achieved by gas reinjection under controlled atmosphere. The proportions typically chosen are 80% N_2 and 20% CO_2.

7.2. Chemical Control Methods

A chemical preservative is an additive incorporated into a food with the aim of slowing down the growth of its microbial flora. Generally, these are compounds used in small doses to avoid any risk of toxicity. Most of them are bacteriostatic under these conditions.

7.2.1. Mineral Preservatives

-a. Sodium chloride (salt)

At sufficient concentrations, chloride has inhibitory properties on many microorganisms, especially Gram-negative bacilli. Only halophilic microorganisms can develop in salted food. Salting is one of the preservation processes for charcuterie products, which are immersed in brine.

b. Nitrates, nitrites

- ❖ A sufficient concentration of nitrates or nitrites (sodium or potassium) inhibits the growth of *Clostridium botulinum* and the production of its toxins. Cases of botulism have become rare since the use of this additive. The inhibitory action is due to nitrites. Nitrates are active because they are reduced to nitrites by bacteria possessing nitrate reductase.
- ❖ The use of nitrite is generally coupled with salt in salting. However, it is not without disadvantages: during cooking and, to a lesser extent, in the digestive tract, nitrites react with amino acids to form nitrosamines, which are known to be carcinogenic.

c. Sulfur dioxide and sulfites

- ❖ Their use is widespread in oenology. These compounds inhibit numerous bacteria and molds, although yeasts are more resistant. They are used for preserving various preparations based on fruit juices, peaches, peeled tomatoes, fruit concentrates, nuts, dried fruits, etc.

- The consumption of sulfur dioxide is not without side effects: headaches and discomfort sometimes felt after excessive consumption of white wine are generally attributed to it.

7.2.2. Organic Preservatives

a. Organic Acids

- **Saturated organic acids:** Organic acids have an inhibitory effect on the growth of microorganisms:
  - By acidifying the food resulting from their addition, which is detrimental to many germs. This is how acetic acid is used to preserve onions, pickles, and fish in marinades.
  - Due to the inhibitory action of their ionized form, as is the case with propionic acid and its salts, which are used to extend the shelf life of cereal-based products.
- **Unsaturated organic acids: t**he presence of a double bond increases the antimicrobial activity of organic acids. The main representative of this group is sorbic acid and its potassium, sodium, or calcium salts. Its action extends to a lesser degree to yeasts and sporulated bacteria like *Clostridium botulinum*. It is used to preserve fats (butter, margarine, mayonnaise), fruit juices, bakery products, and pastries.
- **Benzoic acid and its derivatives:** unlike the previous ones, benzoic acid in its ionized form (thus in an acidic environment) is particularly active against bacteria and yeasts; its inhibitory action on molds is less pronounced.
- **Other organic acids:** the bacteriostatic properties of citric acid, ascorbic acid, and tartaric acid are used in many products alongside lactic acid for fermented products.

b. Phenolic Compounds

- The spectrum of their antimicrobial properties is very broad (bacteria, yeasts, molds, and even some viruses), although the activity of these molecules is low.
- Their interest lies in the fact that they enhance the action of other preservatives such as sorbic acid and are also used for their antioxidant properties: they are therefore additives with multiple functions. Some are active against *Clostridium botulinum*; hydrocinnamic acids inhibit *Pseudomonas*.

Recognition of human illness resulting from the consumption of contaminated foods dates back to ancient times, long before the role of pathogens in foodborne diseases was understood. Ancient civilizations developed dietary guidelines to mitigate the risk of foodborne illnesses. These guidelines often included instructions to eat foods only after they had been cooked thoroughly and served warm, as well as to avoid consuming spoiled foods. These practices were likely intended, at least in part, to protect people from the dangers posed by microbial contamination in food.

The term "Food Poisoning" is often used broadly to describe diseases caused by microorganisms, encompassing both illnesses that result from the ingestion of toxins produced by these organisms and those caused by the infection of the host through the intestinal tract. In the context of foodborne diseases, these illnesses are typically categorized into two main types: poisonings and infections.

1. Definitions

- **Foodborne Intoxications:** or microbial food poisoning, occurs when a person ingests food that contains a preformed toxin. can be the result of either chemical poisoning or the ingestion of a toxicant.
- **Foodborne Infections:** occurs when food or water contaminated with pathogenic enteric bacteria and viruses is consumed. The microorganism multiplies in the food and produces toxins and toxic metabolites, which will be ingested by the organism. While many pathogens can cause these infections, some are more commonly involved than other pathogens.
- **Collective Foodborne Toxicoinfections:** defined by the appearance of at least two similar cases of a symptomatology whose cause can be attributed to the same food origin.

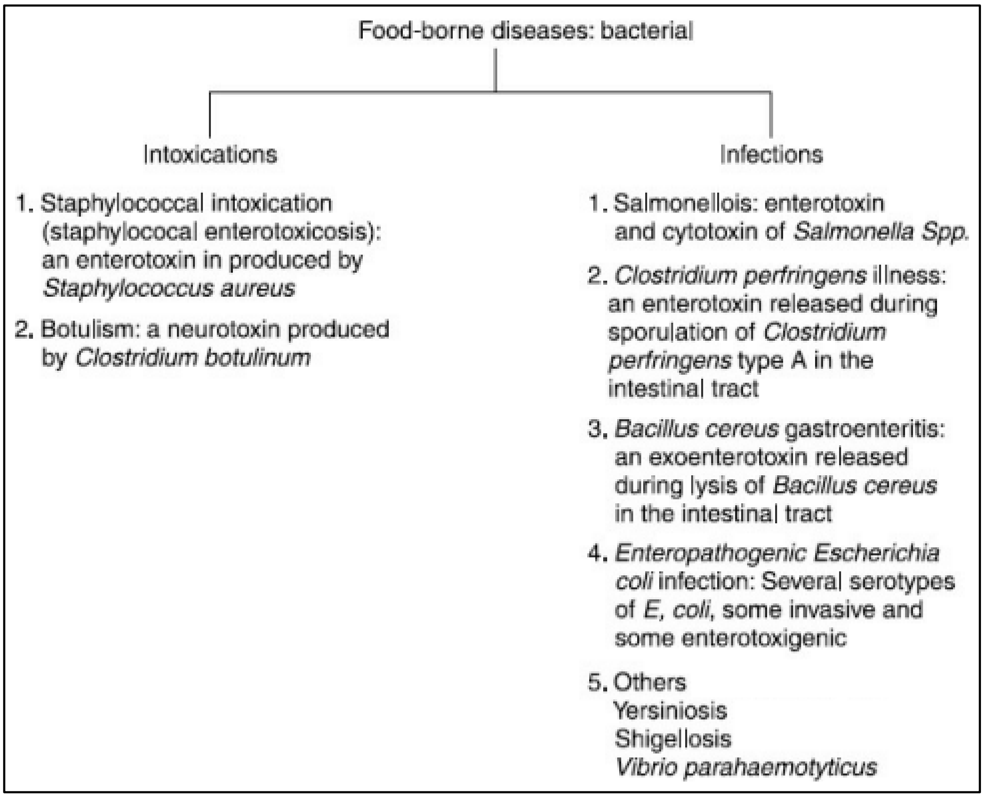

Figure 3. Examples of Bacteria Causing Food-borne Intoxications and Infections.

2. Botulism

Botulism results from consuming food that contains the potent neurotoxin botulin, produced by *Clostridium botulinum*. This toxin primarily causes neurological symptoms, along with some gastric symptoms.

2.1. Taxonomic position and main characters

- *Clostridium botulinum* strains are Gram-positive rods that occur as single cells or in small chains. Many of these strains are motile, strict anaerobes, and they form single terminal spores. The cells are sensitive to low pH (below 4.6), low water activity (A_w 0.93), and moderately high salt concentrations (5.5%). Toxins are produced during the growth of *Clostridium botulinum*. Strains of this bacterium can be classified as either proteolytic or nonproteolytic.
- This bacteria is classified into four groups: I, II, III and IV and secretes one of the most active neurotoxins. There are seven different neurotoxins: A, B, C, D, E, F, G.
- The toxins associated with human foodborne intoxications are produced by *Clostridium botulinum* types A, B, E, and F. Type A strains are proteolytic, while type E strains are nonproteolytic. Types B and F strains can be either proteolytic or nonproteolytic.

- Proteolytic strains can grow at temperatures ranging from 10°C to approximately 48°C, with an optimum temperature of 35°C. Nonproteolytic strains grow optimally at 30°C, within a temperature range of 3.3°C to 45°C.

2.2. Habitat and Transmission

- *C. botulinum* is a soil germ widely distributed in nature in the form of spores in soil, sewage, mud, sediments of marshes, lakes and coastal waters, plants, and intestinal contents of animals and fishes. Fruits and vegetables can be contaminated with spores from soil, fishes from water and sediments, and various other foods from many of the given sources.
- Transmission occurs after ingestion of a sufficient quantity of toxin. This toxin is only produced if the spore survives in the food to form a toxin-producing vegetative form.

2.3. Physiopathology

- The toxins produced by *Clostridium botulinum* are neurotoxic proteins. The toxins associated with food intoxication in humans (Types A, B, E, and F) are extremely potent; even a small amount can cause symptoms and potentially lead to death.
- Toxins are produced in association with non-toxic polypeptides which play a protective role in the digestive tract against the action of acids and proteolytic enzymes.
- Following ingestion, toxin molecules are absorbed from the upper portion of the intestine through the intestinal wall. They then spread via the bloodstream to the peripheral nerves.
- These toxins block the transfer of signals, leading to irreversible paralysis of all involuntary muscles. The toxins move slowly through the body. They reach their receptors at the neuromuscular junctions. These toxins are produced during the exponential phase, and released during the stationary phase.
- It is important to note that toxins produced by nonproteolytic strains are not fully activated until conditions favor their activation.

2.4. Symptoms

- Incubation (the initial stage) varies from a few hours to a few days, generally 12 to 36h, but can be 2 h.
- One of the first signs to appear may be diarrhea, nausea, abdominal pain, severe constipation, tiredness and asthenia.
- 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

(diplopia), difficulty in swallowing, breathing, and speaking, dryness of the mouth, and paralysis of various involuntary muscles. This paralysis can spread to affect the diaphragm, lungs, and heart. Death typically results from respiratory failure due to the paralysis of respiratory muscles.

2.5. Food Association

- The largest number of outbreaks of *Clostridium botulinum* intoxication are associated with fruits and vegetables, particularly low-acid vegetables such as green beans, corn, spinach, asparagus, pepper, and mushrooms, as well as fruits like figs and peaches. Following fruits and vegetables, finfish are the next most commonly associated category of outbreaks. This includes fermented, improperly cooked, and smoked fish, as well as fish eggs. Type E is predominantly associated with fish, while types A and B are associated with vegetables.
- Botulism occurrences from meat, poultry, and dairy products are generally low, likely because these foods are typically heated thoroughly and consumed promptly. However, several outbreaks have been recorded from unlikely sources such as sautéed onions or baked potatoes.
- Additionally, certain condiments like chili peppers, relish, and sauces have also been associated with outbreaks of botulism. These foods can sometimes provide conditions favorable for the growth of *Clostridium botulinum* if not properly handled or stored.

2.6. Epidemiology and prevention

- Type A predominates on the East Coast of the United States, type B in Europe and on the West Coast of North America, types C and D are more common in Africa and the countries of Northern Europe. , type E, generally transmitted by seafood, is the most common in Japan.
- Certain preventive measures can prevent Botulism: fasting animals before slaughter, avoiding contamination of carcasses by soil contamination, applying sufficient heat treatments to preserved foods and finally keeping non-sterilized products at a temperature below +4°C.

3. Staphylococcus Intoxication

One of the most commonly occurring food poisonings is caused by the ingestion of the enterotoxin produced by certain strains of *Staphylococcus aureus* during their growth in food. This toxin is called an enterotoxin because it leads to gastroenteritis, which is the inflammation of the lining of the intestinal tract.

3.1. Taxonomic position and main characters

- *Staphylococcus* are Gram-positive cocci appearing in clusters resembling grapes, pairs, or short chains. These bacteria grow aerobically, possessing catalase and having a fermentative metabolism. On solid media, it generally appears golden or yellow, although some strains may be unpigmented.
- The current classification distinguishes around twenty species; a certain number of them are found in humans, others are present in animals and therefore in foods (meat, dairy products, etc.).
- Among the species found in humans: three species occupy a privileged place: *S. aureus, S. epidermidis* and *S. saprophyticus*, three can coagulate blood plasma and are considered potentially pathogenic. The others, more rarely involved in human pathology, are *S. hominis, S. haemolyticus, S. warneri, S. capitis, S. saccharolyticus, S. auricularis, S. simulons, S. cohnii, S. xylosus, S. intermedius, S. hyicus, S. lugdunensis* and *S. schleiferi*.
- Most enterotoxin-producing *S. aureus* strains are coagulase-positive, producing a thermally stable nuclease and are facultative anaerobes, growing better in the presence of oxygen in complex glucose media but also able to grow anaerobically.
- *S. aureus* produces six serologically distinct enterotoxins (A, B, C1, C2, D, and E) with varying toxicity; type A is most commonly associated with food poisoning. Enterotoxin-producing strains also produce several other toxins. The conditions allowing growth and toxin production vary with the type of food. Generally, the better the food medium is for the bacterium, the wider the range of temperature, pH, or water activity (aw) in which growth can occur.
- The temperature range for *S. aureus* growth and toxin production is about 4 to 46 °C, depending on the food. Growth is most rapid between 20 and 45°C, with growth being four times faster at 45°C than at 20°C.

3.2. Habitat and Transmission

- These germs are very widespread in nature (air, water, soil). *S. aureus* and *S. epidermidis*, are part of the normal flora of many individuals who are "asymptomatic carriers."
- *Staphylococcus aureus*, along with many other staphylococci, is naturally present in the nose, throat, skin, and hair (including feathers) of healthy humans, animals, and birds. It can also be found in infections, such as cuts and abscesses, in humans, animals, and birds. In humans, it is commonly found in cuts on hands and in facial acne lesions.

- Transmission is direct between humans (contact) or indirect through food.

3.3. Physiopathology

- Among *Staphylococcus* coagulase +, only enterotoxin-producing species are involved in food poisoning. *S. intermedius* and S. hyicus can secrete an enterotoxin but it can be abundant and not very active and contamination of food by these two species is unlikely.
- Species *S. aureus* is the only species involved in food poisoning in broad sense. Staphylococcal enterotoxins A to E, G and I to U are different from the others.
- Staphylococcal enterotoxins of the species *S. aureus* have a so-called superantigen activity capable of causing activation of T lymphocytes opening the possibility of massive activation of the immune system, the release of cytokines of inflammation and the occurrence of toxic shock.

3.4. Symptoms

The symptoms are brutal: after two to four hours of incubation they appear: fever (39 to 40.5°C), abdominal pain, nausea, violent and repeated vomiting and diarrhea, hypotension, headaches, confusion, erythroderma, myalgia, thrombocytopenia. Renal failure is common and the syndrome can progress rapidly to death within 48 hours.

3.5. Food Association

- Many foods have been implicated in staphylococcal foodborne outbreaks. In general, *Staphylococcus aureus* grows in the food and produces toxins without negatively affecting the food's acceptance quality. Foods that are protein-rich, handled extensively, poorly support the growth of associated bacteria, or have been temperature abused are often associated with staphylococcal gastroenteritis.
- Frequently implicated foods include ham, corned beef, salami, bacon, barbecued meat, salads, baking products containing cream, sauces, and cheeses.

3.6. Prevention

- To reduce the incidence of staphylococcal food poisoning, it is crucial to minimize the initial load of *Staphylococcus aureus* in food by selecting high-quality raw materials, maintaining strict sanitation, and ensuring proper personal hygiene among food handlers.
- Individuals with respiratory diseases, acute facial acne, skin rashes, or hand cuts should not handle food.
- Heat-treating products to kill live *S. aureus* cells is essential, and preventing recontamination after heating is critical. Rapid chilling of processed and ready-to-eat foods

to 5°C or lower within an hour is vital, and suitable preservatives can be used to inhibit bacterial growth.

- ❖ Additionally, avoiding temperature abuse and prolonged storage at growth temperatures is necessary, as once heat-stable toxins form, reheating does not ensure food safety.

4. Salmonellosis

Salmonellosis occurs after ingesting viable cells from the genus *Salmonella* and is the most frequently occurring bacterial food infection. Before the 1940s, *Salmonella typhi* and *Salmonella paratyphi* were the primary causes of worldwide foodborne and waterborne diseases in humans. However, the implementation of milk pasteurization and water chlorination significantly reduced the spread of typhoid and paratyphoid fever through food and water, particularly in developed countries.

4.1. Taxonomic position and main characters

- ❖ *Salmonella* was named in honor of the American veterinarian Daniel Elmer Salmon.
- ❖ Based on the high degree of relatedness in their DNAs, it has been proposed that the genus *Salmonella* should be classified under one species, *Salmonella enterica*, with six subspecies. Among the six subspecies of *Salmonella enterica,* subsp. enterica includes most serotypes that are frequently associated with foodborne salmonellosis.
- ❖ More than 2200 serovars have been isolated, they are differentiated by their antigens (O antigens) and (H antigens).
- ❖ *Salmonella* cells are Gram-negative, nonsporulating, facultative anaerobic, motile rods that produce gas when growing in glucose-containing media. They typically ferment dulcitol but not lactose, and can utilize citrate as a carbon source. They produce hydrogen sulfide, decarboxylate lysine and ornithine, do not produce indole, and are negative for urease activity. Mesophilic in nature, they grow optimally at temperatures between 35°C and 37°C, with a broader growth range of 5°C to 46°C. They are susceptible to pasteurization temperatures and times, sensitive to low pH levels (4.5 or below), and do not multiply at a water activity (Aw) of 0.94, especially when combined with a pH of 5.5 or lower.

4.2. Habitat and Transmission

- ❖ Salmonella naturally inhabit the gastrointestinal tracts of domesticated and wild animals, birds, pets (including turtles and frogs), and insects. In animals and birds, they can cause salmonellosis and persist in a carrier state.

- ❖ Humans can also become carriers after infection, shedding the pathogens in feces for extended periods.
- ❖ Salmonella have been isolated from soil, water, and sewage contaminated with fecal matter, indicating their ability to survive and spread through environmental contamination.
- ❖ *S. typhi* and *S. paratyphi* are strictly adapted to humans. It is not possible to reproduce typhoid fever in animals.
- ❖ Humans are infected by the oral route.

Table 6. Six Subspecies within the Genus Salmonella

1.	*Salmonella enterica* subsp.[a] *enterica*
2.	*Salmonella enterica* subsp. *salamae*
3a.	*Salmonella enterica* subsp. *arizonae*
3b.	*Salmonella enterica* subsp. *diarizonae*
4.	*Salmonella enterica* subsp. *houtenae*
5.	*Salmonella enterica* subsp. *bongori*

4.3. Physiopathology

❖ Septicemic forms

These are typhoid and paratyphoid fevers due to *S. Typhi*, *S. Paratyphi* A, B and rarely C. In newborns or young children, other serotypes such as *S. panama* or *S. wien* can be responsible for septicemia.

❖ Purely digestive Salmonellosis

Food poisoning and gastroenteritis in infants are due to ubiquitous serovars, mainly *S. enteritidis*, *S. typhimurium*; however, any *Salmonella* regardless of its serovar can be responsible for poisoning.

4.4. Symptoms

❖ Septicemic forms

- For typhoid fever, the incubation lasts 8 to 48 hours. Afterwards, there are two phases: the first the germ enters the intestinal cells causing diarrhea and the second phase manifests itself as septicemia.

- Symptoms include prolonged fever, fatigue, headache, nausea, abdominal pain, constipation or diarrhea. Some patients may experience a rash. In severe cases, it can lead to serious complications or even death.

- For paratyphoid fever, the symptoms are almost the same as typhoid fever except for a sudden onset with mild symptoms and a short duration.

❖ **Purely digestive Salmonellosis**

- The first signs appear 8 to 10 hours after ingestion of the contaminated food.
- Purely digestive salmonellosis manifests itself as diarrhea, fever, and vomiting.

4.5. Food Association

Foods of animal origin, such as beef, chicken, turkey, pork, eggs, milk, and their derived products, have been linked to numerous outbreaks of *Salmonella* infections. These foods can become contaminated directly or indirectly with fecal matter from carriers, including animals, birds, and humans. Furthermore, *Salmonella* has been found in foods of plant origin (due to the use of sewage as fertilizer or washing with contaminated water), seafood, and finfish harvested from polluted waters.

5. *Escherichia coli* Gastroenteritis

Escherichia coli is part of the normal bacterial microflora of the digestive tract of animals and humans. The name Escherichia coli was given in honor of the work of the German pediatrician Theodor Escherich, who first described the bacilli *Bacterium coli* in infant feces in 1885.

5.1. Taxonomic position and main characters

❖ *Escherichia coli* is a Gram-negative bacilli, facultatively aerobic-anaerobic, most often motile, and non-sporulating, belonging to the *Enterobacteriaceae* family. It ferments lactose, indole, and glucose with gas production.

❖ It multiplies at temperatures between 7°C and 50°C, with an optimal temperature of 37°C. Some strains can develop in acidic foods with a pH as low as 4.4, and in foods with a minimum water activity (aw) of 0.95.

❖ Most strains are harmless; however, some, like *E. coli* O157:H7, can cause severe foodborne illnesses. This serotype is significant for public health due to its production of shiga toxins, though other serotypes have also been frequently associated with sporadic cases.

5.2. Habitat and Transmission

❖ *E. coli* is a bacterium commonly found in the digestive tract of humans and animals.

❖ Transmission to humans primarily occurs through the consumption of contaminated food, such as raw or undercooked ground beef, raw milk, and raw vegetables, or through contact with infected individuals or animals.

❖ The main reservoir for this pathogen appears to be cattle. Additionally, other ruminants, such as sheep, goats, and deer, are considered important reservoirs. Infections have also been occasionally found in other mammals (pigs, horses, rabbits, dogs, cats) and birds (chickens, turkeys).

5.3. Physiopathology and Symptoms

❖ Incubation period ranges from 3 to 8 days, with a median duration of 3 to 4 days. Most patients recover within 10 days, but a small proportion, especially young children and the elderly, may develop potentially fatal conditions such as hemolytic uremic syndrome (HUS).

❖ Depending on their serological characteristics, virulence, interaction with the host, and clinical signs, pathogenic *Escherichia coli* have been classified into six serotypes (serovars):

❖ **<u>Enterohemorrhagic *E. coli* (EHEC)</u>**

- Specifically *E. coli* O157:H7, is one of the most formidable serovars of the species *Escherichia coli*, primarily pathogenic to the colon. This strain was first identified in 1982 and is considered a major pathogen responsible for foodborne epidemics.

- EHEC bacteria pass through the small intestine, colonize the colon, and cause watery diarrhea. Following the watery diarrhea, EHEC can lead to hemolytic uremic syndrome (HUS), which is responsible for bloody diarrhea and kidney failure. This severe effect is associated with the production of toxins known as Shiga-like toxins, due to their similarity to those produced by *Shigella dysenteriae*, and are therefore called Shiga toxins.

❖ **<u>Enterotoxigenic *E. coli* (ETEC)</u>**

- It is responsible for numerous cases of diarrhea in travelers, commonly referred to as "traveler's diarrhea," and in children aged 1 to 3 years in developing countries.

- ETEC strains possess two virulence factors that explain their pathogenic effect: the ability to adhere to enterocytes in the small intestine and the production of enterotoxins; one heat-labile enterotoxin and one heat-stable enterotoxin. Infection manifests as watery diarrhea accompanied by abdominal cramps and nausea.

❖ **<u>Enteropathogenic *E. coli* (EPEC)</u>**

- It causes infantile gastroenteritis, particularly in children over 2 years old. These bacteria frequently cause epidemics among infants in developing countries and are rarely the cause of adult diarrhea. EPEC is usually a non-invasive pathogen of the small intestine. It adheres intimately to the surface of enterocytes, causing cellular alterations without producing a toxin. EPEC infections result in watery diarrhea often accompanied by fever, malaise, and vomiting.

❖ **Enteroinvasive *E. coli* (EIEC)**

- It affects both children and adults and shares many similarities with Shigella, including the ability to invade the colonic mucosa and produce toxins. EIEC infections lead to watery diarrhea with high fever and diarrhea.

❖ **Enteroaggregative *E. coli* (EAEC)**

- It strains exhibit a different mode of action compared to the other four types: they adhere to the intestinal wall in a "stacked-brick" formation and produce heat-stable enterotoxins responsible for persistent diarrhea.

❖ **Diffuse-adhering *E. coli* (DAEC)**

- It is less well-defined. They were previously classified with enteropathogenic *E. coli* due to their adhesion to cells but now form a separate group. DAEC are responsible for intestinal clinical signs (diarrhea) in children.

6. Mycotoxins

Many strains of molds, when growing in a suitable environment (including foods), produce metabolites known as mycotoxins, which are toxic to humans, animals, and birds. Consuming foods contaminated with mycotoxins leads to a condition called mycotoxicosis.

6.1. Definition

❖ The term mycotoxin comes from the Greek “mycos” which means fungi and the Latin “toxicum” which means poison.

❖ Mycotoxins are products of the secondary metabolism of molds synthesized during the stationary phase which can develop on the plant in the field or during storage and have toxic potential towards humans and animals.

❖ Many mycotoxins are carcinogenic and, when consumed, can cause cancer in various tissues in the body. Some induce organ toxicity through unknown mechanisms. In recent years, incidents of mycotoxicosis in humans have not been recorded in many countries, particularly in developed ones, due to strict regulation and monitoring of mycotoxin presence in foods.

❖ More than 300 secondary metabolites have been identified but only around thirty have real toxic properties of concern. Here are some examples:

6.2. Aflatoxin

- Three strains of Aspergillus are known for their ability to synthesize Aflatoxins. There is *A. flavus* which mainly produces Aflatoxin B1 and Aflatoxin B2. *A. parasiticus* produces the 4 Aflatoxins (B1, B2, G1, G2). Finally, *A. nomius,* a rare species close to *A. flavus,* is capable of producing Aflatoxins.
- They Are storage mycotoxins (seeds).
- In humans, two human poisoning syndromes have been linked to the ingestion of foods contaminated by aflatoxins: Kwashiorkor and Reye syndrome. Kwashiorkor combines hypoalbuminemia (a protein deficiency malnutrition syndrome) and immunosuppression.
- Reye's syndrome is a rare disease; it is an encephalopathy. Victims are usually between 4 and 12 years old, although a few cases have been reported in adults. The disease first manifests with persistent and severe vomiting. Another evident sign is a change in mental state (confusion); the patient may become aggressive. In the advanced stage of Reye's syndrome, one may observe muscle rigidity or loss of muscle function, seizures, disappearance of reflexes, the onset of breathing difficulties, and coma.

6.3. Patulin

- Patulin was discovered in cultures of *Aspergillus clavatus* in 1942. It is known for its antibiotic properties against Gram-positive and Gram-negative bacteria. patulin is structurally synonymous with expansin, penicidin, claviformin, clavatin, clavacin, mycoin C, and gigantic acid.
- This toxin has been extracted from cultures of *Penicillium patulum, P. expansum, P. glandicola, P. vulpinum, P. paneum, P. carneum, Aspergillus clavatus, A. giganteus, A. terreus, Byssochlamys nivea, B. fulva,* and *Paecilomyces*.
- These mycotoxins notably develop on cereals, legumes, and fruits.
- Patulin constitutes a particular example of a mycotoxin that was once used in veterinary and human therapy due to its antibiotic properties. Neurotoxic effects were described during its therapeutic use in humans in the 1950s. These effects led to the abandonment of this molecule as an antibiotic therapy.

6.4. Ochratoxin

- Ochratoxins A, B, and C are metabolites from various molds of the genera *Aspergillus* or *Penicillium*. Among these ochratoxins, considering the prevalence and toxicity of ochratoxin A (OTA), only the latter will be discussed.

- OTA is produced in the field on grapes and during the storage of numerous foodstuffs (such as cereals, coffee, cocoa, dried fruits, spices, ...). It is also likely to be present in the organs of animals (especially in blood and kidneys) that have consumed contaminated food.
- Human exposure to ochratoxin A through food is associated with the occurrence of a pathology called Balkan Endemic Nephropathy (BEN). Clinical signs include those of progressive renal failure preceded by severe anemia.

6.5. Citrinin

- Citrinin is produced by various species of *Aspergillus* (*A. terreus, A. carneus,* and *A. niveus*) and *Penicillium* (including *P. citrinum, P. verrucosum*, and *P. expansum*). It is also synthesized by the genus *Monascus*, fungi that produces natural colorants traditionally used in Oriental foods.
- This mycotoxin has been found in barley, oats, rye, and wheat. It can also contaminate maize, rice, nuts, peanuts, sunflower seeds, dried fruits, apple juice, and cheeses.
- There is limited data available on the effects of citrinin in humans; it may cause kidney disorders and has been implicated in porcine nephropathy and avian nephropathies.

7. Other Pathogens

7.1. Bacillus cereus

- The genus *Bacillus* includes bacilli, generally mobile, spore-forming. These bacilli are Gram-positive, strict aerobic or facultative anaerobic. The *Bacillus* genus includes around twenty species, two of which are of interest: *B. anthracis*, due to its pathogenicity (animals, humans) and *B. cereus* involved in food poisoning. Depending on their morphology and the position of their spores, the Bacillus genus is divided into 3 groups: Group 1: Bacilli with spores that do not distort the microbial body; Group 2: Spore-deforming bacilli, oval; Group 3: Bacilli with deforming, round spores.
- *Bacillus cereus* belongs to Group 1 of the genus *Bacillus*. It is a Gram + bacillus, spore-forming, aerobic, possessing a catalase and a lecithinase and lacking oxidase. Its optimal growth temperature is between 30 and 37°C, the extreme growth temperatures are, respectively, around 5 and 55°C.
- Spores and cells of *Bacillus cereus* are commonly found in soil and dust and can be easily isolated in small quantities from various foods, including both raw and processed products. Additionally, *Bacillus cereus* is present in the intestinal tracts of about 10% of healthy adult humans under normal conditions.

- *B. cereus* secretes, during the exponential phase of its growth, one of two toxins: either a heat-stable emetic toxin (stable at high temperatures), responsible for vomiting; either a heat-labile diarrheagenic toxin (destroyed or loses its properties at a low temperature).
- There are emetic strains and diarrheagenic strains, both of which can exist in the same food. *Bacillus cereus* poisoning can therefore manifest itself in two ways: An emetic syndrome with nausea, vomiting which occurs half an hour to six hours after ingestion of the food; A diarrheal syndrome with the appearance, after six to fifteen hours of incubation, of watery diarrhea and abdominal cramps without fever. Healing is complete in less than 24 hours.

7.2. *Clostridium perfringens*

- The cells are Gram-positive, motile rods, and spore-formers. They vary in size and can form small chains. *Clostridium perfringens* is anaerobic but can tolerate some oxygen. The vegetative cells are sensitive to low-heat treatments like pasteurization, while the spores are extremely heat resistant, with some surviving boiling for several hours.
- They require several amino acids for growth, making them capable of thriving in many protein-rich foods. The growth temperatures for vegetative cells and spore germination range from 10°C to 52°C, with optimal growth at around 45°C. The cells do not grow well at pH levels below 5.0, NaCl concentrations above 5%, water activity (A_w) below 0.93.
- Spores and vegetative cells are found in soil, dust, the intestinal contents of animals, birds, and humans, as well as in sewage. Many foods, especially raw foods, become contaminated from these sources.
- Among the five types of *Clostridium perfringens*, Type A strains are predominantly involved in foodborne toxicoinfections. The enterotoxin associated with this foodborne disease is a heat-labile protein. This intracellular protein is produced by the cells during sporulation in the intestine and is subsequently released.
- The strains of *C. perfringens* A produce an enterotoxin composed of subunits: one hydrophilic, the other hydrophobic. This distinction is important because only these strains are capable of causing food poisoning. The secretion of the enterotoxin is linked to the germ's sporulation. When food containing large quantities of vegetative forms is ingested, these vegetative forms sporulate in the intestine and release the enterotoxin. The toxin binds to enterocytes by its hydrophilic part, while the hydrophobic part passes into the cell and exerts its biological effects there. The toxin causes superficial lesions in the intestine and induces diarrhea.

- The incubation period is between 8 and 12 hours, and the symptoms manifest as two constant clinical signs: profuse diarrhea and abdominal pain. Generally, there is neither vomiting nor fever. The illness resolves within 24 to 48 hours.

7.3. Campylobacteriosis

- Various Campylobacter species can lead to human gastroenteritis, with *Campylobacter jejuni* and *Campylobacter coli* being the most prevalent causes of diarrheal disease globally.
- *Campylobacter jejuni* is a Gram-negative, motile, nonsporulating, rod-shaped bacterium characterized by small, fragile, and spirally curved cells. These strains are microaerophilic, catalase and oxidase positive, and grow within a temperature range of 32°C to 45°C, with an optimal temperature around 42°C. They prefer amino acids over carbohydrates for growth and are highly sensitive to environmental factors such as oxygen, NaCl concentrations above 2.5%, pH levels below 5.0, temperatures below 30°C, heat (pasteurization), and drying. Despite this sensitivity, they can survive well under refrigeration and remain viable for months when frozen.
- As an enteric organism, *Campylobacter jejuni* is frequently isolated from the feces of animals and birds. Human carriers also shed the bacteria in their feces. Poultry material and contamination of water, sewage, vegetables, and animal-origin foods with *Campylobacter jejuni* is common through fecal matter.
- *Campylobacter jejuni* produces a thermolabile enterotoxin responsible for enteric disease symptoms. After ingestion, symptoms typically appear within 2 to 5 days and usually last for 2 to 3 days, though they can persist for two weeks or more. Asymptomatic individuals can shed the bacteria in their feces for extended periods. The primary symptoms are enteric, including abdominal cramps, profuse diarrhea, nausea, and vomiting. Additional symptoms can include fever, headache, and chills, with some cases reporting bloody diarrhea. It is also possible for individuals to experience a relapse of symptoms after a brief interval.
- *Campylobacter jejuni* has been frequently isolated from various raw foods, including meats (beef, lamb, pork, chicken, and turkey), milk, eggs, vegetables, mushrooms, and clams.

While nature hosts numerous types of microorganisms, typically only a select few are found in a given food under normal conditions. These include microorganisms naturally occurring in raw foods, which provide their ecological niche, as well as those introduced from external sources encountered by the food from production through consumption.

1. Water

We talk about water that can be intended for drinking. They are of several types:

- **Public water supply (tap water):** they come from the capture of surface water or that of groundwater sources; this water undergoes several purifying treatments.
- **Individual catchment water:** are generally intended to supply a house, a hamlet or an industry, mainly in rural areas not served by mains water. Most of the time, this water does not undergo any treatment before use.
- **Bottled waters**: come from groundwater that is naturally well protected and protected from any contamination. These waters do not undergo disinfectant treatments, and are bottled under very specific hygienic conditions.

1.1. Pathogenic microorganisms

The initial microbiological content of water depends on the type of water, its location relative to sources of contamination, the surrounding bedrock and soil constituents, its oxygen and mineral content. Fecal contamination represents the most common and widespread source of public health risk associated with all types of drinking water.

1.1.1. Indices of fecal contamination

The search for signs of fecal contamination is of general application for the control of water and other foods, which are the vehicle for transmission of foodborne illnesses. The most common microorganisms in the intestine are anaerobic (*Bifidobacterium*) and microaerophilic (*Lactobacillus*), but these bacteria cannot be used as indices due to the problems posed by their detection. Then in order of decreasing concentration, we find the *Enterobacteria* among which *E. coli* is the most frequent, the fecal Streptococci and the sulphite-reducing *Clostridium*; These three groups were used as indices.

a. Coliforms and *E. coli*

Among Enterobacteriaceae, Gram bacteria, living in particular in the intestines of humans and animals, coliform bacteria are characterized by their ability to ferment lactose. In addition to their habitat, fecal coliforms have the ability to multiply at 44°C. Finally, *E. coli* generally adds to these properties that of producing indole. The sensitivity that *E. coli* brings to the detection of fecal pollution is good because there are so many of them in the intestinal contents. *Klebsiella* also have good specificity; on the other hand, *Citrobacter* and *Enterobacter* are almost completely devoid of it.

b. Fecal *Streptococcus*

Gram +, catalase -, microaerophilic or anaerobic bacteria, *Streptococcus* are distinguished by their coccoid form, their mode of grouping in pairs or chains. Faecal Streptococcus the characteristic antigenic substance of Lancefield group D and by the fact that their normal habitat being the digestive tract of warm-blooded animals as well as the genus *Enterococcus*. Among these bacteria we distinguish: *Enterococcus faecalis, E. faecium, E. durans, E. hirae* which generally live in the human intestine and *Streptococcus bovis, S. suis and S. equinus* which generally live in the intestines of animals. These bacteria are generally taken into account globally as witnesses of fecal pollution, because all of them have a fecal habitat. Counts of suspected *Streptococcus* are rarely performed independently of coliform counts.

c. Sulphite-reducing *Clostridium*

These bacteria are sometimes used as indicators of fecal contamination. The spore form, much more resistant than the vegetative forms of fecal coliforms and fecal *Streptococcus*, would thus make it possible to detect old fecal pollution. Without debating the real interest of such an indication concerning the date of pollution, it must however be considered that if sulphite-reducing *Clostridium* can certainly be fecal germs, they are also telluric germs and that, therefore, no specificity of fecal origin can be attributed to their detection. From this interpretation, it is beneficial to only look for the species most likely to be of fecal origin: this is particularly the case for *Clostridium perfringens*.

1.1.2. Other microorganisms

There is a wide variety of pathogenic or potentially pathogenic (opportunistic) bacteria for humans in all types of water. These live or survive in the environment, either coming from human waste, eliminated by sick subjects or healthy carriers, or being autochthonous and able to adapt to humans: *Pseudomonas aeruginosa, Salmonella, Shigella,* pathogenic *Staphylococcus*, *Vibrio*, *Yersinia enterolitica*. Their search in the waters is not systematic. Also, hepatitis A virus, Giardia, *Candida albicans* and other yeasts and molds have been implicated as the most common pathogens.

2. Milk and its derivatives

- Milk is a complex source of nutrients, including proteins, carbohydrates, lipids, vitamins and minerals whose primary role is to nourish the newborns of mammalian species. Raw milk can originate from cows, buffalo, sheep, and goats, with cows contributing the largest volume. Pasteurized or market milk, including whole, skim, low-fat, and flavored varieties, as well as cream, undergoes heat treatment (pasteurization) as per regulatory standards. Milk is rich in proteins and carbohydrates (lactose), which provide a nutrient source for the growth of many microorganisms.

2.1. Liquid milk

2.1.1. Pasteurized milk

Milk intended for consumption is usually heat treated to reduce the number of potential pathogenic microorganisms and, at the same time, spoilage microorganisms. The timetemperature combinations used for pasteurized milk vary widely, but the minimum legal requirement in most countries is at least 72°C for at least 16 seconds.

2.1.2. Sterilized milk

In order to create a shelf-stable product that can be stored at room temperature for several months, sterilization is required. To do this without catastrophic effects on flavor, the milk is heated to very high temperatures (> 100°C and > 125°C for UHT milk) for very short holding times (1 to 5 seconds).

2.2. Cream

Fat can be separated from milk by a continuous centrifugation process, which uses the difference in density between the fat and the water phase. Typically, the fat content can vary between 12% and 55%. As with milk, cream is heat treated to ensure safety.

2.3. Butter

During butter making, the cream is stirred to produce a continuous fat phase composed of fat globules. Butter should contain no more than 16% water and no less than 80% milk fat.

2.4. Cheese

It is a food obtained from coagulated milk or from material of exclusively dairy origin (cream, fat, etc.). From the Latin formaticus which means “mold, shape”, made from mainly cow's milk, but also from sheep, goat, etc.

2.5. Powdered milk

Milk powder, formerly called milk flour, is made from dehydrated milk. It can come from whole, semi-skimmed or skimmed milk. It can also be sweetened or contain additives (for example, vitamin D added to milk), we speak of condensed milk. Milk is composed of approximately 87.5% water (with variations depending on the lactation period, animal, species). Its dehydration allows this rate to be lowered to 3% and preserves the proteins, mineral salts and fats of the milk (if it has not been completely skimmed).

2.6. Milk microflora

- ❖ In raw milk, microorganisms originate from various sources including inside the udder, animal body surfaces, feed, air, water, and equipment used during milking and storage. Inside a healthy udder, predominant types include *Micrococcus, Streptococcus*, and *Corynebacterium*. Contaminants from animals, feed, soil, and water typically consist of lactic acid bacteria, coliforms, *Micrococcus, Staphylococcus, Enterococcus, Bacillus*, and *Clostridium* spores, as well as Gram-negative rods. Pathogens such as *Salmonella, Listeria monocytogenes, Yersinia enterocolitica*, and *Campylobacter jejuni* can also originate from these sources. Milking and storage equipment can be significant sources of Gram-negative rods such as *Pseudomonas, Alcaligenes*, and *Flavobacterium*, as well as Gram-positive *Micrococcus* and *Enterococcus*.

3. Meats

- ❖ Meat from slaughtered animals consists of approximately 64-80% water, 16-20% protein, 6-10% fat. The composition of different muscles differs depending on muscle function as well as animal species, breed, age, sex and nutritional status.
- ❖ Following slaughter and dressing, animal and bird carcasses harbor a variety of microorganisms, primarily bacteria originating from skin, hair, feathers, and the gastrointestinal tract. These microorganisms also come from the feedlot and pasture environments (including feed, water, soil, and manure) as well as from the slaughtering facilities (equipment, air, water, and human handlers).
- ❖ Among these, various enteric pathogens such as *Salmonella* serovars, *Yersinia enterocolitica, Campylobacter jejuni, Escherichia coli, Clostridium perfringens*, and *Staphylococcus aureus* may be present, albeit generally at low levels.
- ❖ After boning, chilled raw meat and ground meat retain microorganisms from the carcasses and acquire additional contamination from processing equipment, personnel, air, and water. Certain equipment, like conveyors, grinders, and slicers, can be challenging to clean

and serve as significant sources of microorganisms. Chilled meat typically contains mesophiles such as *Micrococcus, Enterococcus, Staphylococcus, Bacillus, Clostridium, Lactobacillus,* coliforms, and other *Enterobacteriaceae,* including potential enteric pathogens.

❖ However, because meats are stored at low temperatures (-1 to 5°C), psychrotrophs pose the greatest concern. Common psychrotrophs in raw meats include *Lactobacillus, Leuconostoc, Brochothrix thermosphacta*, *Clostridium laramie*, some coliforms, *Serratia, Pseudomonas, Alteromonas, Achromobacter, Alcaligenes, Acinetobacter*, *Moraxella*, *Aeromonas*, and *Proteus*. Psychrotrophic pathogens such as *Listeria monocytogenes* and *Yersinia enterocolitica* may also be present.

❖ Chicken tends to have a higher prevalence of *Salmonella* compared to red meats. Under aerobic conditions, psychrotrophic aerobes, particularly Gram-negative rods like *Pseudomonas, Alteromonas, Proteus*, and *Alcaligenes*, as well as yeasts, can proliferate rapidly. In anaerobic packaging, psychrotrophic facultative anaerobes and anaerobes such as *Lactobacillus, Leuconostoc, Brochothrix, Serratia*, some coliforms, and *Clostridium* are more likely to dominate.

4. Microbiological Analysis

4.1. Total Mesophilic Aerobic Flora (TMAF)

❖ This total germ count can represent the freshness or decomposition state of the product. For the handled product or those subjected to various technological treatments, the enumeration of total germs will allow assessment of the quality of production operations, transportation, etc.

❖ This enumeration is typically conducted using a solid medium. Starting from a stock suspension (the sample to be analyzed), aseptically transfer twice 1 ml (or its dilutions) to the bottom of two pre-prepared and pre-numbered Petri dishes specifically designated for this purpose. Subsequently, fill these two dishes with approximately 15 to 20 ml of melted Tryptone Glucose Extract Agar (TGEA) or Plate Count Agar (PCA) or Nutrient Agar (NA), cooled to 45°C, and gently swirl in a circular and back-and-forth (figure-eight) motion to ensure thorough mixing of the sample with the agar. Allow the medium to solidify for 10 minutes on a bench before adding a second layer of approximately 5 ml of the same agar. Incubate at 37°C for 48 to 72 hours, ensuring:

- First reading at 24 hours;
- Second reading at 48 hours;

- A third reading at 72 hours.

❖ Total germs appear in both cases as lentil-shaped colonies growing in mass; results are expressed in colony-forming units (CFU) per ml or per gram.

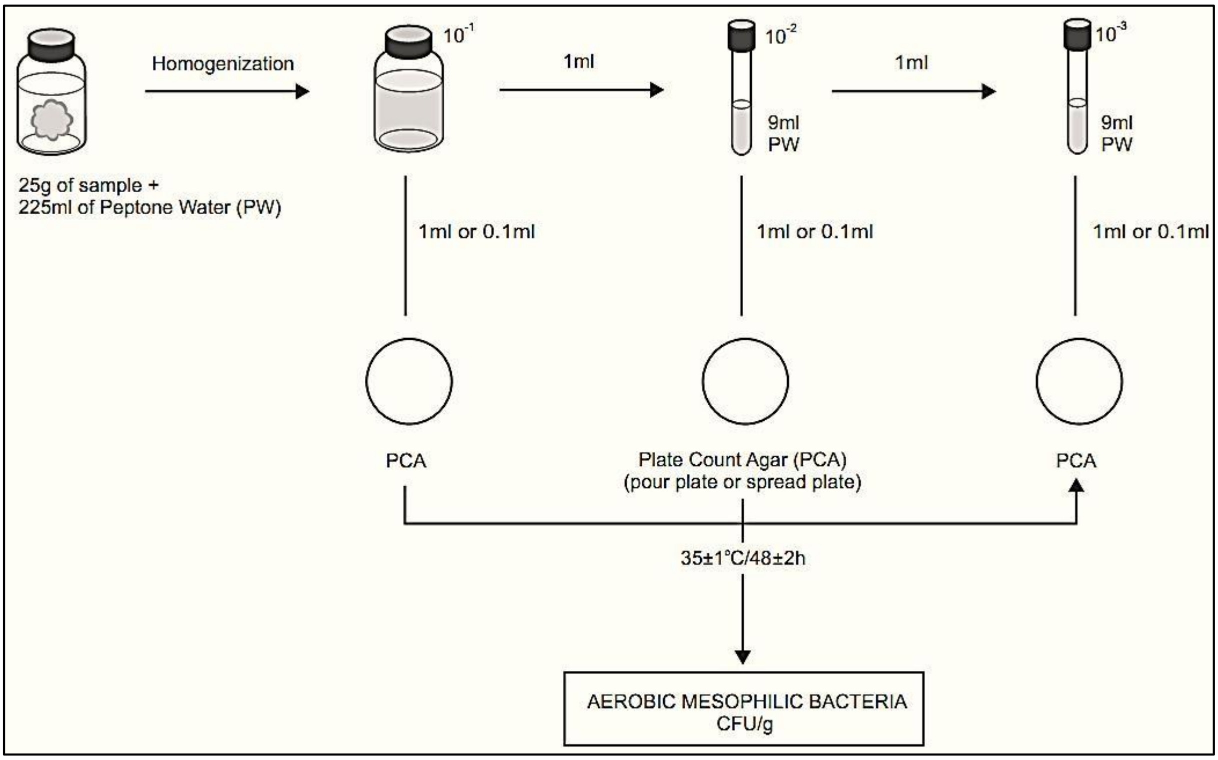

Figure 4. Enumeration of aerobic mesophilic bacteria in foods using the aerobic plate count method.

4.2. Fecal contamination

4.2.1. Enumeration of Coliforms (Colimetry)

4.2.1.1. Colimetry in liquid medium

This method is undertakenin two consecutive steps:

❖ **Presumptive test**: reserved for the detection of total coliforms.

Tests are performed using the Most Probale Number (MPN) method with three-tube dilutions: 3 times 10 ml in 3 tubes containing 10 ml of BCPL D/C medium with a Durham tube; 3 times 1 ml in 3 tubes containing 10 ml of BCPL S/C (simple concentration) medium with a Durham tube; and 3 times 0.1 ml in 3 tubes containing 10 ml of BCPL S/C (simple concentration) medium with a Durham tube. Tubes are considered positive if they show both gas production, microbial turbidity, and a yellow color change of the medium. Results are analyzed using the Mac Grady method.

- **Confirmation test (Mac Kenzie test):** reserved for the detection of thermotolerant coliforms and *Escherichia coli* at 44°C.

BCPL tubes found positive during total coliform enumeration will be streaked onto Schubert medium tubes with a Durham tube. Tubes are considered positive if they show both gas production and a red ring on the surface, indicating indole production by *Escherichia coli* after adding 2 to 3 drops of Kovacs reagent. Results are analyzed using the Mac Grady method.

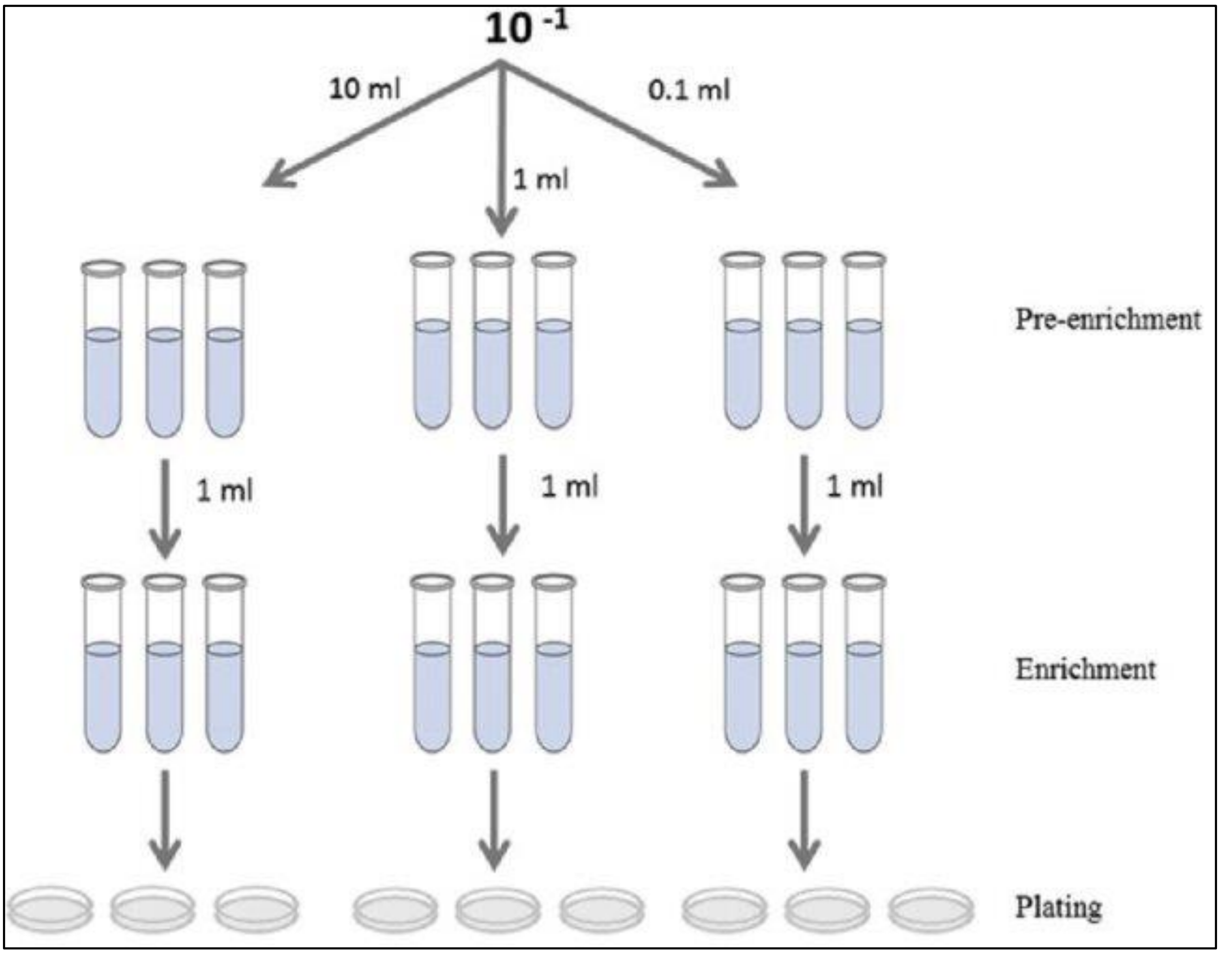

Figure 5. Schematic representation of the MPN method using three-tube dilutions.

4.2.1.2. Colimetry in Solid Medium

- The enumeration of coliforms can be performed by inoculating 1 ml of the product (or the stock suspension) and its dilutions into 15 ml of Violet Red Bile Lactose (VRBL) agar or Bromocresol Purple (BCP) lactose broth.
- 1 ml of the product and its dilutions are introduced into two Petri dishes, to which 15 ml of medium at 45°C is added and mixed. After cooling, 5 ml of the same sterile medium at

45°C is poured on the surface. Once solidified, one dish is incubated inverted for 24 hours at 37°C and the other at 44°C.

- ❖ On this medium, the growth of most non-*Enterobacteriaceae* bacteria is inhibited by crystal violet and bile salts. Lactose utilization is indicated by the red color change of the indicator within the colony. The colonies to be considered are purple to pink-red, approximately 0.5 to 1 mm in diameter, and surrounded by a reddish halo. Lactose-negative colonies remain colorless.

4.2.1.3. Colimetry by Filtration

- ❖ Colimetry can be performed using the enumeration method after filtration. This allows for the enumeration of total coliforms or fecal coliforms. The most commonly used media are TTC lactose agar and tergitol agar or others.
- ❖ Place the membrane on the agar and incubate for 24 hours at 37°C (total coliforms) or for 24 hours at 44°C (fecal coliforms). Purple colonies reduce TTC. Yellow colonies are non-reducers. A yellow halo around the colony indicates lactose fermentation; it is blue with lactose-negative bacteria.

4.2.2. Enumeration of Fecal Streptococcus (Streptometry)

4.2.2.1. Streptometry in Liquid Medium

This enumeration is performed in two steps similar to colimetry:

- ➢ **Presumptive Test:** tests are performed using Most Probale Number (MPN) method with three-tube dilutions and ROTHE medium. Incubate at 37°C for 24 to 48 hours. Tubes showing microbial turbidity are considered positive.
- ➢ **Confirmation Test:** this test confirms the presence of fecal *Streptococcus* potentially detected in the presumptive test. Positive ROTHE tubes are transferred to tubes containing LITSKY EVA medium. Tubes showing both microbial turbidity and a purple (whitish) pellet at the bottom are considered positive. Results are analyzed using the Mac Grady method.

4.2.2.2. Streptometry in Solid Medium

This method is only used when there is a large number of fecal *Streptococcus* present. The medium used is SLANETZ and BARTLEY agar. On this medium, Fecal *Streptococcus* form small red or brown colonies, with or without a white halo.

4.2.2.3. Streptometry by Filtration

Streptometry by filtration is similar to colimetry by filtration. Place the membrane on SLANETZ and BARTLEY agar and incubate at 37°C for 24 hours.

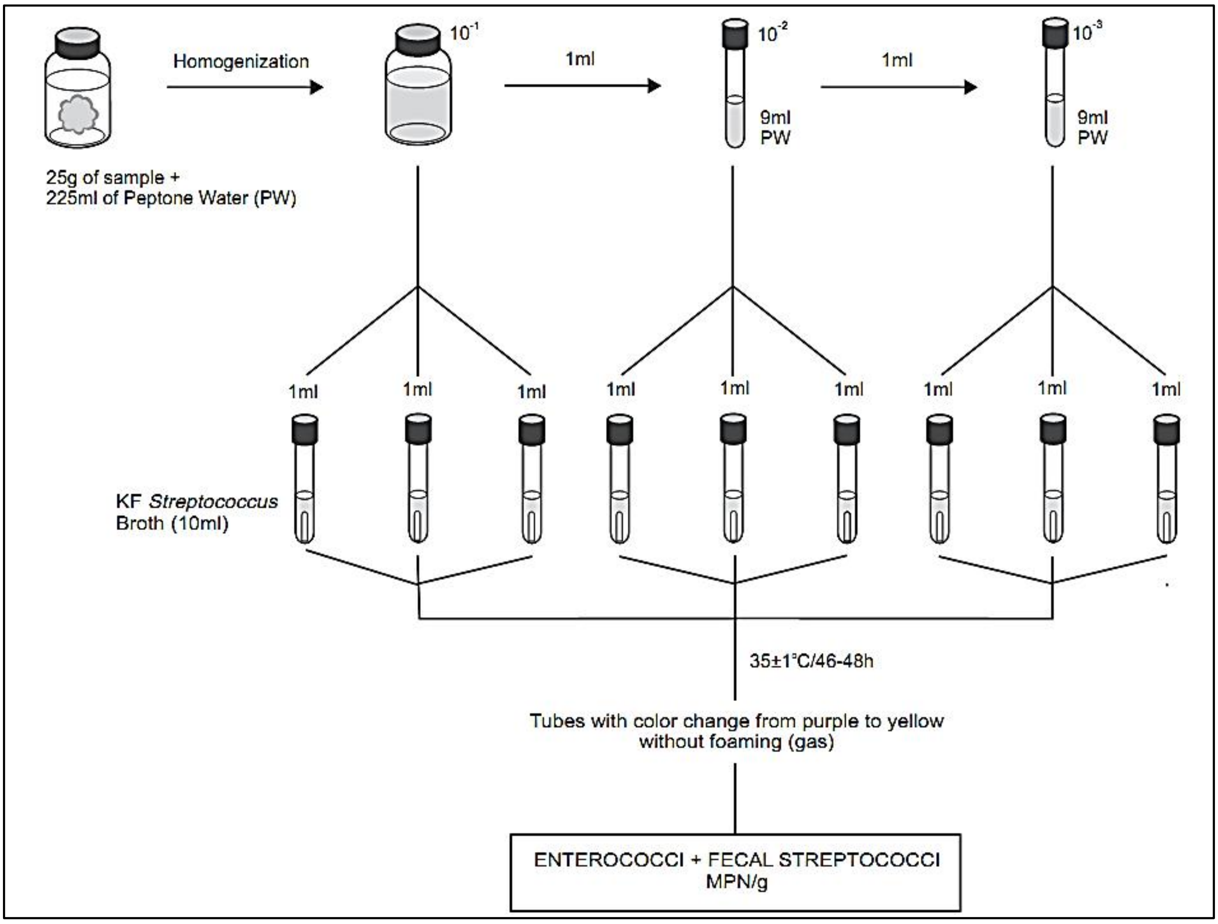

Figure 6. Enumeration of Fecal Streptococci in Foods Using the Most Probable Number (MPN) method.

4.2.3. Enumeration of Sulfite-Reducing *Clostridium* (SRC)

❖ These anaerobic, sulfite-reducing bacteria are normal inhabitants of the intestines of humans and animals. However, they are also frequently found in nature, particularly in soil (soil bacteria) and decomposing organic matter. These bacteria are highly resistant due to their spore-forming nature.

❖ From the stock suspension:

- Take approximately 25 ml in a sterile tube, which will then be subjected to heating at around 80°C for 8 to 10 minutes to destroy any vegetative forms of SRC that may be present.
- After heating, immediately cool the tube under running tap water.
- Distribute the contents of this tube into 4 different sterile tubes, with 5 ml per tube.

- Add about 18 to 20 ml of Meat Liver agar, melted and cooled to 45 +/- 1°C, with the addition of ferric alum (4 drops) and sodium sulfite (0.5 ml).
- Gently mix the medium and the inoculum, avoiding air bubbles and the introduction of oxygen.
- Allow the medium to solidify on the bench for about 30 minutes, then incubate at 37°C for 24 to 48 hours.
- The first reading must be done at 16 hours because SRC colonies often become invasive, potentially turning the tube completely black and making interpretation difficult. The second reading is done at 24 hours, and the third and final reading at 48 hours.
- Count all black colonies with a diameter of 0.5 mm growing in mass. *Clostridium perfringens* produces large black colonies of 3 to 5 mm in diameter.
- The number of sulfite-reducing anaerobic bacteria is expressed per gram or ml of the food product.

4.3. Detection of *Salmonella*

Detection of these bacteria requires several steps:

- **Pre-enrichment:** mix 25 g of the food sample with 225 ml of buffered peptone water. Incubate for 16 to 24 hours at 37°C.
- **Enrichment:** add 10 ml of the pre-enrichment medium to 100 ml of selenite cysteine broth. Incubate at 37°C for 24 hours.
- **Isolation:** Using a drop of the enrichment medium, isolate on SS (*Salmonella-Shigella*) agar.
- After 48 hours of incubation at 37°C, the colonies present as follows:
  - Red colonies, lactose-positive (*Enterobacter, Klebsiella*);
  - Colorless colonies, lactose-negative (*Salmonella, Shigella, Serratia, Proteus morganii, E. hafniae*);
  - Colonies with orange centers (*Proteus rettgeri, Providencia*);
  - Colonies with black centers (*Salmonella, Citrobacter, Proteus vulgaris, Proteus mirabilis*).
- Recognizing the colonies does not identify the bacteria definitively but provides a good presumption. Therefore, further biochemical or other identification is needed (Gram-negative rods, oxidase-negative, inoculation of an API 20E biochemical gallery).

4.4. Detection of *Staphylococcus aureus*

- Isolation: directly isolate from the sample on Chapman medium (other media such as Baird Parker can also be used). This medium is selective for halophilic aerobic bacteria, so many *Bacillus* species can grow on it. Distribute 17 ml of the medium per Petri dish and surface inoculate with 0.1 ml of the product or its dilutions. Incubate for 24 hours at 37°C.
- Colonies of *Staphylococcus aureus* are round, regular, convex, opaque, and golden-yellow pigmented; they are surrounded by a yellow halo due to acidification from mannitol (mannitol-positive). *S. epidermidis* produces white colonies and is mannitol-negative.
- To confirm that the colonies are *Staphylococcus aureus*, perform quick biochemical tests on 2 to 3 colonies from each plate: fresh state (cocci, motility), Gram staining (Gram-positive), catalase test (positive), coagulase test (positive), and inoculation of an API 20 Staph biochemical gallery.

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