2. Food microbiology

Objectives

- To trace sources of microbes in foods
- To explain how microbes cause food spoilage
- To list microorganisms responsible for the spoilage of vegetables, meats, eggs, grains, dairy products, sugary foods and canned foods
- To discuss methods of preservation of food from spoilage
- To distinguish between food intoxication & food infection
- To describe some fermented food products around the world, including those that are made in Ethiopia

Introduction

- Food microbiology is concerned with production and spoilage of food by microbes, prevention of food from microbial spoilage and prevention of food borne diseases.

- Food is a good medium for the growth of microbes.

- Microbes in our foods come from various sources including soil and water, plants and plant products, food handlers, animal hides and air and dust.

- Microbes get into our food they grow in it and may
  - Result in spoilage
  - Produce toxins and diseases

- Microbial growth is controlled by factors related to the food itself (**intrinsic** factors), and also to the environment where the food is stored (**extrinsic factors).**

- The **intrinsic** or food related factors include pH moisture content , water activity or availability, oxidation-reduction potential, physical structure of the food, available nutrients, and the presence of natural antimicrobial agents.

- **Extrinsic** or environmental factors include temperature, relative humidity, gases (CO_2, O_2) present, and the types and numbers of microorganisms present in the food.

2.1. Traditional ferments foods

- Many common foods and beverages are **preserved**, **produced**, or **enhanced** through the direct actions of microorganisms.

- **Fermentation** is the anaerobic catabolism of organic compounds, generally carbohydrates, in the absence of an external electron acceptor.

- Bacteria important in the fermented foods industry are the **lactic acid bacteria (LAB)**, the **acetic acid bacteria**, and the **propionic acid bacteria**.

- Yeasts and molds can also ferment foods.

- Food fermentation can be carried out by microbes naturally present in the food or by starter cultures.

- A starter culture is a selected strain of food-grade microbe of known and stable metabolic activities and other characteristics that is used to produce fermented foods of desirable appearance, body, texture, and flavor.

- Desirable microbial processes can produce significant alterations in raw food.

- Fermentative action of microbes:
  - May reduce pH of the food
  - Increases shelf life of the food
  - Imparts aroma and flavor to foods
  - May increase vitamin content and digestibility
  - May reduce or increase the toxicity of some foods

- There are over 3500 fermented foods in the world.

Food Groups	*Examples*
Dairy products	Cheeses, yogurt, buttermilk, sour cream, dahi, kumiss, kefir, acidophilus milk
Meat products	Salami, pepperoni, chorizo, thüringer, sausage, pickled meat, nahm
Cereal products	Breads, pancake, crackers, pizza, nun, idli, dosa, sour rice, miso
Fruits and vegetable products	Pickled fruits, pickled vegetables, olives, sauerkraut, kimchi, aachar
Legume products	Tofu, fermented soymilk, tempe, soy sauce, koji, mizo, natto, papadam
Fish products	Bagoong, fish sauces, pickled fish, tarama, paak, mamoni, izushi
Beverages	Beer, wine, distilled spirits, coffee, cocoa, tea
Starch crop products	Fermented products from potato, cassava, sweet potato, bananas, plantains
Miscellaneous products	Fermented eggs, ghee (from fermented cream), vinegar, red palm oil, bongkrek, dage

Product	**Starting material**	**Main fermenting microbes**
Cheese	Milk	*Lactobacillus* sp
Yogurt	Milk	*Streptococcus thermophilus* *L. bulgaricus*
Acidophilus milk	Milk	*L. acidophilus*
Butter milk	Milk	*L. lactis, Leuconostoc cremoris*
Ergo	Milk	*Lactobacillus, Streptococcus* *Leuconostoc*
Pickles	cucumbers	*Pediococcus cerevisiae*, *L. Plantarum*
Olives	Green olives	*Leuconostoc mesenteroides* *L. plantarum*
Sauerkraut	Cabbage	*L. mesenteroides, L. plantarum*
Beer	Barley wart	*Saccharomyces carlsbergensis*
Wine	Grapes	*S. ellipsoideus*
Soy sauce	Soybeans	*A. oryzae, Saccharomyces rouxii*

Product (Ethiopian)	**Starting material**	**Main fermenting microbes**
Shamita	Corn flour	LAB (*Lactobacillus*)
Tella	Barley malt	*Saccharomyces* sp. *Lactobacillus* sp.
Borde	Sorghum or millet flour	Lactobacilli, *Saccharomyces*, *Micrococcus, Bacillus*
Bread	Wheat flour	*S. cerevisiae*
Dry sausage	Pork beef	*Pediococcus cerevisiae*
Injera	teff flour	Enterbacteriacae, Lactic acid bacteria , Yeasts
Kocho	False banana	*L. mesentroides* , *Lactobacillus* sp., *Clostridium* sp. & *Bacillus* sp.
Awazae/Datta	pepper	*Bacillus* sp.
Siljo	Broad bean	*Micrococcus* sp., *Lactobacillus* sp and *Bacillus*

Fermented dairy products

- include fermented milk products such as yogurt, cheese, kefir, buttermilk, and sour cream.

- Milk contains lactose that can be hydrolyzed to glucose and galactose.

- These monosaccharides are fermented to the final product of **lactic acid** by the lactic acid bacteria.

- This fermentation **lowers pH** of raw milk (to a pH of less than 5.3 in cheeses and less than 4.6 in other fermented milk products).

Yogurt Production

- In commercial production of Yogurt, nonfat or low-fat milk is pasteurized, cooled to 43° or lower.

- It is then inoculated with a 1:1 ratio of *Streptococcus thermopilus* and *Lactobacillus delbrueckii* subspecies *bulgaricus* (*L. bulgaricus*).

- The two species ferment almost all of the lactose to lactic acid and **flavor** the yogurt with dacetyl (*S. thermopilus*) and acetaldehyde (*L. bulgaricus*).

- Fruits or fruit flavors to be added are pasteurized separately and then combined with the yogurt.

Cheese Production

- About 2,000 varieties of cheese are produced in the world.

- Cheeses can be
  - soft cheese (cottage, cream, Brie),
  - semisoft cheeses (Muenster, Limburger, blue),
  - hard cheeses (cheddar ,Colby, Swiss), or
  - very hard cheeses (Parmesan).

- All cheeses results from a lactic acid fermentation of milk, which results in coagulation of milk proteins and formation of a curd.

- Rennin can also be added to promote curd formation.

- After the curd is formed, it is heated and pressed to remove the watery part of the milk (called the whey), salted, and then usually ripened.

- The cheese curd can be packaged for ripening with or without additional microorganisms.

- The final hardness of the cheese is partially a function of the length of ripening.

- **Soft cheeses** are ripened for only about 1 to 5 months.

- **Hard cheeses** need 3 to 12 months, and **very hard cheeses** like parmesan require 12 to 16 months ripening.

Production of Buttermilk and Sour cream

- **Butter milk** and **sour cream** are produced generally by inoculating pasteurized cream or milk with a lactic starter culture and holding until the desired amount of acidity is attained.

- **Buttermilk** is the milk that remains after cream is churned for the production of butter.

- **Cultured sour cream** is produced generally by fermenting pasteurized and homogenized light cream with a lactic starter.

- These products owe their tart **flavor** to lactic acid and their **buttery aroma** and **taste** to diacetyl.

- **Kefir** is prepared by the use of kefir grains, which contain one or more bacterial species of *Acetobacter*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and one or more yeast species of *Candida*, *Kluyveromyces*, and *Saccharomyces*.

- **Acidophilus milk** is produced by the inoculation of *L. acidophilus* into sterile skim milk.

- The inoculums of 1-2% is added, followed by holding the product at 37°C until a smooth curd develops.

Fermented Meat Products

- **Sausages** are generally made from **pork beef**, or **poultry**.

- The most common are the dry sausages (e.g., salami and pepperoni) and the semidry sausages (e.g., bolognas and summer sausages).

- Sausages are made using a uniformly blended mixture of meat, salt, and seasoning.

- A starter culture of **lactic acid bacteria** is added, and fermentation reduces the pH of the mixture to below 5.

- After fermentation, sausages are often **smoked** and **dried** to a moisture content of about 30%.

- **Dry sausages** can be held at room temperature for extended periods of time.

- **Semidry sausages** have a final moisture content of about 50% and are less resistant to spoilage, so they are generally refrigerated.

- Fish, shrimp, and spices, are also fermented to make fish pastes and fish-flavored products.

Fermented Vegetables

- Include sauerkraut (fermented cabbage) and some types of pickles (fermented cucumbers).
- Peppers, olives, onions, tomatoes, and many fruits are also fermented.
- Vegetables are often fermented in **salt brine** to enhance preservation and **flavor**.
- The salt also helps **prevent the growth of unwanted organisms**.
- Fermentation may also **improve digestibility** by breaking plant tissues.

- **Soy sauce** is complex fermentation product made by fermentation of **soy beans** and **wheat**.

- A culture of the fungus *Aspergillus* is spread on a cooked wheat-soybean mixture, where it grows for 2-3 days.

- This preparation, known as **koji**, is then mixed with **brine** (17-19% NaCl) and **fermentation** proceeds for 2-4 months or more in large vats.

- The *Aspergillus* and various microbes produce fermentation products from the brined koji that contribute to the desirable characteristics of the final product.

- After fermentation, the liquid sauce is **filtered**, **pasteurized**, and **bottled** as soy sauce.

- **Sauerkraut** is fermentation product of fresh cabbage.

- **Olives** are fermented by the natural biota of green olives, which consists of a variety of bacteria, yeasts, and molds.

- **Pickles** are fermentation products of fresh cucumbers, and the fermenting microbes generally consists of the normal mixed biota of cucumbers.

Cucumber

Vinegar

- Vinegar is produced by the conversion of diluted ethanol to acetic acid by **acetic acid bacteria,** such as *Acetobacter* and *Gluconobacter*.

- The usual starting material is wine, fermented rice, or alcoholic apple juice (hard cider).

- Vinegar can also be produced from a mixture of pure alcohol in water. Such vinegar is called **distilled vinegar**.

- Vinegar is used as a flavoring agent in salads and other foods, and because of its acidity, it is also used in **pickling**.

2.2. Food spoilage

- Spoiled food is the one that has been damaged or injured so as to make it undesirable for human use.

- Generally spoiled foods have unpleasant appearance, aroma and taste.

- Food spoilage may be caused by
  - insect damage,
  - physical injury (e.g. bruising and freezing),
  - enzymes activity and
  - microorganisms.

- **Nonperishable foods** have **low water activity** and can generally be stored for considerable lengths of time without spoilage.

- **Perishable foods** (e.g., meats, fish, poultry, eggs, milk, most fruit and vegetables) and **semi-perishable foods** (e.g., potatoes , some apples, and nuts sugar, flour, rice, and dry beans) typically have **higher water activities** and can be **spoiled within a short period of time.**

- **Stable** or **nonperishable** food (e.g., sugar, flour, rice, and dry beans) are more resistant to spoilage.

- The extent of food spoilage depends primarily on its physical and chemical properties.

- Thus, water content, pH, physical structure, carbohydrate content, protein content, vitamin level, oxygen and temperature conditions, etc. are important factors.

Types of microbes associated with foods spoilage

- Different foods differ in their biochemical composition and are subject to spoilage by different microbial populations.
- Digestion of carbohydrates produces acids, alcohols and CO_2.
- Bacteria degrade proteins into amino acids and foul smelling end products such as hydrogen sulfide (gives rotten egg smell to food) and indole & skatole which give food a fecal odour.
- Acids cause souring of food and gas causes sealed food to swell.
- Digestion of fats yields fatty acids, giving a rancid odour or taste to food.
- Food may become slimy due to production of capsules in bacteria.
- There may be color development giving some colour to foods.

Food	Spoilage microbe
Vegetables	***Erwinia***
Fruits	**yeasts** and **molds**
Meat	**bacteria** and **fungi:** result in foul odour due to production of indole, NH_3 and H_2O
Cold stored	Psychrophiles
Fresh iced fish	*Pseudomonas*
Salted and dried fish	Fungi
Eggs	**Bacteria** (*Pseudomonas, Salmonella* and *Staphylococcus*) and **fungi**: result in rotting

Food	Spoilage microbe
Grains	*Bacillus, Rhizopus, Aspergillus flavus* which prodcuces aflatoxin
Dough	LAB (*Lactobacillus*, *Leuconostoc*, & *treptococcus*
Bread	*Rhizopus stolonifer* (bread mold).
Rye, wheat and barley	*Claviceps purpurea:* causes ergot disease.
Sugary foods (cane & sugar beet)	*Bacillus* and *Clostridium* species and osmophilic yeasts.
Beer	**Yeasts** and **bacteria**: cause ropiness, sourness, turbidity
Canned food	**Bacteria** (e.g. *Clostridium*) and **yeasts**

2.3. Food preservation methods

- Inhibit or stop microbial growth in food or remove microbes from food

- Include
  - Removal of microorganisms
  - Low temperature
  - High temperature
  - High pressure
  - Reduced water availability
  - Chemical-based preservation
  - Radiation
  - Microbial product based inhibition
  - Pickling and fermentation

- It is vital to eliminate or reduce the populations of spoilage and disease-causing microbes and to maintain the microbiological quality of a food with proper storage and packaging.

Preservation by removal of microorganisms

- Microorganisms can be removed from water, wine, beer, juices, soft drinks, and other liquids by **filtration**.

- This can keep bacterial populations low or eliminate them entirely.

- Beer is filtered rather than pasteurized to better preserve the flavor and aroma of the original product.

Preservation at low temperature

- **Refrigeration** at 5°C **retards microbial growth.**
  - Extended storage can lead to microbial growth and spoilage.

- Psychrotolerant (cold tolerant) microbes can survive and grow at refrigerator temperatures and cause food spoilage.

- Storage of perishable foods for long periods of time (> several days) is possible only at temperatures below freezing.

- **Freezing at -20^{o}C** is widely used to preserve meats and many fruits and vegetables for weeks or months.

- Long-term storage is done **at – 80^{o}C** (dry ice temperature).

- Slow microbial growth at temperatures below - 10°C has been found in fruit juice concentrates, ice cream, and some fruits.

- **Some** microorganisms are **very sensitive to cold** and their numbers are reduced.

- Thus although refrigeration slows the metabolic activity of most microbes, it does not lead to significant decreases in overall microbial populations.

Treating foods with high temperatures

- **Canning** is sealing food in a container followed by heating to kill all microbes in the food.

- **Canned** food is **heated** in containers called **retorts** at about **115°C** for intervals ranging from **25** to over **100** minutes.

  – Non-acid foods are **not** autoclaved.

- Heating time must be sufficient enough to **kill** all microbes, including endospores.

  – Food may lose nutrient value.

- *C. botulinum* can grow and produce lethal toxins.

- Sometimes canned foods become spoiled.

- This may be due to spoilage before canning, underprocessing during canning, and leakage of contaminated water through can seals during cooling.

- Spoiled food can be altered in color, texture, odor, and taste.

- Organic acids, sulfides, and gases (particularly CO_2 and H_2S) may be produced.

- If spoilage microbes produce gas, both ends of the can will bulge outward. The can may explode.

- Swelling is not always due to microbial spoilage.

- Acid in high-acid foods may react with the iron of the can to release hydrogen and generate a hydrogen swell.

- **Pasteurization:** heating food to a temperature that kills disease-causing microbes and reduces the levels of spoilage organisms.

- Milk, beers and fruit juices are pasteurized .

- Pasteurization can be achieved in 3 ways:
  - at 62.8°C for 30 minutes: conventional **low-temperature holding (LTH) pasteurization**
  - at 71°C for 15 seconds: **high temperature, short-time (HTST)** process
  - at 141°C for 2 seconds: **ultra-high-temperature (UHT)** processing

- Shorter-term processing results in improved flavor and extended product shelf life.

Preservation at low water availability

- Achieved by
  - dehydration (**drying**) of foods by using heat and lyophilization
  - **salting** (adding high concentrations of solutes (salts or sugars) to foods).

- In **lyophilization** (freeze-drying), foods are frozen and water is removed under vacuum to produce freeze-dried foods. It is least damaging to food.

- Milk, meat, fish, vegetables, grains, fruits and eggs are commonly preserved by some form of drying.

- **Salting** preserves meats, fish, sausage and ham.

Chemical-Based Preservation

- Chemical preservatives include simple organic acids, sulfite, ethylene oxide as a gas sterilant, sodium nitrite, and ethyl formate.
- These chemical agents may damage the microbial plasma membrane or denature various cell proteins.
- Other compounds interfere with the functioning of nucleic acids, thus inhibiting cell reproduction.
- The effectiveness of many of these chemical preservatives depends on the food pH.
- Sodium propionate is most effective at lower pH values.

- Chemical preservatives are used with grain, dairy, vegetable, and fruit products.

- **Sodium nitrite** is used to help preserve ham, sausage, bacon, and other cured meats by inhibiting the growth of *Clostridium botulinum* and the germination of its spores.

- This protects against botulism and reduces the rate of spoilage.

- Besides increasing meat safety, nitrite decomposes to nitric acid, which reacts with heme pigments to keep the meat **red** in color.

- Concerns: 1) nitrite can react with amines to form carcinogenic nitrosamines
  2) ethylene or propylene oxides are mutagens.

Preservatives	Organisms Affected	Foods
Propionic acid/propionates	Molds	Bread, cakes, some cheeses, inhibitor of ropy bread dough
Sorbic acid/sorbates	Molds	Hard cheeses, figs, syrups, salad dressings, jellies, cakes
Benzoic acid/benzoates	Yeast and molds	Margarine, pickle relishes, apple cider, soft drinks, tomato ketchup, salad dressings
Parabens	Yeast and molds	Bakery products, soft drinks, pickles, salad dressings
SO_2/sulfites	Insects and microorganisms	Molasses, dried fruits, wine, lemon juice
Ethylene/propylene oxides	Yeasts, molds, vermin	Fumigant for spices, nuts
Sodium diacetate	Molds	Bread
Dehydroacetic acid	Insects	Pesticide on strawberries, squash
Sodium nitrite	Clostridia	Meat-curing preparations
Caprylic acid	Molds	Cheese wraps
Ethyl formate	Yeast and molds	Dried fruits, nuts

Preservation by irradiation

- Both ionizing and nonionizing radiations are used for reducing contamination by bacteria, fungi and insects.
- UV is used to control microbes on the surfaces of laboratory and food-handling equipment, but it does not penetrate food.
- The major method used for radiation sterilization of food is gamma irradiation from a cobalt-60 source.
- Gamma radiation has excellent penetrating power. It must be used with moist foods because the radiation produces peroxides from water in the microbial cells, resulting in oxidation of sensitive cellular constituents.
- Irradiated foods: Fresh pork, Fresh fruits and vegetables, dried spices, meat products and poultry.

- This process of **radappertization**, can extend the shelf life of seafoods, fruits, and vegetables.

- **Radappertization** is the use of gamma rays from a cobalt source for control of microorganisms in foods.

- **Electron beams** can also be used to irradiate foods.
- High energy electrons are produced by electron accelerators.
- They have **low penetration power** and suitable only for sliced meats, bacon, or similar thin products

- This approach does not generate radioactive waste.
- The food does not become radioactive.

High-pressure food preservation

- Prewrapped foods such as fruits, deli meats, and precooked chicken strips are submerged into tanks of pressurized water.
- This process kills many bacteria, such as *Salmonella, Listeria,* and pathogenic strains of *E. coli,* by disrupting many cellular functions.
- It also kills nonpathogenic microorganisms that tend to shorten the shelf life of such products.
- Because the process does not require additives, it does not require regulatory approval.
- It has the advantage of preserving colors and tastes of foods better than many other methods and does not provoke the concerns of irradiation.

Microbial Product-Based Inhibition

- **Bacteriocins** and **antibiotics** can be added to foods to control foodborne pathogens.
- Bacteriocins are bactericidal proteins active against closely related bacteria.
- The only currently approved bacteriocin is **nisin**.
- Nisin can be used in low-acid foods to improve inactivation of *Clostridium botulinum* during the canning process or to inhibit germination of any surviving spores.
- The use of antibiotics may result in the development of antibiotic resistant microbes.

Preservation at low pH by fermentation and pickling

- Over the last several thousand years, fermentation has been a major way of preserving food.
- Microbial growth, either of natural or inoculated populations, causes chemical and/or textural changes to form a product that can be stored for extended periods.
- They produce organic acids (acetic, lactic, propionic acids) that lower the pH of the food.
- The fermentation process also is used to create new, pleasing food flavors and odors.
- The major fermentations used in food preservation are the lactic, propionic, and ethanolic fermentations.

- **Pickling** is the process of acidifying food to prevent microbial growth and spoilage.

- In the process of pickling, vinegar and salt or sugar is added to food to inhibit microbial growth.

- pH 5 or less inhibits the growth of most spoilage microbes.

- Pickled foods include cucumbers, peppers, meat, fish and fruits.

2.4. **Food toxins and their sources**

- The major sources of toxins in foods are bacteria and fungi.
- **Toxins** are poisonous substances that are produced by certain microorganisms.
- Some toxins produce fever, cardiovascular disturbances, diarrhea, and shock.
- Toxins can also inhibit protein synthesis, destroy blood cells and blood vessels, and disrupt the nervous system by causing spasms.

2.4.1. Bacterial Toxins

- Bacterial toxins are of two general types: exotoxins and endotoxins.

- **Exotoxins** are toxic proteins released from the pathogen cell as it grows. Many are enzymes.

- Bacteria that produce exotoxins may be gram-positive or gram-negative.

- Because exotoxins are soluble in body fluids, they can easily diffuse into the blood and are rapidly transported throughout the body.

- Exotoxins work by destroying particular parts of the host's cells or by inhibiting certain metabolic functions.

- Exotoxins are among the most lethal substances known.

- *Neurotoxins* attack nerve cells, *cardiotoxins* attack heart cells, *hepatotoxins* attack liver cells, *leukotoxins* attack leukocytes, *enterotoxins* attack the lining of the gastrointestinal tract, and *cytotoxins* attack a wide variety of cells.

- The body produces antibodies called antitoxins that provide immunity to exotoxins.

- When exotoxins are inactivated by heat or by formaldehyde, iodine, or other chemicals, they no longer cause the disease but can still stimulate the body to produce antitoxins.
- Such altered exotoxins are called toxoids.

- When toxoids are injected into the body as a vaccine, they stimulate antitoxin production so that immunity is produced.

- Diphtheria and tetanus can be prevented by toxoid vaccination.

Table. Food-borne diseases Caused by Exotoxins

Disease	Bacterium	Mechanism
Botulism	*Clostridium botulinum*	Neurotoxin prevents transmission of nerve impulses; flaccid paralysis results.
Traveler's diarrhea	Enterotoxigenic *E. coli* and *Shigella* spp.	Enterotoxin causes secretion of fluids and electrolytes that result in diarrhea.
Clostridium perfringens food poisoning	*Clostridium perfringens*	One exotoxin (cytotoxin) causes massive red blood cell destruction (hemolysis); another exotoxin (enterotoxin) causes food poisoning/diarrhea.
Staphylococcus aureus food poisoning	*Staphylococcus aureus*	Enterotoxin causes secretion of fluids and electrolytes that results in diarrhea.

Endotoxins

- **Endotoxins** are lipid portions (lipid A) of lipopolysaccharides (LPS) that are part of the outer membrane of the cell wall of gram-negative bacteria.

- Thus, endotoxins are lipopolysaccharides, whereas exotoxins are proteins.

- The endotoxins are liberated when the bacteria die and the cell wall lyses, or breaks apart.

- Antibiotics used to treat diseases caused by gram-negative bacteria can lyse the bacterial cells.

- This releases endotoxin and may lead to an immediate worsening of the symptoms, but the condition usually improves as the endotoxin breaks down.

- All endotoxins produce the same signs and symptoms.

- These include chills, fever, weakness, generalized aches, and, in some cases, shock and even death.

- Endotoxins exert their effects by **stimulating macrophages to release cytokines** in very high concentrations.

- At these levels, cytokines are toxic.

- Endotoxins can also **induce miscarriage**.

- Endotoxins can **activate of blood-clotting proteins**, causing the formation of small blood clots.

- These blood clots obstruct capillaries, and the resulting decreased blood supply induces the death of tissues.

- This condition is referred to as *disseminated intravascular coagulation (DIC).*

- Examples of food-borne microorganisms that produce endotoxins are *Salmonella typhi*.

2.4.2. Fungal toxins (mycotoxins)

- Are toxic fungal secondary metabolities
- When ingested, inhaled or absorbed through the skin, can cause disease or death in man and domestic animals, including birds.
- Diseases caused by mycotoxins are called **mycotoxicoses.**
- A particular species of fungus may produce more than one mycotoxin.
- Mycotoxin production occurs only as a result of fungal growth.

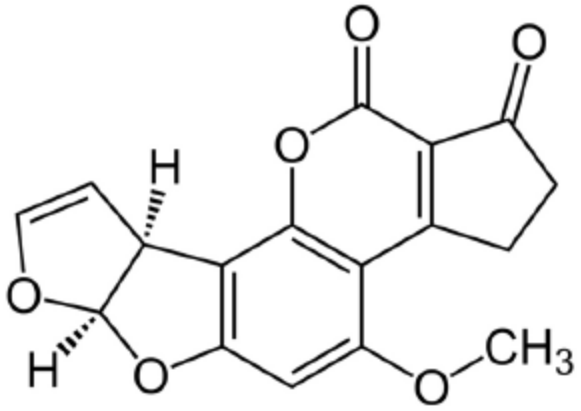
- Mycotoxins may occur in processed foods, or in commodities such as grains or nuts.

- Mycotoxins are chemically **stable** once formed, and persist in food even after the destruction of the fungi that produced them.

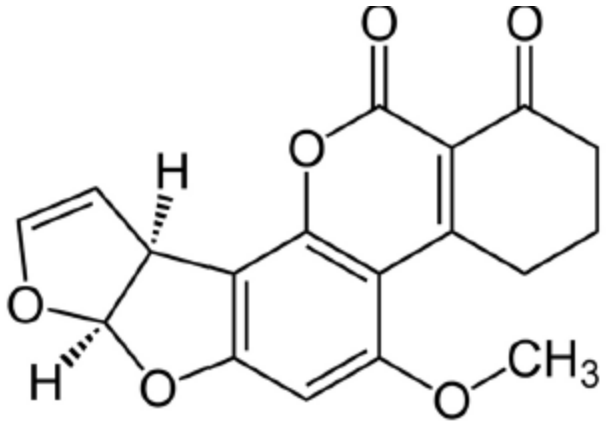
- They may be neurotoxins, teratogens, nephrotoxins, hepatotoxins, immunosuppressive agents, or carcinogens.

- The most important mycotoxins are aflatoxins, ochratoxin A, fumonisins, deoxynivalenol, and zearalenone.

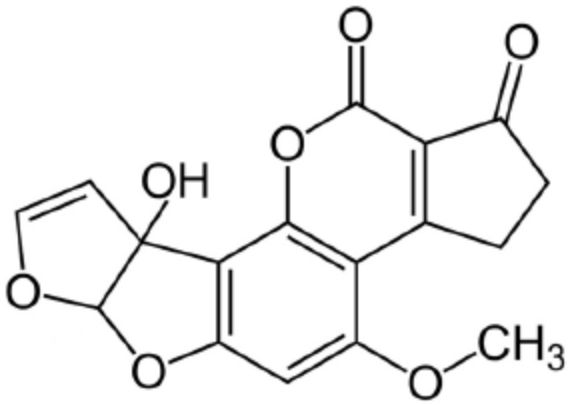
Structures of some mycotoxins



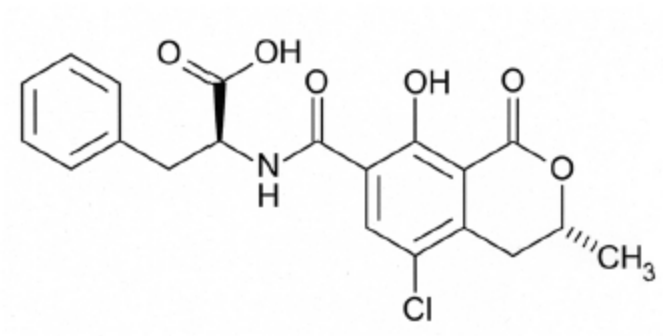
Aflatoxin B_1



Aflatoxin G_1



Aflatoxin M_1



Ochratoxin A

Aflatoxins

- Four naturally occurring aflatoxins: aflatoxins B1 and B2; and aflatoxins G1 and G2.

- When aflatoxins B1 and G1 are ingested by lactating animals, small proportions (1 to 2%) are excreted in milk as aflatoxins M1 and M2, which are hydroxylated derivatives of the parent compounds

- Aflatoxins are produced in foods primarily by ***Aspergillus flavus*** and the closely related species *Aspergillus parasiticus.*

- *A. flavus* occurs in food crops in the tropical and warm temperate zones of the world.

- *A. flavus* is associated with peanuts, maize, and cottonseed and frequently produces aflatoxins in these commodities.

- It also occurs in tree nuts, especially pistachios and Brazil nuts

- Aflatoxins have five toxic effects:
  - acute toxicity (aflatoxicosis)
  - liver carcinogenicity
  - liver cirrhosis
  - immunosuppression
  - growth retardation in children

- **Acute Toxicity:** results in **hepatitis** and characterized by jaundice preceded by fever, vomiting, and anorexia, with ascites and edema in the lower limbs in extreme cases.

- **Liver carcinogenicity:** Liver cancer can result from consumption of much **lower levels** of aflatoxin (e.g. aflatoxin B1) **over longer time periods.**

- **Stunting in Children**. Studies have provided evidence that aflatoxin exposure before birth and in early childhood is associated with stunted growth.

- **Immunosuppression**. Aflatoxins have been shown to suppress the cell-mediated immune response in both cell lines and domestic animals.

- Levels of aflatoxins in foods are highly variable.
- Only a few commodities are at serious risk if good agricultural and manufacturing practice is observed.
- Peanuts, maize, and cottonseed frequently contain unacceptable levels of aflatoxins.
- Under inadequate storage conditions, other grains (e.g., sorghum and rice) may also permit growth of *A. flavus* and aflatoxin production.

Ochratoxin A

- Several ochratoxins are known:
  - ochratoxin A (OTA)
  - ochratoxin C
  - ochratoxin B and others

- Ochratoxins A and B are the only ones so far detected in naturally contaminated foods or feeds.

- OTA is the most toxic compound.

- OTA is produced by three groups of fungi:
  - the ocher-colored aspergilli—*Aspergillus ochraceus, A. westerdijkiae, A. steynii,* and a few, closely related species;
  - the black aspergilli—*A. carbonarius* and *A. niger;* and
  - *Penicillium* species—P*. verrucosum* plus the closely related species *P. nordicum*

- OTA is a chronic nephrotoxin, affecting kidney function.

- OTA also has carcinogenic properties.

- People in Europe and northern North America are exposed to ochratoxin A in barley and wheat and their products, especially bread, and also from meat, especially pork, from animals fed contaminated feed.

- Low levels also occur in beer, wine, coffee, cocoa, chocolate, and dried vine fruits.

- Wheat and barley crops from warmer climates are not infected by *P. verrucosum.*

- OTA intake is much lower in tropical and subtropical regions

- Cereals in tropical and warm temperate climates seldom contain appreciable OTA, as *P. verrucosum* is a cool-climate fungus.

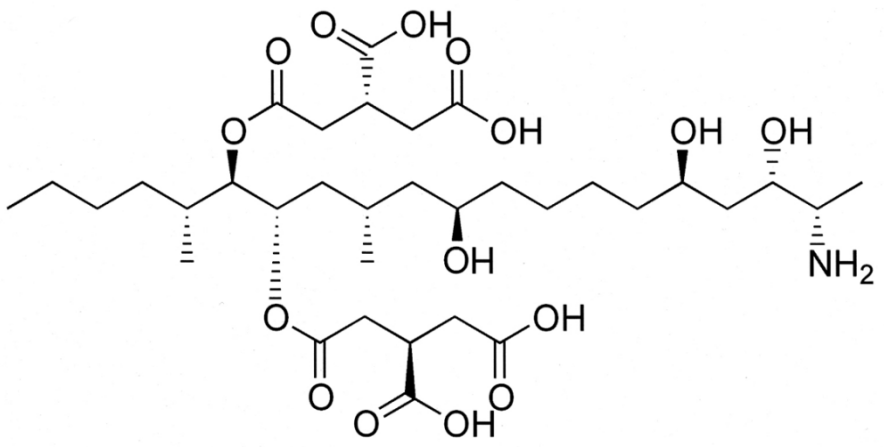
- Tropical foods, such as coffee and cocoa, are sometimes contaminated with OTA as the result of growth by *Aspergillus* species

Fumonisins

- The most important fumonisins is fumonisin B1.
- Fumonisins are produced by *Fusarium verticillioides* and some closely related species.
- These species are systemic in maize worldwide, being always present in the plants and even in healthy kernels.
- Fumonisins are toxic to animals and man.

- They interfere with the function of some membrane proteins, including folate binding.

- Fumonisin levels are reduced by processing at temperatures above 150°C, including frying , baking, roasting, and alkaline cooking.



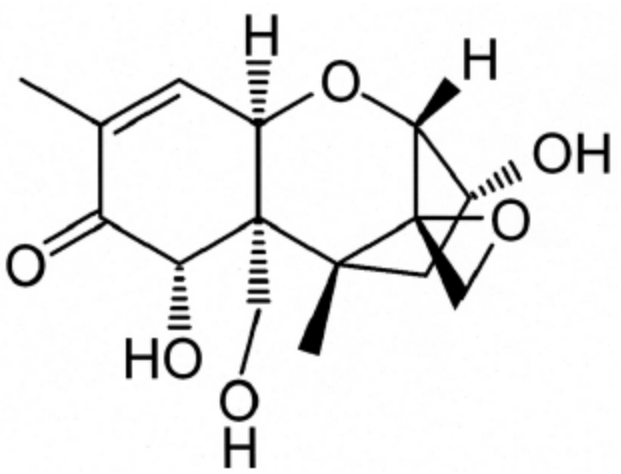
Fumonisin

Deoxynivalenol (vomitoxin)

- DON is mainly produced by *Fusarium graminearum, Fusarium culmorum.*

- *F. graminearum* occurs in maize, and both *F. graminearum* and *F. culmorum* especially wheat and barley.

- DON is prevalent in cooler areas where rainfall is higher.



Deoxynivalenol (DON)

- They cause Gibberella ear rot in maize and Fusarium head blight in wheat, barley, and triticale

- DON is inhibitors of protein synthesis.

- DON can cause gastrointestinal problems and immunotoxicity in humans.

2.5. Food borne diseases

- Food borne diseases are either **intoxications** or **infections**.
- **Food intoxications** are true food poisonings caused by consumption of preformed toxins produced by microbial growth prior to ingestion.
- Incubation period for many intoxications is **short** (2-8 hours).
- **Food infections** are caused by ingestion of food contaminated with pathogens that multiply in the intestine and cause symptoms.
- Incubation periods are generally longer (usually 8 to 48 hours.)
- Food poisoning is common in pasteries, universities and hospitals

Food intoxications

Staphylococcus aureus food poisoning

- It is most common and caused by *Staphylococcus aureus,* which produces a heat-stable toxin called enterotoxin.

- The staphylococci usually enter food from a human source, often infected food handler.

- They are most commonly shed from nasal secretions or infected wounds, boils, and abscesses.

- Protein rich foods (e.g., foods rich in eggs or milk such as cream filled pastries, custards, and salad dressings) serve as excellent culture media for staphylococci.

- Meat and poultry also support the growth of *S. aureus*.

- If contaminated foods are maintained at temperatures between 20 and 35^{o}C for several hours (3-4) the pathogens multiply and release enough toxin to elicit symptoms of intoxication.

- The symptoms appear between 2 to 4 hours after consumption of the food.

- The symptoms include severe nausea, vomiting, cramps, and occasional diarrhea.

- Then illness rarely lasts more than 1 or 2 days and requires no treatment.

- Staphylococcus food poisoning is identified by its
  - characteristic symptoms,
  - the nature of the foods involved,
  - the shortness of the incubation period, and
  - the isolation of the bacteria from the implicated food.

- Important factors contributing to outbreaks:
  - inadequate refrigeration,
  - preparing food for in advance planned service,
  - infected person practicing poor personal hygiene,
  - in adequate cooking or processing and
  - handling food in warm devices at bacterial growth temperatures.

Botulism

- Botulism is caused *Clostridium botulinum,* which produces a highly toxic enxotoxin, a neurotoxin .
- The toxin inhibits nerve function and produces fatal paralysis.
- The toxin is produced when foods contaminated with C. *botulinum* endospores are kept under anaerobic conditions.
- It is very sensitive to heat & can be destroyed by a few minutes boiling.
- It is usually associated with canned foods, in which anaerobic metabolism may produce foul-smelling byproducts, and hydrogen sulfide and carbon dioxide may accumulate and may cause bulge or explosion.

- The symptoms of botulism usually appear between 12 and 36 hours.

- These symptoms are neurological, not gastrointestinal, and include weakness, blurred vision or double vision and eventually flaccid paralysis.

- Without treatment, respiratory or cardiac paralysis and death may occur in 3 to 6 days.

- Diagnosis is done by immunologic identification of toxin in serum or feces.

- *C. botulinum* can also cause infection in infants where endospores are ingested (honey) and upon germination in the intestine the toxin is produced.

Clostridium perferingens food poisoning

- Food poisoning strains belong to type A, whose spores survive 1-5 hours boiling.
- Food poisoning strains exist in soils, water, foods, dust, spices and intestinal tract of man and animals.
- Food poisoning is due to production of an enterotoxin, which occurs together with sporulation.
- Usually older strains produce enterotoxins.
- The enterotoxin binds to intestine and causes it to lose water.
- Foods involved in *C. perferingens* outbreaks are often meat dishes prepared on a day and eaten the next day.

Bacillus cereus food poisoning

- Food poisoning stains produce enterotoxins hemoysin (heat-stable) and β-lactamase (heat-labile) which cause an emetic illness characterized by vomiting and nausea, and diarrhea respectively.
- Vomiting is more severe and acute and appears 1 to 6 hours after ingestion.
- Diarrhea syndrome is mild and develops within 8-16 hours and lasts for 6-12 hours.
- The effects of the toxin are self-limiting and usually disappear within 24 hours of illness onset.
- Vehicle foods consist of cereal dishes, mashed potatoes, vegetables, minced meats, milk and cooked meat, rice dishes, soups and others.
- The emetic type is often associated with boiled or fried rice, while the diarrheal type is associated with a wider range of foods.

Food borne infections

Cholera

- Cholera is caused by the growth of *Vibrio cholerae* (serotype 01) in the intestines.
- It is usually transmitted by water that has been heavily contaminated with feces or vomitus of cholera patients.
- Foods, fingers, and flies can serve as vehicles of cholera outbreaks.
- The pathogen produces **enterotoxin** which attaches to intestinal cells and causes loss of water and salt.
- The rapid loss of water results in a watery diarrhea.

- This causes severe dehydration, thickening of blood, a decrease in blood volume, circulatory collapse (shock) and death if not rapidly treated.

- Cholera can be preliminarily diagnosed by the characteristic appearance of the watery stools, which are virtually free of feces and contain mostly mucus, epithelial cells, and enormous numbers of *Vibrio cholerae*.

- These features give the stools a “rice water” appearance.

- Diagnosis is supported by direct microscopic examination of stool specimen for the presence of the pathogen.

- Treatment is the replacement of fluids and electrolytes.

- Prevention of cholera
  - rapidly treating active cases,
  - properly disposing of sewage,
  - chlorinating water supplies, and,
  - boiling water before it is used for drinking, cooking, or washing dishes (when sewage and water facilities are inadequate).

Escherichia coli gastroenteritis

- Although most *E. coli* strains are avirulent, some strains are pathogens & cause food borne gastroenteritis.
- A few stains (called **enteroinvasive *E. coli***) can cause local invasive disease of the intestine epithelium.
- Most pathogenic *E.coli* strains (called **enterotoxicogenic *E. coli* (ETEC)**)are noninvasive and produce an enterotoxin while growing in the intestine.
- ETEC are among the leading causes of traveler's diarrhea.
- The toxin is physically & antigenically similar to cholera toxin and causes a similar, but milder, watery diarrhea.

- *E. coli* toxin makes people sick, where as cholera toxin kills unlike cholera.

- Gastroenteritis is caused by ingestion of 10^6-10^{10} viable cells per gram of food.

- Enterotoxicogenic strains are distinguished from the normal flora by serological tests & by demonstration of enterotoxin production.

- The most severe cases of *E. coli* infection are caused by **enterohemorrhagic** strains of the bacteria.

- These *E. coli* produce a toxin, called **verotoxin**, which causes bloody diarrhea usually accompanied by severe abdominal cramps.

- Occasionally, the toxin damages the kidneys, resulting in blood in the urine and a condition known as hemolytic uremic syndrome (HUS).

- HUS is the most common cause of acute renal failure in children, and *E. coli* 0157:H7 is the strain that is most often responsible.

- Outbreaks are associated with undercooked or raw meat.

Shigellosis (Bacillary dysentery)

- characterized by fever, bloody diarrhea, and fecal leukocytosis.
- caused by any of four *Shigella* species: *S*. *dysentriae*, *S*. *filexneri, S. boydii* and *S*. *sonnei*.
- *S. dysentriae* causes a more severe disease than the others.
- Shigellosis can be initiated with a low infectious dose (10 to 100 bacteria).
- **Transmission**: person-to-person with poor personal hygiene. Contaminated foods are also vehicles.
- The bacteria are extremely sensitive to dry environments and will not survive on many dry foods.

- Shigella infections range from asymptomatic cases to life-threatening dysentery.

- Shigella multiplies in the epithelium of the colon, where it produces & releases endotoxin that triggers inflammation and local damage.
- The toxin is absorbed into blood stream & causes fever.
- *Shigella* also produces an exotoxin that causes diarrhea.

- *Shigella* can be isolated from fecal specimens or from rectal swabs on selective, differential, or enrichment media that discourage overgrowth by normal flora.

- Treatment is usually limited to supportive therapy and the replacement of fluids and electrolytes, and the disease is allowed to run its course.
- If severe, usually ampicillin or tetracycline is prescribed. Recovery confers serotype specific immunity.

Salmonellosis

- There are more than 2000 serovars of *Salmonella* that cause gastroenteritis in humans. These fall into 3 species.
  - *Salmonella typhi* (agent of typhoid fever),
  - *Salmonella paratyphi* (agent of paratyphoid fever),
  - *Salmonella enteriditis* (causes gastroenteritis of short duration).

- These bacteria are shed from animals, and less frequently humans.

- Foods obtained from animal sources (eggs, poultry, milk and sausage) pose high risk for *Salmonella* contamination.

- The bacterium invades the mucosa of the large and small intestines, and produces enterotoxin & cytotoxin causing inflammation, moderate fever, vomiting, diarrhea, abdominal pain, chills and nausea.

- The symptoms usually appear 6 to 36 hours after infection.

- Most patients recover in 2 to 4 days. In infants, the elderly and immunocompressed persons, the infection is often fatal.

- The average mortality rate is 4%, but higher (15%) in old age.
- The bacterium invades the bloodstream & spreads to other body sites.

- The infectious dose is 10^7-10^9 cells/gram of contaminated food.

- The leading causes for outbreaks are
  - improper cooling of cooked food,
  - lapse of a day or more after cooking,
  - inadequate cooking or heating,
  - ingestion of contaminated raw foods and
  - cross contamination

Vibrio parahaemolyticus gastroenteritis

- *V. parahaemolyticus* is a halophile most commonly found in marine environments.
- It causes gastroenteritis following ingestion of contaminated seafood.
- The disease is associated with eating contaminated raw or inadequately cooked seafood (oysters, shrimp, crabs, and fish).
- Symptoms are similar to those foods borne illness caused by *Salmonella*, *Shigella* and enteroinvasive *E. coli.*
- Special media containing increased concentration of NaCl is used for identification.

- Preventive measures:
  - adequately cooking the sea food,
  - storing seafoods at low temperatures (the pathogens rapidly multiply to infections concentrations between 20 & 35^{o}C), and
  - avoiding the cross-contamination of cooked food by raw seafood.

Yersiniosis

- This is a gastroenteritis caused by *Yersinia enterocolytica* following ingestion of feces-contaminated food or water or by direct contact with contaminated feces.

- The bacterium is usually isolated from cakes, pan cakes, vegetables and milk.

- It produces **heat-stable enterotoxin** (stands 100^{o}C for 20 minutes) that causes diarrhea or severe abdominal pain (or both), usually accompanied by a fever.

- High incidence occurs in young and olds.

Campylobacter gastroenteritis

- It is caused by *Campylobacter jejuni* .
- Transmitted to humans by ingestion of contaminated food, raw milk, or water.
- Poultry is the major source followed by raw milk.
- *Campylobacter jejuni* is the single common cause of acute diarrhea in man.
- It causes abortion in cattle & sheep.
- It can spread to people by direct contact with fecal material from infected pets, including cats & dogs.
- Gastrointestinal symptoms usually diarrhea (often with blood) begin 3 to 5 days after infection.

- Although most infections are self-limiting, relapses occur in 20% of cases.

- *C. jejuni* is fastidious & must be grown in special media in a microaerophilic environment.

- Growth is optimal at 42^{o}C.

- Severe infections are treated with fluid & electrolyte replacement therapy and with erythromycin.

Listerosis

- Listerosis is caused by *Listeria monocytogenes.*
- The bacterium is primarily an opportunistic pathogen of pregnant women and their fetuses and immunocompromised individuals.

- Infection occurs following consumption of foods, particularly from vegetables, meat, or milk (soft cheeses), that have been contaminated with the organism.

- Because the pathogen grows at refrigeration temperatures, infectious doses can be generated if lightly contaminated foods are stored for prolonged periods.

- *Listeria* lives within human macrophages.

- Listerosis is usually an asymptomatic infection in healthy adults, but in immunocompromised individuals or cancer patients it may cause meningitis.

- Infection in pregnant women usually leads to infections of the fetus, resulting in spontaneous abortion, stillbirth, or infection of the newborn.

- Pregnant women, the young and old, and immunocompromised individuals are especially vulnerable to *L. monocytogenes* infections.

Review Questions

1. What does food microbiology study?
2. What effects do microbes bring if they contaminate food?
3. What is the source (origin) of microbes that contaminate foods?
4. List intrinsic & extrinsic factors that control the growth of microbes in foods
5. List 10 fermented foods and their respective starting materials. What are the main microbes that fermented them?
6. Describe how cheese is produced
7. What is food spoilage? What causes it?
8. Distinguish between perishable and Non-perishable foods.
9. Which microbes cause the spoilage of vegetables, fruits, meat, eggs, grains, bread, injera and sugary foods?

10. Describe the major approaches used in food preservation.

11. What types of chemicals can be used to preserve foods?

12. Nitrite is often used to improve the storage characteristics of prepared meats. What toxicological problems may result from the use of this chemical?

13. Under what conditions can ultraviolet light and gamma radiation be used to control microbial populations in foods and in food preparation? What is radappertization?

14. In principle, how do bacteriocins such as nisin function? What bacterial genus produces this important polypeptide?