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Theoretical Food Microbiology

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المرحلة الرابعة – الدراساتين الصباحية والمسائية
الفصل الدراسي الاول

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Lec (1): The relationship between food and microorganisms and the new branches of food microbiology

One of the most important discoveries in history of biology occurred in 1665 with the help of simple microscope. **Antony Van Leeuwenhock** discovered the world of microorganisms; he discovered the smallest structure units called them: **Animalicules**. He observed them in the food residues obtained from his teeth, in juice and vinegar and in rain drops. This was the first report about the relationship between food and microorganisms. After Leeuwenhock discovery, the scientific community of that time became interested in the origins of the tiny living things until the second half of nineteenth century. Many scientists and philosophers believed that some forms of life could arise spontaneously from non-living matters and they called this process: **Spontaneous generation theory (SGT)**. They believed that snakes and mice could be born from moist soil; flies from manure, maggots (fly larvae), worms, m-ms from spoiled meat and food. In order to refute such theory, **Francisco Redi** filled three flasks with meat, one left open, the second sealed, and the third covered with a piece of gauze. Maggots appeared only in opened flask after flies entered the flask and laid their eggs, but the other flasks showed no maggots. However a putrid flavour appeared in the sealed flask as a result of meat putrefaction by anaerobic bacteria (food spoilage process).



In order to protect food from microbial spoilage, **Spalanzani** suggested heating the food extract to kill food m-ms (food preservation process).

English scientist **Tyndall** didn't agree with Spalanzani, because the heat didn't kill all food m-ms, because the raw food usually contaminated with two groups of m-ms (Food contamination): A) Heat-labile vegetative cells B) Heat-resistant bacterial spores.

In order to overcome such problem Tyndall suggested the discontinuous heating (Tyndallization) of food, in which the food heated three successive days every short period of time in order to promote the germination of bacterial spores which resist the heat on first or second day and converted to vegetative cells on third day to be sensitive to the heat. After tyndallization, the European scientists used oil baths, salt solution and steam pressure (autoclave) in order to increase the temperature above water-boiling point to kill heat-resistant bacterial spores (Food sterilization).

1795-Appert discovered **food preservation by canning** by placing meat in glass bottles and boiled it.

In spite of all these experiments, the deep scientific works on food and industrial microbiology was made by the French chemist **Louis Pasteur 1857-1887**

The main works of Pasteur in this field are:

1- Pasteur demonstrated that m-ms are present in **air** and can contaminate the food, to prove this Pasteur filled several short-necked flasks with beef extract and then boiled their contents; some were left open for few days. These open flasks were found to be contaminated with microbes. The others sealed after boiling were free of microbes.

2- Pasteur placed the meat broth in flask and bents the neck into S-shape curve (**Swan-neck flask**) and closed its opening with a cotton plug in the lowest point of neck. The contents of this flask were boiled, the meat content didn't decay even after months, and he concluded that the

cotton plug trapped air-born m-ms to protect food from microbial spoilage.

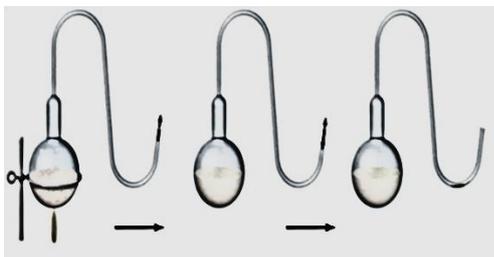
3- Pasteur made a heavy works on food spoilage and on fermentation, he stated that **raw food material contain many m-ms** causing the spoilage of food product produced from this raw material. He found in the French wine rod-shaped m-ms in addition to the oval-shaped yeast used for production the product. So rod-lactobacilli contaminating the grape produced lactic acid instead of alcohol and spoiled the wine.

4- To kill rod bacteria causing spoilage of wine, Pasteur suggested the **Pasteurization** of the product.

5- Heavy works on all types of fermentations, Pasteur proposed a (**Germ theory of fermentation**) He proved that for each fermentation there is a special m-m cause it.

6- Based on the study of butyric-acid fermentation caused by anaerobic *Clostridium butyricum*, Pasteur made his famous historical statement for first time: **Life without oxygen.**

7- In addition to his works in food and industrial microbiology, Pasteur strongly proved that: no living things created from non-living matter and the spontaneous generation theory is wrong.



Parallel with Pasteur's works, other groups of scientists made an emphasis on the role of food m-ms in the **food-borne diseases** and **food poisonings**, while third group focused on the **beneficial face** of food m-ms and used these m-ms as pure bacterial starters to produce improved foods. Storch in 1885 used for first time a pure starter to

Lec2: Food-borne diseases

For centuries and till now the food is playing an important role in the distribution of many dangerous human diseases. According to the World Health Organization (WHO) reports about half of human microbial diseases in the under-development countries are due to the contamination of food and water with pathogenic microorganisms (m-ms) According to health risk the food could be divided into two groups:

1- High-risk foods: are those foods that are most likely to be the vehicles of the food-poisoning and disease-causing pathogens under favorable conditions support the multiplication of pathogenic bacteria such as:

A) All cooked meat, poultry, fish, eggs and their products.

B) All foods which do not exposed to food treatments.

C) Ready to eat foods (fast foods).

2- Low-risk foods: such as preserved foods (Sterilization, canning, drying etc.), acid foods such as vinegar, high acid juices $\text{pH} < 4$. Foods with high sugar, salt, fat content. Generally the most common diseases caused by contaminated food are shown in the following table:

Viruses in human foods

Foods may serve as vehicles for transmission of viral diseases such as infectious hepatitis. Enteric viruses also may be transmitted via contaminated food. It is clear that epidemiologists have paid a little

attention to food-borne viruses because such studies are expensive and complicated, so the studies on food-borne viruses still very poor.

Viruses especially those of human origin, have been found in many types of foods including sea foods, milk, eggs, meats and vegetables; some of these foodstuffs are eaten raw (oyster, fish, eggs & vegetables) or treated by methods not enough to inactivate or destroy the food viruses. Foodstuffs may become contaminated with viruses by two ways:

1) Primary Contamination: some human viruses have been found in animals which then products of this animal may act as a vehicle for transmission to the human.

2) Secondary Contamination: This occurs after the time of slaughtering or harvesting. The main source of secondary contamination is via food handlers during food preparation or processing. Contamination may also occur through polluted wastewater effluent used for food crop irrigation, surfaces, flies, cockroaches and rodents. In many cases poliomyelitis and hepatitis viruses were isolated from ground beef, raw milk, raw hamburger, fish, poultry, vegetables and fast meals and almost the source is food handler and insects. Viruses may survive for weeks in flies fed with feces of poliomyelitis patients. Cockroaches may also serve as potential vector for enteric viruses.

Parasites in human foods

Foods are unsuitable for growth and multiplication of parasites. However food served as vehicles to transmit the parasites from human or animals to human. Food may be contaminated with parasites in farms of production, in food plants, in food stores, in markets, in kitchens of public restaurants or house kitchen etc.

Food handlers, domestic cats and dogs, flies and cockroaches play the important role in the contamination of food and prepared meals with many parasitic protozoa and worms such as:

1) *Entamoeba*: The main source of food contamination is the feces of patients and carriers of this parasite, particularly villagers in the farm and food handlers in the restaurants.

Three species were isolated from food: *Entamoeba coli*, *E. nana* and *E. histolytica*; the latter is the most common species contaminated our foods, causing amoebic dysentery. This parasite transmitted from feces of food handlers by unclean hands, flies, cockroaches and rodents. This parasite in the cyst stage can survive in the food for long time resist the washing, disinfection, low-temperature treatment. Only high-temperature treatment of food destroys this parasite and its cyst.

2) *Giardia lamblia*: World-wide distribution parasite. The carriers of this parasite in U.S.A and Europe form more than 20% of population; for this reason the new standards of water and food included detection of this parasite. Food handlers are the main source of food contamination through feces which transmitted by insects and rodents.

3) *Toxoplasma gondii*: This parasite causes toxoplasmosis in human, the main reservoirs of this parasite are dogs and cats; through contaminating the food, meals, kitchen tables and utensils, directly by their feces or via flies and cockroaches. Cooking at 70°C. is fair enough to destroy this parasite, but our problem is the contamination of open prepared meals in our restaurants and houses with this parasite.

Therapeutic Foods and Probiotics

We daily swallow thousands beneficial m-ms with our meals with raw vegetables and fruits with bakery products, dairy products, fermented foods, etc. These m-ms contribute in the improvement of our health. According to this fact, at present time some therapeutic foods supplied with living m-ms are produced commercially as probiotics. Probiotic is described as a "Live microbial food" or human foods supplied with living

m-ms that are beneficial to the human health. In some countries such as Japan, probiotics are used instead of antibiotics in all sanatoriums and aged-people houses to control human diseases.

The main beneficial impacts of probiotics are:

- 1) Inhibition of intestinal pathogens
- 2) Anti-constipation agent
- 3) Synthesizing many vitamins such as folic acid, niacin, riboflavin, vitamin B6 & B12.
- 4) Stimulation of immune response.
- 5) Decreasing the serum cholesterol.
- 6) Reduction of blood pressure in hypertensive.

The main m-ms used for probiotic production are:

- 1) Bifidobacterium spp. B. bifidum, B. lactis, B. infantis
- 2) Lactobacillus spp. L. lactis, L. bulgaris, L. acidophilus, L. plantarum.
- 3) Enterococcus faecium
- 4) Streptococcus thermophilus
- 5) Saccharomyces cerevisiae and S. boulardii

Lec 3: Microbial Contamination of Food

The raw food usually produced, contaminated with microorganisms (m-ms), because the plants and animals from which the raw food is produced grow in natural environments in contact with soil, water rich in m-ms in addition to the microbial flora of plant and animal from which produced, which are considered as: natural sources of food contamination. Foods also get heavy contamination during handling and processing.

I- Natural Sources of Food Contamination:

1- The Water: The water is in continuous contact with foods from the field or farm of production to the table of food in the restaurants and houses (FFTT) as a: a) Irrigation Water b) Cleaning Water c) Processing Water d) Cooling Water.

2- The Soil, Manure and Sewage: The soil is rich in microbial spores and continuously contaminate the raw food with bacterial and fungal spores, particularly heat-resistant spores that resist the heat treatment of food. Sewage and animal manure used as fertilizers contaminate the food with very dangerous bacteria, viruses and parasites.

3- The animals and plants: The plants particularly leafy vegetables loaded with m-ms because in contact with sources of contamination such as: soil, sewage, water, manure, rodents etc. These m-ms reach the food produced from raw plants.

4- The air: The air of open production field usually poor in m-ms and not suitable for growth of m-ms

However the air of closed food plants, restaurants, food stores; loaded with m-ms and may be served as a source of contamination of food and

4th Biology 2021-2022 Food Microbiology Dr. Marwa H. Alkhafaji & Dr. Alyaa R. Hussein prepared meals. In such case it is necessary to disinfect the air of closed buildings by bactericidal chemicals or bactericidal U.V. lamps and using special cooling-system and other sanitary particles (replace the oxygen by nitrogen in food stores).

II- The food contamination during handling and processing

In addition to the natural sources of contamination, the food expose to the heavy contamination during a long way from the field of production to the table of eating (FFTT)

The most important stage of these four stages is the stage of food processing in the food plant for this reason a strict constructions and rules of food plant sanitation was issued for long time.

Food Plant Sanitation

Food exposed to all sources of contamination in the food plant: air, water, soil, sewage, rodents, insects, food handlers, surfaces, equipments, containers, etc. so plant manager hold a big responsibility for production a food with following characteristics:

1) Clean food 2) Fresh food 3) Pure food 4) Safe food 5) Food with normal character 6) Food with high nutritive value.

In order to achieve these goals, there are many quality control section established in each plant:

- ✘ 1 Pest control section
- ✘ 2 Chemicals control section
- ✘ 3 Cleaning and Sanitizing control section
- ✘ 4 Microbiological control section
- ✘ 5 Food handlers, employees, workers health section

Microbiology - Quality control in food plant

Microbiological section of quality control responsible for microbiological examinations of the following items:

I) Sites and materials of food plant

1- Microbiology of processing water and drinking water

In addition to pathogenic m-ms it is necessary to examine the iron and manganese bacteria, slime producing bacteria and other undesirable bacteria.

Chemical composition also needed particularly the hardness of water, iron & manganese content and excessive organic matter.

- 2) Microbiology of products: Raw materials, ingredients, additives, end product, packaging materials, etc.
- 3) Microbiology of sewage and waste-treatment units.
- 4) Microbiology of food plant and equipments: Floor, ceiling, walls, windows, doors, sinks, air, processing surfaces and equipments ...etc.
- 5) Microbiology of food stores, cartons, bags, containers, cars, trucks ...etc.

II) Microbiological examinations of food handlers, workers, employees. Food handlers are very dangerous source of food contamination especially in our country; because they are closely contact with the food from the field of production to the fork of consumer (FFF). **The main microbiological tests in food plant**

The tests are determined according to the food type from this list of tests:

- 1) TBC : Total Bacterial Count
- 2) TYMC: Total Yeast Mold Count

- 3) TPBC: Total Psychrophilic Bacterial Count
- 4) TTBC: Total Thermophilic Bacterial Count
- 5) TSC : Total Spore Count
- 6) TCC: Total Coliform Count and Fecal E. coli
- 7) Enterococci
- 8) Clostridium perfringens
- 9) Test for Pathogenic Bacteria
- 10) Test for Parasites
- 11) Test for Viruses
- 12) Test for Bacterial Toxins
- 13) Test for Mycotoxins

Food Adulteration

The famous world-wide organization Food and Drug Administration (FDA) considered the food product in food plants as (Adulterated food) in the one of the following cases:

- 1) If the food contains: Filthy, Putrid, or decomposed substances.
- 2) If the food contains: Poisonous or unsafe chemicals & food additives
- 3) If food has been prepared, handled or packed under unsanitary conditions whereby become contaminated with m-ms. and chemicals.
- 4) If food is a product of diseased animal or an animal was died not by slaughtering.
- 5) If food container is composed of poisonous substances.

Lec (4): **Microbial Spoilage of Food**

Microbial spoilage is any organoleptic change in food makes it unacceptable by consumer. The change usually occurred in the flavor, color, odor, taste and texture.

In spite, the differences between the signs of spoilage according to the type of food, there are three main signs of microbial spoilage are common in most foods:

- I) **Color change**: Many colors may be appeared on the surface of the spoiled food as a diffusible exopigments or colored colonies with endopigments. This food discoloration caused by bacteria, yeasts and molds.

1- Bacterial Discoloration

2) Yeast Discoloration

A) White-Pale colonies of non-pigmented yeasts ex. Saccharomyces cerevisiae.

B) Red-Pink colonies of pigmented yeasts ex. Rhodotorula.

3) Mold Discoloration

Wide range of water-soluble and insoluble pigments and color mold colonies on food surfaces.

- II) **Sliminess**: Activity of m-ms in the food may be lead to form a slime on the food surface

- III) **Changes in flavours and odors**:

The normal acceptable flavor of food may be changed to non-acceptable flavor in spoiled food; the change is occurred by two mechanisms:

- 1- Microbial destruction of natural flavor compounds of food; as in the destruction of natural flavor of butter di-acetyl to off-flavor acetyl-methyl carbinol by Pseudomonas fragi, or destruction of natural fruit organic acid to unacceptable alcohol, ethers, ketones, aldehydes.
- 2- Microbial production of unacceptable off-flavors and off-odor in the food as in the following examples:

Indicator Bacteria of Food Contamination & Microbiological Standards of Food

We should examine the food and food plants for contamination indicators for the following purposes:

- 1) To detect the food is healthy or not healthy for human consumption.
- 2) For assessment the sanitary practices of food plant.
- 3) The official standards are meeting the produced food. (For compatibility with official standards and sanitary constructions).

Routine tests for selected food pathogens or toxins such as salmonella or staphylococcal enterotoxins are necessary whenever epidemiological outbreaks are occurred.

- 1) In most cases such as infectious hepatitis, detecting methods for causative agent are not available to food microbiologists.

- 2) For other food-borne infections such as shigellosis, the methods may be unreliable, especially when the pathogens are distributed in food heavily contaminated with other m-ms.
- 3) In addition tests for pathogens directly are very dangerous for laboratory workers.

For all these reasons and difficulties all microbiological labs firstly test species which are more readily cultivated, enumerated, not dangerous to the workers and environment, normal inhabitants of human and animal intestine which indicate the exposure of food to wastes and conditions containing pathogenic m-ms and their toxic products. These species so used are called (Indicator Bacteria).

Such indicators also help in assessing the quality of food and the presence of dirty particles such as insects parts, hairs, rodents excretion.. etc.

Microbiological Standards of Food

The main purposes of microbiological standards of food are to give assurance that:

- 1) The food will be acceptable from the public health standpoint.
- 2) The food will be acceptable from the nutritive value and normal characteristics.
- 3) The food will be acceptable from an esthetic viewpoint. (Not filthy with fecal materials, parts of vermin, pus cells, mycelia...)
- 4) The food will have keeping quality that should be expected.

In order to issue food microbiological standards it is important to take into consideration the following principles. (1,2,3,4 example1) (5example2).

- 1- Determination the higher limit of m-ms permitted in each food.

- 2- Determination the types and higher limit for each type of m-ms in each food.
- 3- All types of food must be free of any pathogenic and toxigenic m-ms (in some cases except staphylococci).
- 4- For the same food may be more than one standard according to the state of food (Fresh, canned, minced, dried, frozen..etc.).
- 5- In some cases as in the milk, the food divided into many grades: A, B, C) according to the microbial content, and a standard for each grade.
- 6- The standards may be not identical for all countries, all town; because different conditions between these countries as follow:
 - A- Level of food productivity and available foods.
 - B- Economical situation
 - C- Sanitary situation
 - D- Nutrition habits
 - E- Emergencies outbreaks such as war and catastrophes.

There are many organizations responsible for microbiological standards of foods such as:

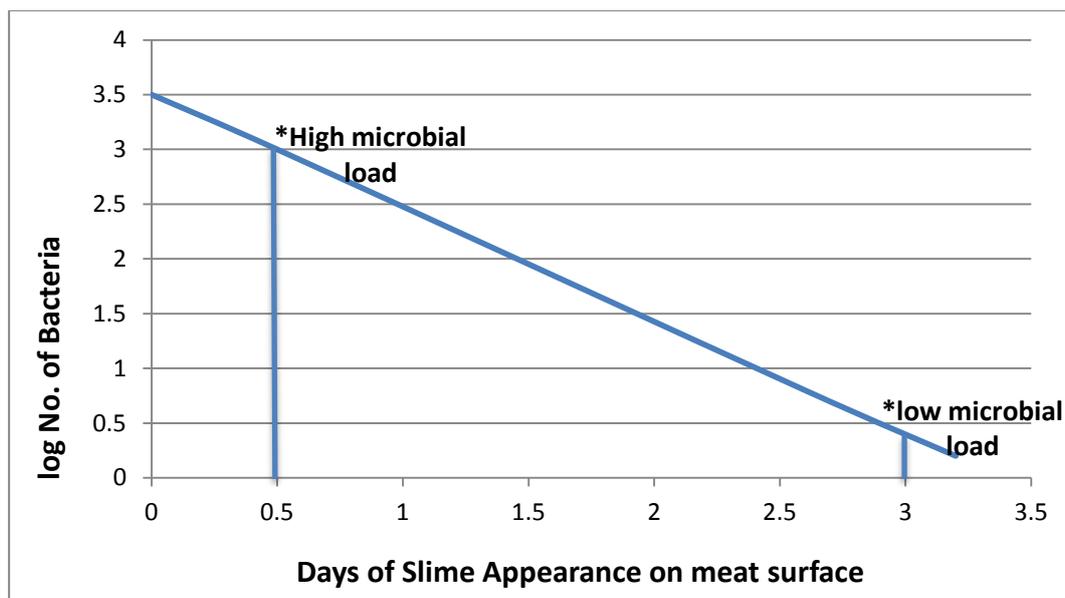
- 1) WHO: World Health Organization - U. N.
- 2) FAO : Food Agriculture Organization - U. N.
- 3) CAC : Codex Alimentarius commission
- 4) FDA : Food & Drug Administration
- 5) APHA: American Public Health Association
- 6) EPA : Environmental Protection Agency
- 7) EOF : European Organization of Food
- 8) COSQC: Central Organization for Standardization and Quality Control. (Local organization).

Lec (5) Intrinsic & Extrinsic Factors Affecting Microbial Spoilage of Food

There are many factors related to the food itself (**Intrinsic**) and factors surrounding the food (**Extrinsic**) strongly affect the type and time of food spoilage, some of these factors are:

I- The number and kind m-ms in food

Generally when the number of m-ms on food surface is high; the spoilage will be occurred faster and vice versa, for example slime appeared on the meat surface with high load faster than on the meat with low number of bacteria. This is not always true, because in spite of a high number of m-ms there is no microbial group or type which can act on a food with a special chemical composition as in the proteolytic group in protein food and the lipolytic group in fatty food.



II- Chemical composition of food

M-ms need simple sources of carbon, nitrogen and energy for growth and activity, so food with simple chemical composition and simple sources of carbon such as glucose and nitrogen as amino acids spoiled faster than complex food. According to the main chemical constituent, the foods are divided into three big groups:

1- Protein Foods:

The main spoilage in this food is: Putrefaction which occurred as follows:

Protein food $\xrightarrow{\text{True proteolytic m.o}}$ **Polypeptides** $\xrightarrow{\text{Proteolytic m.o}}$ **Amino acids**

Organic acid, Amines, Gases, Putrid compounds

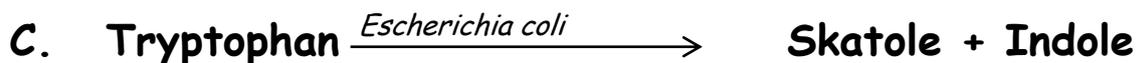
amino acid break down

The main pathways of amino acid break down which lead to putrid compounds production:

A. Deamination



B. Decarboxylation



2- Sugar Foods

The common spoilage is fermentation. The type of fermentation is determined according to the sugar content:

1- **Low sugar food (Meat)** $\xrightarrow{\text{Bacterial oxidation}}$ **Organic acid+ Gases**

2- **Medium sugar food (Milk)** $\xrightarrow{\text{Organic acid fermentation}}$ **Lactic A+Org. A.+Gases**

3- **High sugar food (Juice)** $\xrightarrow{\text{Alcoholic fermentation by yeast}}$ **Alcohols**

4- **Very high sugar food (Honey)** $\xrightarrow{\text{very slow alcoholic fermentation}}$ **Little amount of Alcohol (Yeasty honey)**
by osmophilic yeast

3- Fatty Foods

The main spoilage is: Rancidity; there are two main types of rancidity:

- 1) Oxidative Chemical Rancidity
- 2) Microbial Hydrolytic Rancidity

III- Food pH:

Most foods are with neutral pH suitable to activity of most of bacteria and spoiled faster than some foods with low or high pH. Food pH strongly affects the type of m-ms growing in that food and consequently the type of spoilage as shown in the following table:

IV- Food Moisture:

Water is necessary to the growth of bacteria and to the enzymatic activity in the food, particularly the food free water which could be expressed as Water activity a_w ($a_w = P_1/P_2$) P_1 : vapor pressure of solution, P_2 : vapor pressure of solvent (usually in food is water).

Food with a_w more than 0.90 is spoiled by bacteria while less than 0.90 spoiled by mold and yeasts.

V- Other factors less important such as:

- 1- Aerobic & anaerobic conditions.
- 2- Biological structure of food particularly the surface of food which protects the entrance of m-ms inside the food.
- 3- The presence of antimicrobial substances in the food.

4- Some food treatments that stimulate the microbial growth, for example:

- A- Washing with raw water without drying or disinfectants.
- B- Non-efficient heating that promotes the spore germination, not killing them.
- C- Mincing or slicing the food: such treatment lead to promote the microbial growth in the minced food because:
 - 1) Increasing the food surfaces for microbial growth.
 - 2) Increasing the oxygen content inside of food.
 - 3) Outflow the food juices rich in nutrient from the inner tissues to form optimal medium for microbial growth.
 - 4) Increasing the sources of contamination from mincing and slicing machines; rest food in these machines forms permanent sources of contamination, knife working surfaces, etc..

Investigation and inspection of food disease outbreaks

Gastro-intestinal disturbance resulting from the ingestion of food (Food illness) have a variety of causes:

- 1- Overeating 2- Allergies 3- Nutritional Deficiencies 4-Toxic Plants
- 5- Toxic Animals 6- Toxic Chemicals 7- Bacterial Intoxication
- 8- Bacterial Infection 9- Food Mycotoxins 10- Fungal Infections
- 11- Food Viruses 12- Food Parasites 13- Unknown Causes.

However for long time, thousands researches proved that m-ms are the main cause of food illness.

For example the following table has been issued by U.S. disease center:

Disease agent	Percentage %
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A- Microbial agents	65
1- <u>Staphylococcus aureus</u>	25
2- <u>Clostridium perfringens</u>	15
3- <u>Clostridium botulinum</u>	6
4- <u>Salmonella</u>	10
5- Other Bacteria	3
6- Viruses& Parasites	6
B- Chemicals & Unknown	35

The most important food microbial diseases are:

food microbial disease	Causative agent
Staphylococcal Food Poisoning (SFP)	<u>Staphylococcus aureus</u>
Botulism	<u>Clostridium botulinum</u>
Clostridium perfringens F. P.	<u>Clostridium perfringens</u>
Salmonellosis A- Typhoid fever (human) B- Paratyphoid fever(zoonotic)	<u>Salmonella</u> sp.
Shigellosis, Bacillary dysenteriae	<u>Shigella</u> sp.
Food poisoning	Enterotoxigenic <u>E. coli</u>
Enterocolitis	<u>Yersinia enterocolitica</u>
Food poisoning	<u>Vibrio parahaemolyticus</u>
Food poisoning	<u>Bacillus cereus</u>
Food poisoning	<u>Entamoeba histolytica</u> , <u>Cryptosporidium</u> , <u>Giardia</u>
Chemicals, toxic plants, toxic animals	Arsenic , <u>Vicia faba</u> , Shark

The main purposes of the investigation of an outbreak of food born disease are to determine:

- 1- How the foodstuff became contaminated?
- 2- How the growth of toxigenic or infectious m-ms could take place.
- 3- How can we prevent the repetition of this case?

This requires:

- 1) Location of the outbreak
- 2) Identification of the causative agents
- 3) Route of transmission
- 4) Demonstration of the opportunity for growth of pathogen
- 5) Proof that pathogen has infected the publicity given an outbreak.

The investigation team includes:

1- Field group:

- A- Persons consumed suspected food, physicians and nurses who are treating the victims.
- B- Collecting samples of suspected food, specimens from food handlers and patients and transmitting them to lab.
- C- Inspects the site where the food was stored, prepared, served and sold.

2- Lab. group: Microbiological and chemical tests.

- 3- The person in charge who can explain the data from all sources to determine the cause and the source of disease outbreak.

Collection the Food Inspection

1- Collection of food samples

2- Collection of specimens from human sources

- a- Fecal samples b- Blood samples c- Vomit

3- Laboratory testing:

A- Test for botulism

B- Test for Staphylococcal food poisoning

C- Test for Salmonella and Shigella

D- Test for C. perfringens & B. cereus

E- Test for Mycotoxins

F- Test for Parasites: Entamoeba histolytica, Cryptosporidium, Giardia lamblia

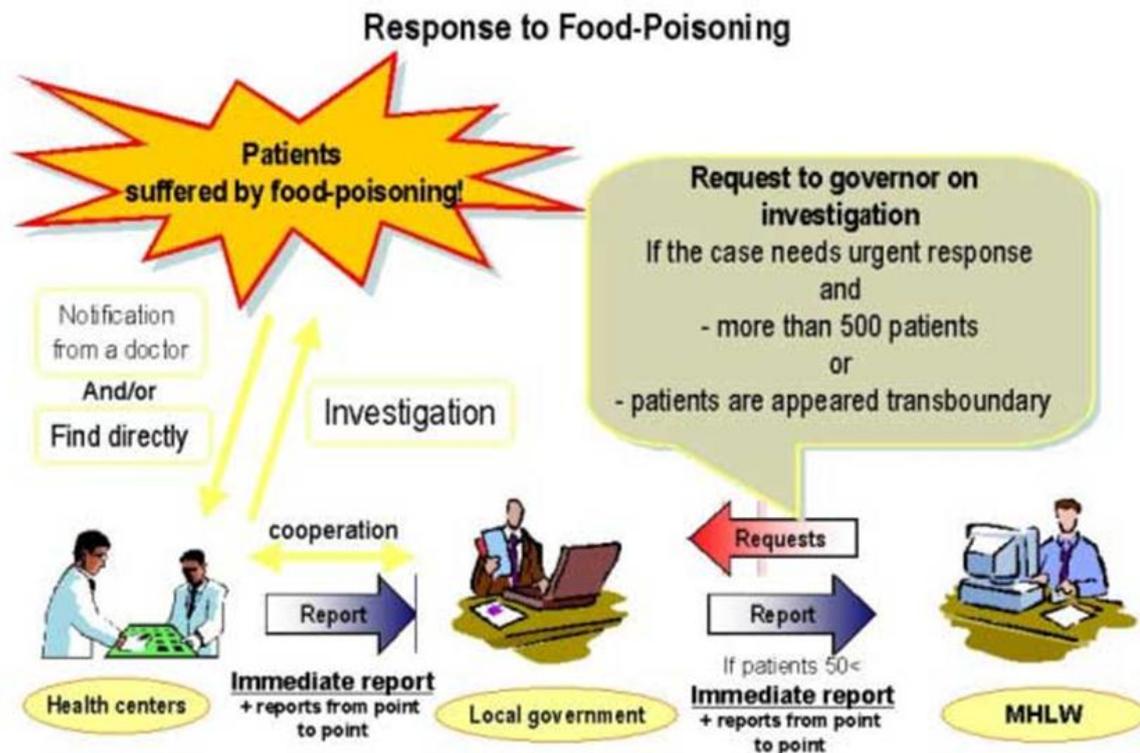
G- Test for viruses

H- Test for toxic plants & animals

I- Test for pesticides and heavy metals.

The practices employed to protect food from dangerous m-ms:

- 1- Keep food free from contamination with pathogens and food handlers and other sources.
- 2- Prevention the microbial growth in food
- 3- Rejection suspected food
- 4- Educate the public about the prevention of food borne illness.

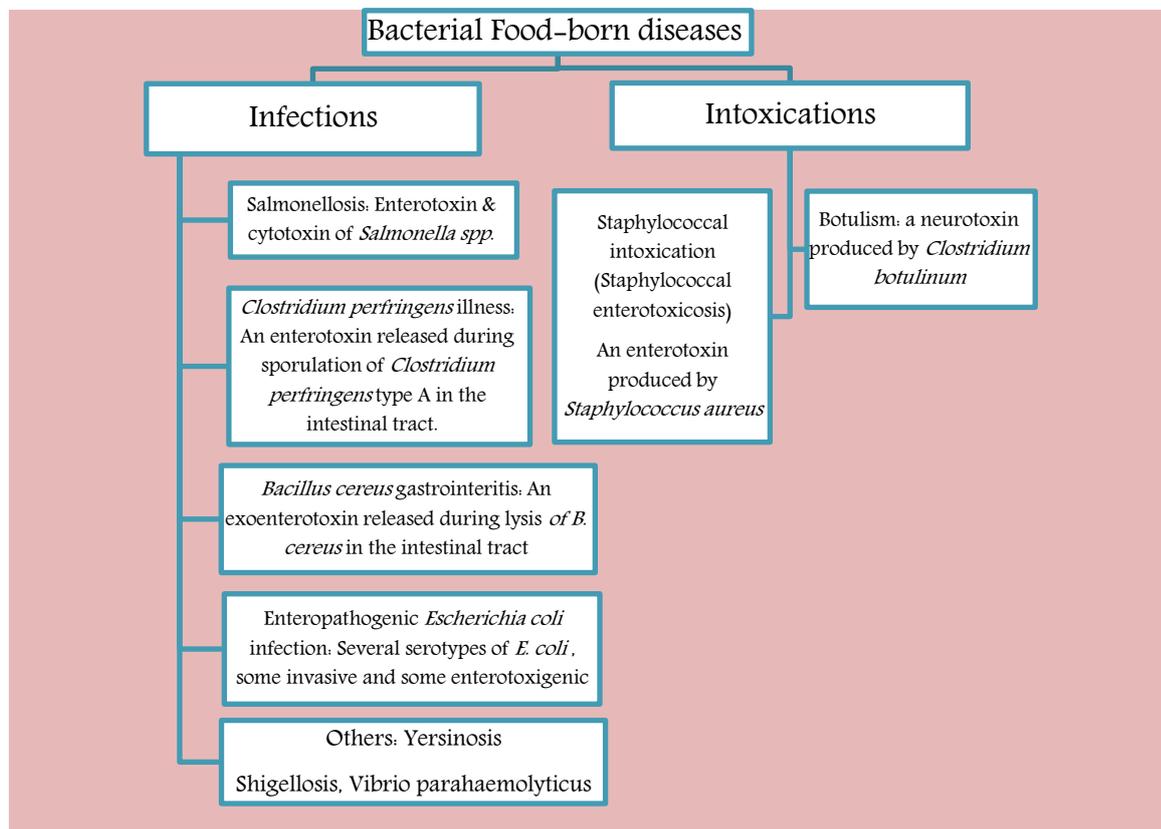


Lec (6) Food-borne Infections and Intoxications

Food-borne Diseases: have a variety of causes, e.g. overeating, allergies and nutritional deficiencies; actual poisoning by chemicals, toxic plants or animals; toxins produced by bacteria; infestation by animal parasites and infection by microorganisms.

FBD is an acute illnesses associated with the recent consumption of food.

These illnesses often are grouped together because they may have similar symptoms.



1- **Botulism:** Caused by the presence of toxin produced by *Clostridium botulinum* in food.

2- **Staphylococcal intoxication:** Caused by a toxin produced by *Staphylococcus aureus* in food.

Staphylococcal Food Poisoning (SFP)

Staphylococcal Food Poisoning is an intoxication caused by the ingestion of an enterotoxin formed in food during growth of certain strains of *Staphylococcus aureus*. Most enterotoxin producing S. aureus cultures are coagulase-Positive (CoPS), produce a thermo-stable nuclease, however not all CoPS are necessarily enterotoxigenic. Some of the toxigenic cocci are very salt-tolerant (10-20% NaCl), they also are fairly tolerant to dissolved sugars (50-60% sucrose).

At least 10 of the coagulase-Negative Staphylococcus spp. (CoNS) produce enterotoxins, for example: S. epidermidis & S. haemolyticus.

Incidence in food

(milk and milk products including pasteurized milk, yoghurt, chocolate milk, fermented milk, cream filled pastries, poultry, fish, shellfish, meat and meat products, non meat salads, egg and egg products, vegetables and cereal products have been involved).

Temperature growth range

In general growth occurs in range 7-47 °C and enterotoxins are produced between 10 °C & 46 °C with the optimum between 40 °C & 45 °C. These minimum and maximum temperatures for growth and toxin production assume optimal conditions stable to the other parameters.

Effect of salts

Although S. aureus grows well in culture media without NaCl, it can grow well in 7-10% conc. of NaCl and some strains can grow in 20%. The maximum conc. that permit growth depends on other parameters such as temp., pH & aw.

Effect of pH & water activity (aw)

Regarding pH, *S. aureus* can grow over the range 4-9.8 but its optimum is the range 6-7. With respect to aw the staphylococci are unique in being able to grow at values lower than any other non halophilic bacteria. Growth has been demonstrated as low as **0.83** under otherwise ideal conditions, although 0.86 is the generally recognized minimum aw.

Symptoms of SFP include diarrhea, **vomiting**, and abdominal pain. They are usually not life threatening. Symptoms may appear rapidly, sometimes in as little as **30 minutes**. However, it typically takes up to **six hours** for symptoms to manifest. Most cases of SFP do not require treatment because the disease will pass on its own. Most people get over the food poisoning in about two days. According to the U.S. Food and Drug Administration (FDA), SFP related deaths are very rare. However, there is increased risk for this complication among the **elderly, small children,** and people with weakened immune systems (**Immunocompromized**).

The minimum quantity of an enterotoxin needed to cause illness in humans is about **20 ng**.

The repertoire of *S. aureus* SEs/SEls comprised 22 members, excluding molecular variants: (i) the classical SEA, SEB, SEC (with the SEC1, SEC2 and SEC3, SEC ovine and SEC bovine variants), SED and SEE, which were discovered in studies of *S. aureus* strains involved in SFP outbreaks, and classified in distinct serological types ; and (ii) the new types of SEs (SEG, SEH, SEI, SER, SES, SET) and SEls (SEIJ, SEIK, SEIL, SEIM, SEIN, SEIO, SEIP, SEIQ, SEIU, SEIU2, and SEIV). The toxic shock staphylococcal toxin (TSST-1), initially designated as SEF, lacks emetic activity

These SE are **proteins** have a remarkable ability to resist **heat** and **acid**, therefore, they may not be completely denatured by mild cooking of contaminated food, SE ability to induce **emesis** and gastroenteritis, they are resistant to inactivation by gastrointestinal proteases including

pepsin, trypsin, rennin and papain, thus they can easily outlast the bacteria that produce them.

Botulism

Botulism is an **intoxication** caused by ingestion of food containing the **neurotoxin** (botulinum toxin), which is produced by *Clostridium botulinum*, a Gram positive, anaerobic, spore forming rod with oval to cylindrical terminal spores. **Seven** antigenically distinct toxin types (A, B, C, D, E, F, G) have been identified. Types **A, B, E, and F** cause natural disease in humans. The vast majority of disease is caused by types A, B, and E; type F rarely occurs, Types C and D cause natural disease in birds, horses, and cattle. The **species** is further subdivided on the basis of **proteolytic activity**: the main strains of concern in foods are proteolytic types A, B, F and G, and non proteolytic type E as are some strains B and F.

Growth & Toxin production

Toxin production by *Clostridium botulinum* depends on the ability of the cells to **grow in a food and autolyze there**, for the types **A, B, E&F** toxins apparently are synthesized as large, comparatively **in-active** proteins which become **fully toxic** after some **hydrolysis**. Therefore the factors that influence spore germination, growth and hence toxic production are of special interest. These factors include the composition of the food especially its nutritive properties, moisture, pH, salt content, temperature and time of food storage.

The concentration of sodium chloride necessary to prevent growth & toxin production in food depend on the composition of the food and the temperature. A pH near neutrality favors *C. botulinum*, the minimal pH at which growth and toxin production will take place depends on the kind of food & the temp. A pH of 4.5 or lower will prevent toxin production in most foods.

The optimal temp. for toxin production & growth of the proteolytic strains is about 35°C while 26-28°C is usually given for the optimal temp. for the non proteolytic strains.

Salmonellosis

Salmonellosis is a **foodborne infection** caused by *Salmonella* bacteria. Most people infected with *Salmonella* develop **diarrhea, fever, vomiting, and abdominal cramps 12 to 72 hours** after infection. In most cases, the illness lasts four to seven days, and most people recover without treatment. In some cases, the diarrhea may be so severe that the patient becomes dangerously dehydrated and must be hospitalized.

Incriminated food: Poultry, pork, beef and fish (seafood), if the meat is prepared incorrectly or is infected with the bacteria after preparation. Infected eggs, egg products, and milk when not prepared, handled, or refrigerated properly tainted fruits and vegetables.

#Typhoid fever occurs when *Salmonella* bacteria enter the lymphatic system and cause a systemic form of salmonellosis. Endotoxins first act on the vascular and nervous apparatus, resulting in increased permeability and decreased tone of the vessels, upset thermal regulation, vomiting and diarrhea. Septic shock may also develop.

Paratyphoid fever is an enteric illness caused by one of the following three serotypes of *Salmonella enterica* subspecies: *S. Paratyphi A*, *S. Paratyphi B* and *S. Paratyphi C*. Like *S. Typhi* they are transmitted by means of contaminated water or food.

The paratyphoid fever bears similarities with typhoid fever, and the two are referred to by the common name enteric fever. The course of paratyphoid is more benign.

***Bacillus cereus* food borne illness**

- This is a food borne illness caused by consumption of enterotoxins produced by some strains of *Bacillus cereus*.
- The organism produces the following enterotoxins which are involved in a food borne intoxication

A- Two diarrhoeal enterotoxins:

B- **Emetic toxin**

- **Incriminated foods:** meat, eggs and dairy products, Cereal dishes e.g. rice, spice, mashed potatoes, herbs, vegetables, minced meat, cream and milk pudding have been involved in *B. cereus* poisoning.

***Clostridium perfringens* intoxication**

This is a food borne intoxication caused by *Clostridium perfringens* enterotoxin (CPE) produced in the gastrointestinal tract by enterotoxigenic strains of *C. perfringens*.

- The organism is found in the soil, dust, water, sewage marine sediments, decaying materials, intestinal tracts of humans and other animals.
- This organism is a spore-forming, anaerobic, gram positive bacillus. Food poisoning strains have a variety of origins including human and animal feces, abattoirs, sewage and flies.
- Spores produced by these organisms can resist boiling for 4 or more hours.
- If the spores are present as contaminants on raw meat they may resist boiling or steaming, and on slow cooling the spores will germinate into rapidly multiplying bacterial cells, which produce large amounts of toxin. *Clostridium* food borne intoxication is caused by the ingestion of food containing large numbers of

vegetative cells of enterotoxigenic *C. perfringens* type A and some type C and D strains.

- These cells multiply in the intestine and sporulate releasing *Clostridium perfringens* enterotoxin (CPE).
- Sometimes CPE may be pre-formed in food, and once the food is consumed, symptoms may occur within 1-2 hours. *Clostridium perfringens* enterotoxin (CPE) is synthesized during sporulation.
- CPE is **heat labile** (destroyed at 60°C for 10 min) and its activity is enhanced by trypsin.
- Note: The food poisoning strains are heat resistant and survive heating at 100°C for 1 hr).
- **Incriminated food:** The food involved are those that are prepared one day and served the next day. Foods that have been involved include red meats, chickens, fish, pork, fruits, vegetables, spices etc. The heating of such foods is inadequate to destroy heat resistant endospores, Upon cooling and warming the endospores germinate and grow. Cooking kills the vegetative cells of *Cl. perfringens* but activates surviving spores, which will germinate and multiply.
- Food poisoning occurs when the level reaches 10^7 - 10^8 cells/g of food,
- Growth is enhanced by anaerobic conditions achieved after removal of oxygen by cooking

Symptoms of Diseases: Symptoms appear 6-24 hours after ingestion of a large number of viable vegetative cells up to 5×10^8 /g food, but not after ingestion of spores. **Symptoms** include nausea, intestinal cramps, pronounced diarrhea, vomiting is rare and the illness takes a duration of 1-2 days.

Lec 7: Listeriosis

Listeriosis caused by **Listeria monocytogenes** which is a Gram-positive rod, non-spore-forming, motile with peritrichous flagella, facultatively anaerobic, grows at 0-45 °C (**psychrotrophic**).

Food requirements: the nutritional requirements of *Listeria* are typical of those for many other gram-positive bacteria. They grow well in many common media such as Brain Heart Infusion (BHI).

The bacteria are able to grow in the presence of 10% salt. **Listeria monocytogenes** is a special problem since it can survive adverse conditions. It is salt tolerant surviving concentrations as high as 30% for 100 days at 39.2 °F. *Listeria* is now recognized as an important food borne pathogen.

Symptoms: host **Influenza-like symptoms** such as fever, headache and occasionally gastrointestinal symptoms or symptoms may be so mild that they go unnoticed.

Listeriosis in humans is not characterized as a unique set of symptoms because the course of the disease depends on the state of the host. Non-pregnant healthy individuals who are not immunocompromised are highly resistant to infection by the bacteria. Immunocompromised humans such as pregnant women or the elderly are highly susceptible to virulent *Listeria*. Abortion, premature birth or stillbirth is often the consequence of listeriosis in pregnant females. When the newborn is infected at the time of delivery listeriosis symptoms typically are those of meningitis and they begin 1-4 weeks after birth. Following invasion of macrophages virulent strains of *Listeria* may then multiply, resulting in disruption of these cells and septicemia. At this time the organism has access to all parts of the body. Death is rare in healthy adults; however, the mortality rate may approximate 30% in the immunocompromised, newborn or very young.

Mode of transmission and associated foods: Foods involved include raw milk, soft cheese, meat-based paste, fresh and frozen meat, poultry and sea food products and raw fruit and vegetables.

Reservoir/source: the *Listeria* bacteria are widely distributed in nature and can be found in decaying vegetables and in soils, animal feces, sewage, water, silage and faeces of numerous wild and domestic animals. Other sources may be infected animals and people.

Other bacteria that cause food poisoning (less common bacteria)

1. Enteropathogenic *E.coli*:

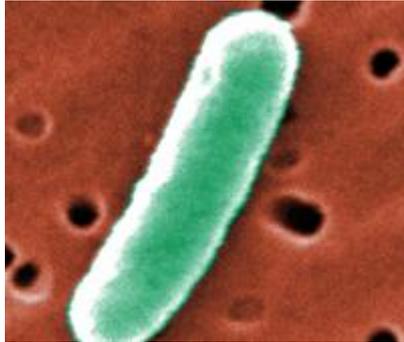
A- ETEC (which cause syndroms like cholera)

B- EHEC.O157H:7

2. *Shigella*-Endotoxins: (shiga toxins).
3. *Yersinia enterocolitica* (Endotoxins) causes Yersiniosis.
4. *Vibrio parahaemolyticus* . (Causes Gastroenteritis).
5. *Campylobacter jejuni* . (Causes Campylobacteriosis).

1-A Enterotoxigenic *Escherichia coli* (ETEC)

Enterotoxigenic *Escherichia coli* (ETEC) are bacteria that cause disease in humans and domestic animals by producing a **heat labile** or **heat stable toxin** or both.



Photomicrograph of Enterotoxigenic *E. coli*

Enterotoxigenic *Escherichia coli* (*E. coli*), or ETEC, is an important cause of bacterial diarrheal illness. Infection with ETEC is the leading cause of travelers' diarrhea and a major cause of diarrheal disease in lower-income countries, especially among children. ETEC is transmitted by food or water contaminated with animal or human feces. Food sources reported for ETEC-related diarrhea include fresh fruits and vegetables (especially lettuce), parsley, basil, scallops, shrimp, crab meat, tuna paste, prepared salads, and soft cheese. Contaminated water and food have been implicated as vehicles for transmission of ETEC infection . Infection can be prevented by avoiding or safely preparing foods and beverages that could be contaminated with the bacteria, as well as washing hands with soap frequently.

What are the symptoms of ETEC?

The main characteristic of the disease is a watery diarrhea. It begins about 14 to 50 hours after the bacteria have been ingested. The diarrhea may be mild and last only a few days or it may be more severe and prolonged, lasting up to three weeks. Generally the diarrhea does not contain blood or mucus. Other symptoms include: Abdominal cramps, Low-grade fever, Nausea, Headache and Muscle aches. The pathogenesis of ETEC-induced diarrhea is similar to that of cholera and includes the production of enterotoxins and colonization factors.

The clinical symptoms of ETEC infection can range from mild diarrhea to a severe cholera-like syndrome. The effective treatment of ETEC diarrhea by

rehydration is similar to treatment for cholera, but antibiotics are not used routinely for treatment except in traveler's diarrhea.

1-B **Enterohemorrhagic *E. coli* (EHEC):** (Incubation period: **72-120** hrs).

Escherichia coli are Gram negative enteric bacilli that are carried normally in the intestine of humans and animals. *E. coli* **O157:H7** is the most common serotype of EHEC.

Suspected foods: Cattle are the main source of infection. This disease is often associated with ingestion of inadequately cooked hamburger meat, raw milk, cream and cheeses made from raw milk.

Pathogenesis: EHEC strains may produce one or more types of cytotoxins, which are collectively referred as **Shiga-like toxins (SLT)** since it is similar to Shiga toxin produced by *Shigella dysenteriae*. SLTs were previously known as verotoxin. The toxins kill colonic epithelial cells.

Symptoms (Clinical features): Initial symptoms may be diarrhea with abdominal cramps, which may turn into bloody diarrhea in a few days. No fever occurs.

2- *Shigella*

Shigella is a species of enteric bacteria that causes disease in humans and other primates. The disease caused by the ingestion of *Shigella* bacteria is referred to as **Shigellosis**, or 'bacillary dysentery' which is most typically associated with diarrhea (often bloody) and other gastrointestinal symptoms. ***Shigella*** is easily spread person-to-person because of its relatively tiny (compared to other bacteria) infectious dose. Infection can occur after ingestion of fewer than 100 bacteria. Another reason ***Shigella*** so easily cause infection is because the bacteria thrive in the human intestine and are commonly spread both by person-to-person contact and through the contamination of food. *Shigella* bacteria are generally transmitted through a fecal-oral route. Foods that come into contact with human or animal waste can transmit *Shigella*. Thus, handling toddlers' diapers, eating vegetables from a field contaminated with sewage, or drinking pool water are all activities that can lead to **shigellosis**.

3-*Yersinia enterocolitica*

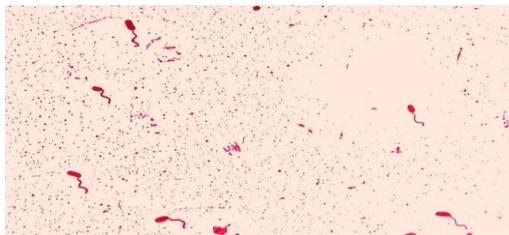
Yersinia enterocolitica: is a Gram-negative bacillus-shaped bacterium that causes an infectious disease called **yersiniosis**. *Yersinia enterocolitica* is a member of the *Yersinia* family of bacteria.

Common symptoms of **yersiniosis** in children (who most often contract the disease) are fever, abdominal pain, and diarrhea, which is often bloody. Symptoms typically develop 4 to 7 days after exposure and may last 1 to 3 weeks or longer. In older children and adults, right-sided abdominal pain and fever may

be the predominant symptoms and may be confused with appendicitis. **Yersiniosis** is most often acquired by eating contaminated food, especially raw or undercooked pork products. The preparation of raw pork intestines may be particularly risky. Drinking contaminated unpasteurized milk or untreated water can also transmit the infection.

4- *Vibrio parahaemolyticus*

V. parahaemolyticus is a Gram-negative curved rod, facultatively aerobic, non-spore forming, oxidase positive bacterium. It also has one polar flagellum contributing to its motility.



Vibrio parahaemolyticus is a self-limiting, enterotoxic bacterium, typically causing **acute gastroenteritis** in humans. More severe cases of infection can occur in immune-compromised individuals, which can lead to septicemia and death, although this is very rare. Moderate to severe skin infections can also result from open wound exposure to *V. parahaemolyticus* in warm seawater, *Vibrio parahaemolyticus* is typically transmitted to human hosts through the consumption of raw and undercooked shellfish including clams, muscles, and oysters, or drinking contaminated water.

5- *Campylobacter jejuni*

These are small, curved-spiral Gram negative bacilli with polar flagella, appearing mainly in comma shape. *Campylobacter* are harbored in reproductive and alimentary tracts of some animals.

Suspected foods: Transmission to humans occurs via a fecal-oral route, originating from farm animals, birds, dogs, and processed poultry, with chicken preparation comprising 50-70% of all campylobacter infections. The organism is transmitted to man in milk, meat products and contaminated water. Undercooked poultry and unpasteurized dairy are most often implicated as a source of it. **The infective dose—that is, the amount of bacteria that must be ingested to cause illness—is relatively small. Ingestion of as few as 500 organisms**, an amount that can be found in one drop of chicken juice, has been shown to cause human infection.

Campylobacter jejuni has come to be understood as one of the leading causes of bacterial gastroenteritis. When a person is infected and develops symptoms, the illness is called **Campylobacteriosis**. "the incubation period varies from 2 to 7 days, a characteristic that is probably inversely related to the dose ingested." Campylobacteriosis symptoms can range from diarrhea and lethargy that lasts a day to severe diarrhea and abdominal pain (and occasionally fever) that lasts for several weeks. Diarrhea and abdominal pain are the most common symptoms and the vast majority of cases are mild. Other typical symptoms of *C. jejuni* infection include fever, nausea, vomiting, abdominal pain, headache, and muscle pain.

Campylobacter jejuni grows poorly on properly refrigerated foods, but does survive refrigeration and will grow if contaminated foods are left out at room temperature. The bacterium is sensitive to heat and other common disinfection procedures; **pasteurization of milk, adequate cooking of meat and poultry, and chlorination or ozonation of water will destroy this organism.**



Mycotoxins

Mycotoxins are natural chemical substances (secondary metabolites) produced by fungi (molds) growing as contaminants on some food crops (in field and in storage), in particular cereals, nuts and fruit. Their presence in crops and in foods and animal feeds produced from them is undesirable, as they are toxic and have a number of adverse effects on health, both in humans and animals. Mycotoxins can affect the immune system, nervous system, liver, kidneys, and blood, and some mycotoxins are known to be carcinogens (cancer-causing). There is a major difference between the toxic metabolites of fungi and the toxins of most bacteria associated with food poisoning, the fungal toxins have relatively low molecular weight compounds, although their chemistry may be very complex, while the bacterial toxins are macromolecules such as polypeptides, proteins or lipopolysaccharides.

The chemical and biological properties of the mycotoxins, as well as their toxic effects are extremely variable. Some mycotoxins were shown to be mutagenic, teratogenic, or/and carcinogenic.

Exposure to mycotoxins

The Food and Agriculture Organization (FAO) estimates that 25% of the world's food crops, overall, are affected by mycotoxins. Considering that these food crops include cereals, nuts, fruit and vegetables which comprise a significant part of the European consumer's diet, there is potentially a significant exposure to mycotoxins. Exposure of consumers to mycotoxins is mainly via plant foods. However, an additional potential exposure may be via foods of animal origin such as milk, cheese and meat, as a result of consumption of contaminated feed by food animals. This illustrates the need to control levels of mycotoxins in animal feed as well as food.

The most common mycotoxins are **aflatoxins, ochratoxin A, etc...** Many foods and feeds including corn, wheat, barley, rice, oats, nuts, milk, cheese, peanuts and cottonseed can become contaminated with mycotoxins since they can form in commodities before harvest, during the time between harvesting and drying, and in storage.

The poisoning by mycotoxin is referred to as **mycotoxicosis**. A wide range of adverse and toxic effects in animals are produced by mycotoxin in addition to being food borne hazards to humans.

TYPES OF MYCOTOXIN Mycotoxins have been reported to be carcinogenic, tremorogenic, haemorrhagic, teratogenic, and dermatitis to a wide range of organisms and to cause hepatic carcinoma in humans. More than a 100 species of filamentous fungi are known to cause toxic responses under naturally occurring conditions by producing mycotoxins.

Mycotoxins can enter the human and animal food chains by **direct contamination** when the food has been contaminated by toxigenic fungi while growing or after harvest, or **indirect contamination**, for example in milk from cows fed with contaminated food. More than 300 mycotoxins are known, of which about 20 are serious contaminants of crops used in human foods and animal feeds. Mycotoxin contamination of foods and feeds depends highly on environmental conditions that lead to mould growth and toxin production.

1- Aflatoxin

Aflatoxins B1, B2, G1 and G2 are produced by three molds of the **Aspergillus species: A. flavus (A+fla+toxin), A. parasiticus and A. nomius** and various species of **Penicillium, Rhizopus, Mucor and Streptomyces**, which contaminate plants and plant products.

2-Ochratoxin-A (OTA)

Ochratoxin-A: produced by the fungi **Aspergillus ochraceus** and **Penicillium verrucosum**. Isolates of *Aspergillus niger* as well as *A. carbonarius* are capable of producing OTA. OTA generally appears during storage of fresh produce (in cereals, coffee, cocoa, dried fruit, spices, and also in pork) and occasionally in the field on grapes. It may also be present in some of the internal organs (particularly blood and kidneys) of animals that have been fed on contaminated feeds.

3-Fusariotoxins (*Fusarium* toxins)

Fungi belonging to the genus **Fusarium** are associated with the production of Fusariotoxins.

4-*Alternaria* toxins

Mycotoxins produced by fungi belonging to **Alternaria** species are referred to as *Alternaria* toxins. They commonly occur during the pre- and post harvest stages of fruits and vegetables. The most important toxin-producing species is **Alternaria alternata**, which usually contaminates cereals, sunflower seeds, olives, and fruits.

5-*Claviceps purpurea*/ergot toxins

Sclerotia of fungi belonging to the genus *Claviceps* produce ergot alkaloids. A sclerotium is a dark-colored, hard mycelial mass that establishes itself on the seed or kernel of the plant. Usually, wild grass species are considered to favor the cross-contamination of *C. purpurea* onto the cultivated grass. Ergot reduces yield because seeds or kernels are replaced by sclerotia. Ergot produced by the mold **Claviceps purpurea**, which can cause **ergotism** in humans and other mammals who consume grains contaminated with its fruiting structure (ergot **sclerotium**).

Claviceps purpurea

6-Patulin

It is a mycotoxin produced by a variety of molds, in particular, **Penicillium expansum**. Most commonly found in rotting apples. In addition, patulin has been found in other foods such as grains, fruits, and vegetables. While not considered a particularly potent toxin, a number of studies have shown patulin to be genotoxic, which has led some to theorize that it may be a carcinogen. Patulin has shown antimicrobial properties against some microorganisms. It is heat-stable, so it is not destroyed by pasteurization or thermal denaturation. Patulin has also become important to apple processors as a method for monitoring the quality of apple juices and concentrates. The presence of high amounts of

patulin indicates that moldy apples were used in the production of the juices. Patulin is being considered as a "possible toxin" and is regarded as the most dangerous mycotoxin in fruits, particularly apples, pears, and their products. Patulin is mainly associated with surface-injured fruits, which renders them vulnerable to fungal infection, mainly by *Penicillium* spp .

7-Citrinin

It is produced by the mold ***Penicillium citrinum*** , Citrinin acts as a nephrotoxin .It causes mycotoxic nephropathy in livestock and has been implicated as a cause of Balkan nephropathy (referred to as "yellow rice disease") in humans. Citrinin is used as a reagent in biological research. It induces mitochondrial permeability pore opening and inhibits respiration. It has been found on human foods, such as grains and cheese.Citrinin often occurs as a common contaminant of food and feed (fruits, barley, maize, cheese, dietary supplements).

Production of Mycotoxins

Several factors influence mold development, including moisture, temperature, aeration and substrate. Moisture is probably the most important of these factors but the type of mold and whether or not a toxin is produced will depend on the interplay of all these factors.Physical damage, such as breakage and stress cracks in grain, will increase the likelihood of fungal growth. High moisture corn, especially if ground, is highly susceptible to fungal invasion and toxin formation. To prevent production of the toxin on harvested products, care should be taken to prevent physical damage at harvest and to reduce the moisture level soon after harvesting. Rapid ensiling or the addition of organic acids will aid in preventing the formation of additional mycotoxin.

Lec8:

General Principles of food Preservation

Foods for human consumption can be divided into 4 plant and 4 animal origin as well as other additive materials.

Food from plants

Cereals and cereal products.

Sugar and sugar products.

Vegetables and vegetable products.

Fruit and fruit products.

Food from animals

Meat and meat products.

Poultry and eggs.

Fish and other sea food.

Milk and milk products.

To the list of plant origin it may also contain spices and other flavoring materials, like sodium chloride which is a mineral and flavoring material, an essential nutrient and a chemical preserver. Some foods may be fortified with minerals such as iron and calcium which are added to flour. Some of the coloring and flavoring materials used in food are synthetic. Vitamins usually are presented in foods but may be added or consumed separately after chemical synthesis or production by M.O.

Food preservation: is a set of physical, chemical and biological processes that are performed to prolong the shelf life of foods and at the same time retain the features that determine their quality, like color, texture, flavor and especially nutritional value. Food preservation is achieved by destroying enzymes and microorganisms using heat (blanching, pasteurization), or preventing their action by: removal of water, or increasing acidity or using low temperatures.

Most kinds of foods are readily to be decomposed by M.O unless special methods are used for preservation.

Methods of food preservation

Method	Procedure	Antimicrobial material	Typical surviving microbes
Physical	High temp.	-	Thermophil bacteria , spores
	Low temp.	-	Psychrophiles
	Water content	-	Xerophiles
	Irradiation	-	spores
	Filtration	-	Depending on further preservative methods
Chemical	salting	NaCl	Halophilic bacteria
	Curing	Nitrites	G+ve bacteria
	Smoking	Phenoles ,acids	Depending on further preservative methods
	Preservatives	Organic acids	Bacteria tolerate acids
Biological	Fermentation	Organic acids	Depending on ecological factors

Principles of food preservation:

To accomplish the preservation of foods by the various methods, the following principles of prevention or delay of decomposition should be involved:

1. CONTROLLING MICROORGANISMS BY:

- A.** Keeping microorganisms out of food, e.g.: Asepsis.
- B.** Removing microorganisms from food, e.g.: Filtration.
- C.** Delaying microbial growth, e.g.: low temp., drying, anaerobic conditions or chemicals.
- D.** Killing microorganisms or spores, e.g. Heat or irradiation.

2. CONTROLLING ENZYMES BY:

- A.** Inactivating endogenous enzymes, e.g.: blanching.
- B.** Preventing or delaying chemical reactions in the food, e.g.: prevention of oxidation by means of antioxidants.

3. CONTROLLING INSECTS, RODENTS, BIRDS AND OTHER PHYSICAL CAUSES OF FOOD DETERIORATION.

It's very important in food preservation (i.e. preservation of spoilage) is the lengthening, as much as possible of the lag phase and the phase of positive acceleration.

Food protection with low temperature

Physical Methods: Non-Thermal processing

The application of extreme heat treatments used for food preservation affect the nutritional and organoleptic properties of food. In recent years, the consumers demand for fresher, higher quality and safe food has increased. Therefore, nonthermal methods of food preservation for the inactivation of microorganisms and enzymes as an alternative to thermal processes are being used. However, the high resistance of certain enzymes and microorganisms to nonthermal processes, especially bacterial spores, limit their application. During nonthermal processing, the temperature of foods is held below the temperature normally used in thermal processing; therefore, a minimal degradation of food quality is expected. Nonthermal process of food preservation improves food quality and enhances safety levels. Overall, most nonthermal preservation techniques are highly effective in inactivating vegetative cells of bacteria, yeast, and molds. Bacterial spores and most enzymes are however, difficult to inactivate with these procedures. Thus their use is limited to foods where enzymatic reactions do not affect food quality or where spore germination is inhibited by other prevailing conditions, such as low pH.

Low Temperature Preservation

Storage at low temperatures prolongs the shelf life of many foods. In general, low temperatures reduce the growth rates of microorganisms and slow many of the physical and chemical reactions that occur in foods. Low temperatures are used to preserve food by lowering microbial activity through the reduction of microbial enzyme activity. However, psychrotrophic bacteria are known to grow even at commercial refrigeration temperatures (7°C). These bacteria include members of the genera ***Pseudomonas***, ***Alcaligenes***, ***Micrococcus*** and ***Flavobacterium***. Some of the fungi also grow at refrigeration temperatures. Slow freezing and quick freezing are used for long-term preservation. Freezing reduces the number of microorganisms in foods but does not kill all of them. In microorganisms, cell proteins undergo denaturation due to increasing concentrations of solutes in the unfrozen water in foods, and damage is caused by ice crystals.

Refrigeration

Refrigeration slows down the biological, chemical, and physical reactions that shorten the shelf life of food. Exposure of microorganisms to low temperatures reduces their rates of growth and reproduction. This principle is used in refrigeration and freezing. Microbes are not killed at refrigeration temperature for a considerable period of time. In refrigerators at 5°C, foods remain unspoiled but in a freezer at -5°C the crystals formed

tear and shred microorganisms. It may kill many of the microbes. However, some are able to survive. **Salmonella** spp. and **Streptococcus** spp. Survive freezing. For these types of microorganisms rapid thawing and cooking is necessary. Refrigerators should be set to below 12°C to control the growth of micro-organisms in foods. This lowered temperature also reduces the respiration rate of fruits and vegetables, which retards reactions that promote spoilage. All perishable foods should be refrigerated as soon as possible, preferably during transport, to prevent bacteria from multiplying. Refrigeration is generally used to: **i)** reduce spoilage during distribution of perishable foods, **ii)** increase the holding period between harvesting and processing; and **iii)** extend the storage life of commercially processed foods. All foods are not benefited from cold temperatures. For example, bananas turn black when refrigerated.

Freezing

Freezing is also one of the most commonly used processes commercially and domestically for preserving a very wide range of food stuffs including prepared food stuffs which would not have required freezing in their unprepared state. For example, potato waffles are stored in the freezer, but potatoes themselves require only a cool dark place to ensure many months storage. Freezing makes water unavailable to microorganisms. The chemical and physical reactions leading to deterioration are slowed by freezing. White or grayish patches on frozen food caused by water evaporating into the packages air spaces called freezer burn occurs which causes deterioration of taste and appearance. This occurs in fruits, vegetables, meat, poultry and fish. While many home freezers are held at -10°C, commercial freezers are under -18°C. At this temperature, the growth of micro-organisms is almost stopped. Deteriorative microbial reactions will still occur, but over a much longer time. In addition, deteriorative enzymatic reactions will still take place during frozen storage. Uncooked fruits and vegetables must be blanched before freezing to prevent these reactions. During freezing, the water in food forms ice crystals. The rate of this phenomenon has a big impact on the quality of frozen foods: Slow freezing (e.g. home freezer) forms large ice crystals which puncture cell walls and cellular fluid is released and also results in shrunken appearance of thawed food. In this process the freezing is done for 3-72 h. This method is used in home freezer and temperature is lowered to -15 to -29°C. Rapid freezing During rapid freezing small, numerous ice crystals are formed and cell structure is not changed. In this process the temperature of food is lowered to about -20°C within 30 min. This process blocks or suppresses the metabolism. The shelf life of frozen foods is largely dependent on storage conditions. Under ideal conditions, frozen foods can have a shelf life of one year. However, if foods are continuously exposed to warmer temperatures, such as the opening and closing of freezer doors, then heat shock occurs. Heat shock is when ice melts and re-forms into larger ice crystals. The best example is ice cream, which has a gritty texture if large ice crystals have developed.

Advantages of freezing are generally good retention of nutrients and prevention of microbial growth by low temperature and unavailability of water. However, **disadvantages of freezing** are loss of some B-Group vitamins and vitamin C due to

blanching of vegetables prior to freezing and unintended thawing can reduce product quality.

Preservation by Freeze Drying

The process of freeze drying or lyophilization is commonly used these days for preservation. The food is deep frozen, after which the water is drawn off by a vacuum pump in a machine. The dry product is then sealed in foil and is reconstituted with water. This method is very useful for storing, transporting and preserving bacterial cultures. Drying or dehydration involves the removal of water from the food by controlled processes. This may be done by evaporation due to heating of the product, e.g., drying of fruits, osmotic dehydration, e.g. brining of fish and sublimation, or freeze drying e.g. in the drying of coffee. There are two distinct stages in this technology. In the first stage, the removal of surface water depends solely on the state of the air surrounding the food, such as its temperature, relative humidity and speed. In the second phase of drying, the moisture within the food moves to the surface. As the air is heated, its relative humidity decreases, resulting in more absorption of water. Here the rate of drying is dependent on the time the moisture takes to get to the surface. The heating of the air around a food product can, therefore, cause it to dry more quickly. The principle of sublimation is used in freeze drying and lyophilization. This is the process in which a solid changes directly to a vapor without passing through the liquid phase.

Food Protection at High Temperature

The killing of microorganisms by heat is supposed to be caused by the **denaturation of proteins** and especially by the **inactivation of enzymes required for metabolism**.

Physical Methods-Thermal Processing

Heat kills microorganisms by changing the physical and chemical properties of their proteins. When heat is used to preserve foods, the number of microorganisms present, the microbial load, is an important consideration. Various types of microorganisms must also be considered because different levels of resistance exist. For example, bacterial spores are much more difficult to kill than vegetative bacilli. In addition, increasing acidity enhances the killing process in food preservation.

HIGH TEMPERATURE

Three basic heat treatments are used in food preservation: **pasteurization**, in which foods are treated at about 62°C for 30 minutes or 72°C for 15 to 17 s; **hot filling**, in which liquid foods and juices are boiled before being placed into containers; and **steam treatment under pressure**, such as used in the canning method. The heat resistance of microorganisms is usually expressed as **the thermal death time**, the time necessary at a certain temperature to kill a stated number of particular microorganisms under specified conditions.

Pasteurization

It is the process of heating a food-usually a liquid-to or below its boiling point for a defined period of time. The purpose is to **destroy all pathogens, reduce the number of bacteria, inactivate enzymes and extend the shelf life of a food product**. Pasteurization treatment is able to kill most heat resistant non spore forming organisms like ***Mycobacterium tuberculosis*** and ***Coxiella burnetti***. Foods with a pH of less than 12.6, such as milk and spaghetti sauce, can be pasteurized. Permanent stability that is, shelf life of about two years is obtained with foods that can withstand prolonged heating, such as bottled juices. There is a greater loss of flavour from foods that are exposed to a longer time-temperature relationship. Therefore, temporary stability (that is, limited shelf life) is only obtained with some foods where prolonged heating would destroy its quality. These foods, such as milk, usually require subsequent refrigeration. **"High Temperature Short Time" (HTST) and "Ultra High Temperature" (UHT)** processes have been developed to retain a food's texture and flavour quality parameters. Pasteurization is not intended to kill all microorganisms in the food. Instead pasteurization aims to reduce the number of viable pathogens so that they are unlikely to cause disease. Pasteurization involves a comparatively low order of heat treatment, generally at temperature below the boiling point of water.. Desired pasteurization can be achieved by a combination of time and temperature such as heating food to a low temperature and maintain for a long time i.e. **Low temperature long time LTLT** -62.8 ° C for 30 minute, or by heating food to a **high temp** and maintain for a **short time**: **HTST**-71.7 ° C for 15 second. Pasteurization is used when more rigorous heat treatment might harm the quality of the food product, as the

market milk and for the main spoilage organisms which are not heat resistant, such as yeast in fruit juice. It also kills the pathogens .

Ultra heat pasteurization

In this process milk is heated to 120-138 ° C for 2-4 seconds and followed by rapid cooling. This treatment kills all the spoilage microorganisms. UHT pasteurized milk is packaged aseptically resulting in a shelf stable product that does not require refrigeration until opened. Heat resistance of microorganisms and their spores It is expressed in terms of their thermal death time (TDT).

Thermal Death Time (TDT) It is the time taken to kill a given number of microorganisms or spores at a certain temperature under specified conditions.

Thermal death point It is the temperature necessary to kill all the organisms in ten minutes. Heat resistance of different microorganisms is different. Microorganisms are more heat resistant than their spores. Heat resistance of vegetative yeast is 50-58 ° C in 10-15 min and the ascospores is 60 ° C for 10-15 min. However, yeast and spores are killed by pasteurization.

Heat resistance of microorganisms

Heat resistance of mold is 60° C in 5 to 10 min and asexual spores are more heat resistance than the ordinary mycelium and require a temperature 5-10°C higher for their destruction. *Aspergillus*, *Mucor*, *Penicillium* are more resistant than yeast. Heat resistance of bacteria and bacterial spores is different. Cells high in lipid content and capsule containing bacteria are harder to kill. Higher the optimal and maximal temperature for growth , the greater the resistance to killing.

D value

It is the **decimal reduction time**, or the time required to destroy 90% of the organisms. Mathematically, it is equal to reciprocal of the slope of the survivor curve and is a measure of the death rate of a microorganisms.

Effect of pasteurization

+ The positive effects of pasteurization are the destruction of pathogenic microorganisms to increase the safety of market milk for human consumption, improved keeping quality and inactivation of certain naturally occurring enzymes.

- The negative effects are: certain preformed products of microbial origin are not inactivated during pasteurization, e.g. Staphylococcal toxins and aflatoxins. There is small loss of native aroma particularly in case of fruit juices. In case of milk, it destroys the natural microbicidal property of milk by inactivating different natural occurring antimicrobial substances like lysozyme, lactin & lactoferrin.

Blanching

It is a kind of pasteurization generally applied to fruits and vegetables, primarily to **inactive natural food enzymes**. It is a common practice when such food products are to be frozen, since frozen storage itself would not completely arrest enzyme activity. Peroxidase and catalase are the most heat resistant enzymes; the activity of these enzymes is used to evaluate the effectiveness of a blanching treatment. If both are inactivated then it can be assumed that other significant enzymes also are inactivated. The heating time depends on the type of fruit or vegetable, method of heating, the size of fruits or vegetable or the temperature of the heating medium.

Rapid changes in colour, flavor and nutritive value occur as a result of enzyme activity. Blanching is a slight heat treatment, using hot water or steam, that is applied mostly to vegetables before canning or freezing. The main objectives of blanching are to inactivate enzymes, to remove the tissue gases, to clean the tissue, to increase the temperature of the food. Blanching is also used before canning for different reasons, because enzymes will inevitably be destroyed during canning. Blanching induces a vacuum in canned goods, and it is also used to control the fill into containers (for example, spinach).

Sterilization

Sterilization destroys all pathogenic and spoilage microorganisms in foods and inactivates enzymes by heating. All canned foods are sterilized in a retort (a large pressure cooker) and called commercial sterilization which indicates that no viable organisms are present. This process enables food to have a shelf life of more than two years. Foods that have a pH of more than 4.6, such as meat and most vegetables must undergo severe heating conditions to destroy all pathogens. These foods are heated under pressure to 121°C for varying times. Severe conditions are applied primarily to ensure that **Clostridium botulinum** spores are destroyed during processing. These spores produce the deadly botulinum toxin under anaerobic conditions (that is, where there's no oxygen). The spores are destroyed by heat or are inhibited at pH values of less than 4.6. Therefore, a food with a pH of less than 4.6 that is packaged anaerobically, such as spaghetti sauce, doesn't need to undergo such a severe heat treatment. The destruction of vegetative and sporeforming organism and pathogens is secondary objective of commercially sterilized foods.

Nicolas Appert, a Parisian confectioner by trade, established the heat processing of foods as an industry in 1810. The food product is washed, sorted, and graded and then subjected to steam for three to five minutes. This last process called blanching, destroys many enzymes in the food product and prevents further cellular metabolism. The food is then peeled and cored, and diseased portions are removed. For canning, containers are evacuated and placed in a pressurised steam steriliser, similar to an autoclave at 121°C. This removes especially **Bacillus** and **Clostridium** spores. If canning is defective, foods may become contaminated by anaerobic, bacteria which produce gas. These are species of **Clostridium**, and coliform bacteria (a group of Gram-negative non spore-forming rods which ferment lactose to acid and gas at 32°C in 48 hours). Canning cooking fruits or vegetables, sealing them in sterile cans or jars, and boiling the containers to kill or weaken any remaining bacteria as a form of pasteurization. High-acid fruits like strawberries require no preservatives to can and holding for only a short boiling cycle,

whereas marginal fruits such as tomatoes require longer boiling and addition of other acidic elements. Many vegetables require pressure canning. Food preserved by canning or bottling is at immediate risk of spoilage once the can or bottle has been opened. Lack of quality control in the canning process may allow ingress of water or micro-organisms. ***Clostridium botulinum*** produces an acute toxin within the food and may lead to severe illness or death. This organism produces no gas or obvious taste and remains undetected by taste or smell. Food contaminated in this way include Corn, beef and Tuna. In canning process heat is applied to food that is sealed in a jar in order to destroy any microorganisms that can cause food spoilage. Proper canning techniques stop this spoilage by heating the food for a specific period of time and killing these unwanted microorganisms. During the canning process, air is driven from the jar and a vacuum is formed as the jar cools and seals. Water-bath canning and pressure canning are two approved methods of canning.

DRYING

One of the oldest methods of food preservation is by drying, which reduces water activity sufficiently to delay or prevent bacterial growth. Drying is done to produce concentrated form of foods, inhibits microbial growth and autolytic enzymes, retains most nutrients. Drying can cause loss of some nutrients, particularly thiamine and vitamin C. Sulphur dioxide is sometimes added to dried fruits to retain vitamin C, but some individuals are sensitive to this substance. Most types of meat can be dried. Many fruits can be dried; for example, the process is often applied to apples, pears, bananas, mangos, papaya, and coconut and grapes . Drying is also the normal means of preservation for cereal grains such as wheat, maize, oats, barley, rice, millet and rye. Drying is an excellent way of preserving several of the seasonal fruits for use during the off season. There are several types of dryers which are used. These include: drum dryer, cabinet dryer, tunnel dryer, rotary dryer, spray dryer and solar dryer.

Advantages of drying are many

- i) Long Shelf Life – Since most microorganisms responsible for food spoilage are unable to grow and multiply in the absence of moisture, spoilage due to microbial degradation is limited in dried foods. Furthermore, enzymes which catalyse undesirable changes in foods need moisture to be effective.
- ii) Reduced Weight – This results in reduced transportation, storage and shipping costs.
- iii) Convenience – The production of convenience items with novelty appeal for niche markets makes drying an attractive option.
- iv) Concentration of nutrients – The removal of most of the water from a food results in a highly concentrated source of nutrients.
- v) No refrigeration is required for dried products – Savings in energy and storage costs together with the long shelf life provide a lucrative processing alternative for tropical countries.

Disadvantages of Drying

Disadvantages of Drying are few and mainly relate to oxidation, which usually accompanies drying. This results in losses of micronutrients such as carotene and ascorbic acid and minimal loss in protein as a result of browning reactions. Reduced consumer appeal is often linked with the latter. There might also be changes in flavour and texture if drying is not properly controlled, particularly with regard to maximum temperatures.

Factors Affecting Heat Resistance of Microorganisms:

1. **Temperature time relationship:** the time of killing cells or spores under a given set of conditions decrease as the temp. is increased.
2. **Initial concentration of spores or cells :** the more spores or cells present, the greater the heat treatment necessary to kill all of them.

Microbial load	D-value after minutes						
	1	2	3	4	5	6	7
Food A 10^6 /gram	10^5	10^4	10^3	10^2	10^1	1	0
Food B 10^3 /gram	10^2	10^1	0				

3.

The condition under which the cells have been grown and spores have been produced and their treatment, therefore, will influence their resistance to heat.

- A. Culture medium:** The medium in which grown takes place is especially important. The effect of nutrient in the media, their kind, and the amount vary with the organism, but in general the better the medium for growth, the more resistant the cells or spores.
- B. Temperature of incubation:** The temp. of growth of cells and the temp. of sporulation influence their heat resistance. In general, resistance increases as the incubation. Temp. is raised toward the optimal for the organism and for many microorganisms increases, further as the temp. approaches the maximum for growth.
- C. Phase of growth or age:** Bacterial cells show their greatest resistance during the lag phase but almost as greatest resistance during their stationary phase. very young spores are less resistance than the mature one.

D. Desiccation: Dried spores of some bacteria are harder to kill by heat than are those kept in moist.

4. Composition of the substrate in which cells or spores are heated is so important that it must:

a. Moisture: almost heat is much more effective killing agent than dry heat. Dry heat material require more than heat sterilization with moist.

b. Hydrogen ion concentration (pH): Cells or spores are most heat resistance in substrate that is at or near neutrality. An increase in acidity more effective than increase in alkalinity.

c. Other substrates: Low concentration of salt has protective effect on some spores. Sugars protect some organism or spores. Its protection high for osmophilic. The protective effect of sugar may be related to resulting degree in a_w . A reduced a_w dose result in an increase in observed heat resistance.

Effect of Temperature on the Microorganism:

1. Denaturation of protiens.
2. Inhibition of enzyme activity.
3. Destruction of nucleic acid and membranes.

Effect of Temperature on the food :

1. Fish meat :

Thiamin → Canning, Boiling, Frying, Broiling (Roosting)
56 – 77%, 50 -70%, 20 – 50%, 10 -40%

Riboflavin → 0 – 40%

Folic acid → 50 -90%

B₆ → 70%

2. Egg (boiling):

Thiamin → 15%

Riboflavin → 7%

Folic acid → 20-50%

3. Milk:

Thiamin → 10% pasteurization

20-30% Drying

30-50% sterilization

Food Preservation by Chemicals

Since ancient times, man used natural chemical materials in food as a flavor and prevent spoilage such as spices, salt, sugar, vinegar and garlic. After a period of time from using chemicals as food preservative, some medical reports showed that these chemicals are **carcinogenic** or **mutagenic**, and all chemicals are harmful to food and consumer. Another point is that chemicals which considers safety, doesn't know what will happen when reacts with water, metal or food, and what will happen from the interactions between these substances in the human gut and configuration of new materials which may be harmful to human. For these reasons, memorandum issued annually by the associations and health organizations of food, a new list of chemical substances added to the allowed foods and amending the previous lists (reduce material, deleted items, reduce the concentration of substances previously regarded safe, added new substance to the list).

One of these organizations is the **WHO (World Health Organization)** which published (key or guide of the safe use) of food additives, also the **Food and Drug Administration (FDA)** made the following characters on each chemical allowed in food use **Generally Recognized As Safe (GRAS)** and has set this organization that any material added to food must be generally safety, so would be unable to give the resolution seriously that this chemical material safety, because we can not know what will the consumer take in various countries of other chemical products in their food or drink and what is happening between them (e.g nitrite added to canned food, and may be the consumer will take the pesticides with their food, the result from its interaction _nitrous acid which is carcinogenic).

E numbers:

Are codes for substances that are permitted to be used as food additives for use within the European Union and Switzerland. The "E" stands for "Europe". They are commonly found on food labels throughout the European Union. Safety assessment and approval are the responsibility of the European Food Safety Authority.

E100–E199 (colours).

E200–E299 (preservatives).

E300–E399 (antioxidants, acidity regulators).

E400–E499 (thickeners, stabilizers, emulsifiers).

E500–E599 (acidity regulators, anti-caking agents).

E600–E699 (flavour enhancers).

E700–E799 (antibiotics).

E900–E999 (glazing agents and sweeteners).

E1000–E1599 (additional chemicals).

General Characters of Chemicals Added to foods:

1. Inhibitory or lethal to food microorganisms.
2. Should not cause any harm to the consumer.
3. Stop any chemical activity, analyzing, enzyme activity and anti-oxidant in food.
4. Does not affect the nature of food and taste.
5. Cheap and available.

The effect of chemical preservatives in microbial cells:

A- Direct effect: through its effect on cell membrane directly damaging the components of the cell so that it will cause changing in permeability of microorganisms and chemical composition and their ionization.

B- Indirect effect: effects on the environment of microorganisms through its impact on the food itself by making it unable to use for microorganism growth by reducing the water activity, which is necessary to make toxic compounds.

Use of chemicals in food preservation

Preservatives are substances that limit, slow down or stop the growth of microorganisms (bacteria, yeasts, molds) or present within the food and thus prevent the deterioration of the goods or food poisoning. They are used in cooked food, wine, cheese, fruit juices and margarines. Chemical preservatives prevent microbial growth without loss of nutrient but some people are sensitive to some chemical preservatives. Chemical food preservatives are the best and the most effective for a longer shelf life and are generally fool proof for the preservation purpose. Chemical substances are the artificial preservatives that stop or delay the growth of bacteria, spoilage and its discoloration. These artificial preservatives can be added to the food or sprayed on the food. Several kinds of chemicals can be used for food preservation including propionic acid, sorbic acid, benzoic acid, and sulfur dioxide. These acids are acceptable because they can be metabolized by the human body. Acids such as citric acid, acetic acid (vinegar) and ascorbic acid are also known to confer protection against product deterioration. In these cases, the pH of the product is shifted to being low, that is, more acidic, where very few moulds, yeast and bacteria are able to grow and multiply. Food additives such as benzoate and sorbate are quite commonly used in the fruit drink industry to protect against microbial spoilage, while nitrites are used in meat processing. The most commonly used preservatives are the acids such as sorbic acid, benzoic acid and propionic acid. These check mainly the growth of yeasts and molds. Sorbic acid is used

for preservation of syrups, salads jellies and some cakes. Benzoic acid is used for beverages, margarine, apple cider etc. Propionic acid is an ingredient of bread and bakery products. Sulphur dioxide, as gas or liquid is also used for dried fruits, molasses and juice concentrates. Ethylene oxide is used for spices, nuts and dried fruits. Some antibiotics can also be used, depending upon local laws and ordinances. Tetracycline, for example, is often used to preserve meats. Storage and cooking normally eliminates the last remnants of antibiotic. Antioxidants are also the chemical food preservatives that act as free radical scavengers. In this category of preservatives in food comes the vitamin C, BHA (butylated hydroxyanisole). Unlike natural food preservatives some of the chemical food preservatives are harmful. **Sulfur dioxide and nitrites** are the examples. **Sulfur dioxide** causes irritation in bronchial tubes and **nitrites** are carcinogenic. All of these chemicals act as either antimicrobials or antioxidants or both. They either inhibit the activity of or kill the bacteria, molds, insects and other microorganisms. Antimicrobials prevent the growth of molds, yeasts and bacteria and antioxidants keep foods from becoming rancid or developing black spots. They suppress the reaction when foods come in contact with oxygen, heat and some metals. They also prevent the loss of some essential amino acids and some vitamins. These substance works on the principle of **controlling water activity, pH and osmotic pressure of food.**

WATER ACTIVITY

Water is the most important factor in controlling the rate of deterioration of a food. It is the availability of water for microbial, enzymatic, or chemical activity that determines the shelf life of foods. This water availability is measured as water activity (a_w). a_w of milk is 0.97, honey is 0.5-0.7 and dried fruits is 0.5-0.6. Food spoilage micro-organisms, in general, are inhibited in food where the water activity is below 0.6. However, if the pH of the food is less than 13.6, micro-organisms are inhibited when the water activity is below 0.85. The water activity (a_w) values for inhibition of some of the microorganism are ***Cl.botulinum*** 0.97 , ***Ps. fluorescence*** 0.97, ***E.coli*** 0.95, ***B.cereus*** 0.93 and ***S.aureus*** 0.86. Generally, Intermediate moisture food (IMFs) possesses water activities that range from 0.6 to 0.85. This enables the food to be stable at room temperature, because the growth of most micro-organisms is inhibited at these levels. Binding the water that's present preserves intermediate moisture foods, for example, cookies, cake and bread. This reduces the availability of the water for deteriorative reactions. Water is immobilized by adding permissible humectant additives such as glycerol, glycols, sorbitol, sugars and salts. The addition of some chemicals inhibits microbial growth in foods. These chemicals include not only those classified as preservatives . Salt, sugars, wood smoke and some spices also inhibit the growth of micro-organisms.

TYPES OF ARTIFICIAL/CHEMICAL FOOD PRESERVATIVES

These are generally grouped as :

1. Antimicrobial agents
2. Antioxidants
3. Chelating agent

In antimicrobial the benzoates, sodium benzoate, sorbates and nitrites are generally used. Antioxidants include the Sulfites, Vitamin E, Vitamin C and Butylated hydroxytoluene (BHT) and the chelating agent like the disodium, ethylenediaminetetraacetic acid (EDTA), polyphosphates and citric acid.

Some common chemical preservatives

a) Nitrates and nitrites: Nitrates are converted to nitric acids which form stable red colour in meat. Nitrites have inhibitory action against ***Clostridium botulinum*** in meat products but forms carcinogenic nitrosamine. It is also used to preserve meats such as sausage, ham, bacon, beef, etc. The side effects are: allergy, asthma, nausea, vomiting and headaches and sodium nitrite can be converted to nitrous acid in the body and cause cancer.

b) Sulfites (sulfur dioxide and metabisulfite): Sulfites form sulphurous acid which is active antimicrobial compound and can kill the microbial cell by reduction of sulphide linkage, formation of carbonyl compounds and inhibiting the respiratory mechanism. These are used to prevent fungal spoilage and browning of peeled fruits and vegetables. The commonly used level of sulphites is 0.005 -0.2% . The side effects are allergy , asthma, nausea, vomiting, joint pain, palpitation, and headaches.

c) Sodium benzoate or benzoic acid: They are more effective against yeast and molds and are used at a concentration of upto 0.2%. The action of benzoates against microorganisms are by inhibiting enzymes necessary for oxidative phosphorylation, inhibiting membrane protein function and also by destroying membrane potential. They are added to carbonated drinks, margarine, flour, pickles, fruit purees, and fruit juices. The side effects are severe allergic reaction and cancer.

d) Propionates: Calcium and sodium propionate are effective against mold and bacteria at 0.1-0.2% but not effective against yeast at this concentration. The inhibitory action is due to cytoplasm acidification and destabilization of membrane proton gradient.

e) Sorbate: Sodium, calcium or potassium salt is used at the concentration of 0.05-0.2%. It is more effective against yeast and mold than bacteria. The activity of sorbic acid increases as the pH decreases. Sorbic acid and its salts are tasteless and odourless when used at levels below 0.3%. It is used in non-alcoholic drinks, alcoholic drinks, processed vegetables and fruits and dairy desserts.

f) Butylated Hydroxytoluene (BHT) and Butylated Hydroxyanisole (BHA): They serve as antioxidants that prevent the oxidation of fats (rancidity). They are used in preserving fresh meat, pork, sausages, potato chips & crackers, beer, baked goods, drink powder, dry cereals, and frozen pizza. These compounds have several side effects and they can cause cancer and liver disease.

g) Mono-glycerides and Diglycerides: They are used as preservatives for cookies, cakes, pies, bread, peanut butter, roasted nuts, shortening, and margarine. They may cause cancer and birth defects.

Salt and sugar: Salt and sugar have long been used as effective means of extending shelf life of various products as these solutes bind water, leaving less water available for the growth of microorganisms. Essentially the water activity (a_w) of the product is reduced. Since most microorganisms require a high water activity, they are unable to survive.

Natural Antimicrobial Compounds

Natural food preservatives have been used and recognized to mankind since long time. Apart from the preservatives, in all-natural preservation, freezing, pickling, deep frying, salting and smoking also come. Organic substances such as salt, sugar, vinegar and alcohol. These are utilised in both raw and cooked food stuff to increase the shelf value of food so that aroma, taste as well as the foods itself may be stored for a longer period of time. Some preservatives targets enzymes in fruits and vegetables that continue to metabolize after they are cut. For instance, citric and ascorbic acids from lemon or other citrus juice can inhibit the action with the enzyme phenolase which turns surfaces of cut apples and potatoes brown.

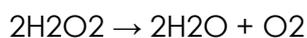
Natural Food Preservatives have many advantages. They do not alter the color of the food and gives the required flavour. Artificial preservatives are responsible for causing a lot of health trouble pertaining to respiratory tract, heart, blood and other. **There is no main health concern associated with the use of natural food preservatives.** These compounds naturally do the process of osmosis and are completely safe for consumption.

Activated lactoferrin (ALF, Activin) Lactoferrin is an antimicrobial protein present as normal component of fresh milk. It has antibacterial, antifungal and antiviral activity. It also occurs in saliva, tears, and some other body fluids. Activin is activated lactoferrin and is a more potent antimicrobial than plain lactoferrin. It has been accorded **Generally Recognised As Safe (GRAS)** status by the U.S. Food and Drug Administration. It has antimicrobial activity due to its capacity to chelate Fe^{2+} along with HCO_3^- . It binds to cell surfaces and has a high affinity for the outer membrane proteins (OMP) of Gram negative bacteria. It also inhibits growth and neutralizes endotoxins. It has been approved at a level of 65.2 ppm for beef carcasses, and may be applied either as a mist or by spraying. It is not acidal agent but acts primarily by preventing pathogens from establishing a niche on meat surfaces.

Ozone (O₃) This gaseous compound possesses antimicrobial activity. Like chlorine, it is the most powerful oxidant available for conventional water treatment and is highly reactive. It is 1.5 times more potent than chlorine. It is effective in solution and in its gaseous form as it is unstable it must be generated on site and used. Because it is more effective in killing ***Cryptosporidium parvum*** than chlorine, its use in water treatment systems is increasing. It is normally supplied from ozone generators. The cell target for O₃ is the membrane where it disrupts permeability functions. Ozone is GRAS for bottled water use, and for use on a variety of fresh foods, but its strong oxidizing power does not recommend its use for red meats. A typical concentration used is 0.1-0.5 ppm, which is

effective against Gram-positive and Gram-negative bacteria as well as viruses and protozoa. Ozone treatment can be used in vegetables, fruits, beef etc. to destroy pathogens like ***E.coli O157:H7***, ***S. typhimurium***, ***Giardia lamblia***, etc.

Hydrogen peroxide (H₂O₂) Hydrogen peroxide is a strong oxidizing agent and it is formed to some extent by all aerobic organisms, and it is enzymatically degraded by the enzyme catalase :



It is used as a sterilant for food-contact surfaces of olefin polymers and polyethylene in aseptic packaging systems. Hydrogen peroxide vapors have microbiocidal properties. The antimicrobial effect of hydrogen peroxide attributes to a strong oxidizing effect on the bacterial cells and to the destruction of basic molecular structure of cellular proteins. H₂O₂ also prevent spores of ***Bacillus cereus*** from swelling properly during the germination process. It is used in the treatment of vegetables, fruits and fruit juice in the form of vapors.

Sodium chloride and sugars

These compounds are grouped together because of the similarity in their modes of action in preserving foods. **NaCl** has been employed as a food preservative since ancient times. The early food uses of salt were for the purpose of preserving meats. This use is based on the fact that at high concentrations, salt exerts a drying effect on both food and microorganisms. Salt (saline) in water at concentrations of 0.85–0.90% produces an isotonic condition for non-marine microorganisms. Because the amounts of NaCl and water are equal on both sides of the cell membrane, water moves across the cell membranes equally in both directions. When microbial cells are suspended in high salt concentration such as 5% saline solution, the concentration of water is greater inside the cells than outside. In diffusion, water moves from its area of high concentration to its area of low concentration. In this case, water passes out of the cells at a greater rate than it enters. The result to the cell is plasmolysis, which results in growth inhibition and possibly death. This is essentially what is achieved when high concentrations of salt are added to fresh meats for the purpose of preservation. Both the microbial cells and those of the meat undergo plasmolysis (shrinkage), resulting in the drying of the meat, as well as inhibition or death of microbial cells. Enough salt must be used to effect hypertonic conditions. The higher the concentration, the greater are the preservative and drying effects. In the absence of refrigeration, fish and other meats may be effectively preserved by salting. The inhibitory effects of salt are not dependent on pH, as are some other chemical preservatives. Most nonmarine bacteria can be inhibited by 20% or less NaCl, whereas some molds generally tolerate higher levels. Organisms that can grow in the presence of and require high concentrations of salt are referred to as **halophiles**; those that can withstand but not grow in high concentrations are referred to as **halodurics**. **Sugars** are involved in the preservation of food. Sugars, such as sucrose, exert their preserving effect in essentially the same manner as salt. One of the main differences lies in relative concentrations. It generally requires about six times more sucrose than NaCl to affect the same degree of inhibition. The most common uses of sugars as preserving agents are in the making of fruit preserves, candies, jams, jellies, fruit juices,

condensed milk etc. The shelf stability of certain pies, cakes, and other such products is due in large part to the preserving effect of high concentrations of sugar, which, like salt, makes water unavailable to microorganisms. Microorganisms differ in their response to hypertonic concentrations of sugars, with yeasts and molds being less susceptible than bacteria. Some yeasts and molds can grow in the presence of as much as 60% sucrose, whereas most bacteria are inhibited by much lower levels. Organisms that are able to grow in high concentrations of sugars are designated **osmophiles**; **osmoduric** microorganisms are those that are unable to grow but are able to withstand high levels of sugars. Some osmophilic yeasts such as **Zygosaccharomyces rouxii** can grow the presence of extremely high concentrations of sugars.

Flavoring agents

Many flavoring agents possessing definite antimicrobial effects are used in foods.

Flavor compounds generally have more antifungal activity than antibacterial. The non-lactic, Gram-positive bacteria are the most sensitive, and the lactic acid bacteria are rather resistant. The minimal inhibitory concentrations (MIC) of many flavoring compounds are 1,000 ppm or less against either bacteria or fungi. All were pH sensitive, with inhibition increasing as pH and temperature of incubation decreased. **Diacetyl** is one of the most effective flavoring agents, which is produced by **Lactobacillus**, **Leuconostoc** and **Streptococcus**. It is somewhat unique in being more effective against Gram-negative bacteria and fungi than against Gram positive bacteria diacetyl reacts with the arginine binding proteins of gram negative bacteria. **Menthol**, which imparts a peppermint like aroma inhibits **S. aureus** at 32 ppm, and **E. coli** and **C. albicans** at 500 ppm. **Vanillin and ethyl vanillin** are inhibitory, especially to fungi at levels < 1000 ppm.

Spices and essential oils

Many spices possess significant antimicrobial activity. The antimicrobial activity is due to specific chemicals or essential oils, Such as eugenol and Allicin. Antimicrobial substances vary in content from the allicin of garlic (with a range of 0.3–0.5%) to eugenol in cloves (16-18%). Cinnamon and clove oils are also highly effective against **Aspergillus parasiticus** aflatoxin production. Plant EOs such as cumin, caraway and coriander have inhibitory effects on organisms such as **Aeromonas hydrophila**, **Pseudomonas fluorescens** and **Staphylococcus aureus**. Basil has high activity against **B. cereus**, **Enterobacter aerogenes**, **Escherichia coli**, and **Salmonella**. Lemon balm and sage EOs have adequate activity against **L.monocytogenes** and **S. aureus**. Oregano and thyme EOs had comparatively high activity against enterobacteria. Minimum inhibitory concentration (MIC) of oregano and thyme at a range of 190 ppm and 440 ppm, respectively for **E. cloacae** and for lactic acid bacteria MIC of oregano and thyme at a range of 5 ppm and 440 ppm, respectively,

Acetic and lactic acids

The organic acids are commonly used as preservatives. These acids are present in the fermented foods such as pickles, sauerkraut, and fermented milks due to their production within the food by lactic acid bacteria. lactic acid bacteria, produce acetic, lactic, and propeonic acids during fermentation. These acids posses' antimicrobial effect which is

due to both the depression of pH below the growth range and specific toxicity by the undissociated acid molecules. Lactic acid function as a permeabilizer of the outer membrane of Gram-negative bacteria and thus possibly acts as a potentiator of other antimicrobials. Organic acids are employed to wash and sanitize animal carcasses after slaughter to reduce their carriage of pathogens and to increase product shelf life.

Antibiotics

Antibiotics are secondary metabolites produced by microorganisms that inhibit or kill a wide spectrum of other microorganisms. These antibiotics are produced by molds and bacteria of the genus **Streptomyces**, **penicillium** and a few by **Bacillus**. Subtilin and tylosin have been used as heat adjuncts for canned foods, Chlortetracycline and oxytetracycline at 7ppm concentration has been applied to poultry. Tetracycline has been permitted for fresh and other sea foods. The antibiotics may be applied as a dip or an ice.

Antimicrobial peptides

Antimicrobial peptides were first isolated from natural sources in the 1950s when nisin was isolated from lactic acid bacteria for potential application as a food preservative. Subsequently, antimicrobial peptides were isolated from other natural sources, such as plants, insects, amphibians, crustaceans, and marine organisms. Antimicrobial peptides (AMPs) are widely distributed in nature and are used by many if not all life forms as essential components of nonspecific host defence systems. Antimicrobial peptides present a promising solution to the problem of antibiotic resistance because, unlike traditional antimicrobial agents, specific molecular sites are not targeted, and their characteristic rapid destruction of membranes does not allow sufficient time for even fast-growing bacteria to mutate. **Lactoferrin** bovine and **activated lactoferrin (ALF)**, an iron binding glycoprotein present in milk, has antimicrobial activity against a wide range of Gram-positive and negative bacteria, fungi, and parasites. **Lactoferrin** has been applied in meat products and approved for their application in preservation of beef in USA. **Biopreservation is the use of natural or controlled microbiota or antimicrobials as a way of preserving food and extending it's shelf life**. Beneficial bacteria or the fermentation products produced by these bacteria are used in biopreservation to control spoilage and render pathogenic inactive in food. It is a benign ecological approach which is gaining increasing attention and **lactic acid bacteria (LAB)** are the important among them. Lactic acid bacteria have antagonistic properties which make them particularly useful as biopreservatives. When LABs compete for nutrients, their metabolites often include active antimicrobials such as lactic and acetic acid, hydrogen peroxide, and peptide bacteriocin. Some LABs produce the antimicrobial **nisin** which is a particularly effective preservative. These days LAB bacteriocins are used as an integral part of hurdle technology. Using them in combination with other preservative techniques can effectively control spoilage bacteria and other pathogens, and can inhibiting the activities of a wide spectrum of organisms, including inherently resistant Gram-negative bacteria.

Food Radiation

Radiation is often used in medicine and pharmacy field, but still remains a concern for using in food processing. After 1963, many researches appeared of using radiation, but the damage on the food led to expanded meetings held between different agencies which was related with radiation of food, these organizations were:

1. **WHO:** world health organization.
2. **FDA:** food and drug administration.
3. **IAEA:** international atomic energy agency.

The three organizations approved:

1. There is a group of food which is **not allowed to radiate** (such as **sugar**).
2. Limitation of the portions that are used for each food and the period of exposure to radiation.

Radiation on food measured by a unit called Rad (is energy absorbed by 1 gm irradiated food).

$$1 \text{ KRad} = 1000 \text{ Rad}$$

$$1 \text{ mega Rad} = 10^6 \text{ Rad}$$

The newer unit of absorbed dose is the Gray (**1 Gray = 100 Rad**).

Irradiation in food preservation

Generally **alpha, beta and gamma radiation** particles are used for the preservation of food. These radiations are of high frequency with a high energy content and they have the power to break molecules into oppositely charged units termed as ions. These radiations, are, therefore, called ionizing radiations. The treatment has a range of effects, including killing bacteria, molds and insect pests, reducing the ripening and spoiling of fruits and at higher doses inducing sterility. The technology may be compared to pasteurization. It is sometimes called '**cold pasteurization**', as the product is not heated. Irradiation is useful only for foods of high initial quality. A spoiled food cannot be reverted to un-spoiled state. Irradiation is not effective against viruses. It cannot eliminate toxins already formed by microorganisms. The radiation process is unrelated to nuclear energy, but it may use the radiations emitted from radioactive nuclides produced in nuclear reactors. Irradiated food does not become radioactive. National and International expert bodies have declared food irradiation as 'wholesome'. The food is exposed to controlled levels of ionizing radiation in the form of gamma radiation, X-rays and electron beams to kill harmful bacteria, pests, or parasites, or to preserve its freshness. The particle sources are readily available in the form of radio-isotope Cobalt 60. This is the most suitable gamma-particle emitter. The penetration power of different radiation particles is different. Alpha particles are stopped by a sheet of paper, beta particles can penetrate through 1-2 cm thick sheet and gamma particles can penetrate through 30-120 cm thick

sheet. UV-radiation are also used as an alternative means of treatment of foods but penetration power is very low. Ultraviolet radiation is valuable for reducing surface contamination on several foods. This short-wavelength light has been used in the cold storage units of meat processing plants.

Mechanism of microbial inactivation by radiation

Radiations causes disruption of internal metabolism of cells by destruction of chemical bonds, DNA cleavage results in loss of cells ability to reproduce . Free radicals are formed upon contact with water containing foods. These free radicals react with cellular DNA causing radiation damage. DNA is considered –radiation sensitivell portion of cells. Interaction between free radicals and DNA is responsible for –killing effect of ionizing radiation (IR). The ionizing radiations can cause chemical reaction and alterations of chemicals in tissues and which can be toxic or fatal to humans in high dose. Much of the reactivity of ionizing radiations in organism is with water and produces superoxide radicals (O_2^-), hydroxyl radicals (HO^-), hydroperoxyl radicals (HOO^-) and hydrogen peroxide. These are produced during high energy collisions of gamma rays and heavy elements (i.e. Tungsten). Alpha particles are hazardous when an alpha–emmitting isotope is inside the body. **Gamma Radiations are the most widely used type of ionizing radiation as they have good penetrating effect upto 20 cm in food depending on exposure time and emitted in all directions continuously.** The lethal dose for vegetative bacteria is 0.50-10 kiloGrays Kgy, bactrial spores is 10-50 Kgy, human beings and animals is 0.005-0.01 Kgy and for insects is 0.1-1.0 Kgy. There are many benefits of irradiations. These are to reduce or eliminate harmful food borne pathogens e.g. ***E.coli O157:H7, Camplyobacter, Salmonella, Trichinella, Listeria*** and many others, delays ripening of fruits and vegetables, eliminates insects in fruits and vegetables, inhibits sprouting in onions, potatoes. These irradiations also replace the need for chemical fumigation. This method is cheaper than freezing and refrigeration.

Irradiation dose reduced microbial spoilage (1.5 - 3 kGy) and eliminated pathogenic microbes (3-7 kiloGrays kGy) to improve shelf-life of meat, poultry and sea foods under refrigeration. It also reduced number of microorganisms in spices to improve hygienic quality (10 kGy) .

Food Irradiation Processes

Radurization: (0.75-2.5 Kgy) This method mimics pasteurization. It inhibits sprouting and delays ripening, kills insects and it is used for shelf life extension,

Radication: (2.5-10KGy) This method will eliminate spoilage microorganisms and non spore forming pathogens. Food will not get spoiled but still may contain some pathogens

Radapperization: (10-50 kGy) This method is also called radiation sterilization. Here reduction of microorganisms occurs to the point of sterility.

Effect of Radiation on Microorganisms :

Gram negative bacteria are generally more sensitive than Gram positive forms, bacterial spores are strongly resistant, yeasts tends to be rather more resistant than molds and smallest viruses required doses of >200 kGy to achieve a million-fold reduction in their numbers. **The principal targets of irradiation are nucleic acids and membrane lipids.**

Principles underlying destruction of microorganisms by irradiation

Several factors should be considered when the effects of radiation on microorganisms and these are discussed in the following subsections:

A. Type of microorganisms:

Gram positive bacteria are more resistant to irradiation than Gram negative. In general spore formers are more resistant than non-spore formers, with respect to the radio sensitivity of molds and yeast, the later have been reported to be more resistant than the former, with both groups in general being less sensitive than Gram positive bacteria.

1. High sensitive: (*Salmonella*, *Shigella*, *proteus*, and *pseudomonas*).
2. Medium sensitive: (*staphylococcus aureus*).
3. Resistant: (*Micrococcus radiodurans* and *Micrococcus radiophilus*).

B. Number of microorganisms:

The larger number of cells, the less effective dose.

C. Composition of food:

Microorganisms in general are more sensitive to radiation when suspended in buffer solution than in protein – containing media.

D. Presence or absence of oxygen:

The radiation resistance of microorganisms is greater in the absence of oxygen than in its presence.

E. Physical state of food:

The radiation resistance of dried cells is in general, considerably higher than for moist cells.

F. Age of microorganisms:

Bacteria tend to be most resistant to radiation in the lag phase.

Effect of irradiation on food quality:

The most sensitive nutrients to irradiation is vitamins followed by proteins and nitrogenous compounds (Denaturation) change in viscosity and destruction of amino acids and toxic compound result followed with fatty foods and sugars and toxic compound also produce. In general, it will change chemical composition, nutrient value, change in color and odor.

pH CONTROL

Almost every food, with the exception of egg whites and soda crackers, has a pH value of less than 7. Foods can be broadly categorized on the basis of their pH as high acid, acid, medium acid or low acid. Examples of each category include:

- High acid (pH: 3.7) : apples, lemons, raspberries
- Acid (pH: 3.7 to 4.6) : oranges, olives, tomatoes (some)
- Medium acid (pH: 4.6 to 5.3) : bread, cheese, carrots
- Low acid (pH: over 5.3) : meat, fish and most vegetables

Most micro-organisms grow best in the pH range of 6.5 to 7.5. Yeasts and moulds are capable of growing over a much broader pH range than bacteria. Most spoilage yeast and molds grow at pH value greater than 2.0. Few pathogens will grow below pH 4.0. This information is important, because it will help in determining food stability with respect to microbial spoilage.

Osmotic pressure

The principle of osmosis is applied. Foods are preserved by adding salts and sugars to them. These chemicals remove the water out of microbial cells causing them to shrink in hypertonic environment, thus stopping their metabolism. Jams, jellies, fruit syrups, honey etc. are preserved by high sugar concentration. Fish, meat beef and vegetable products are preserved with salt.