FE 305 FOOD MICROBIOLOGY
Food Preservation

Dr. Hüseyin BOZKURT

Food Preservation Methods

- All foods are originally living tissues of organic origin.
  - While the vitality of foods such as meat, fish and chicken is terminated during consumption, vital activities in other foods such as vegetables and fruits do not end but continue.
  - Foods are prone to decomposition due to their organic structure and to spoilage by saprophytic and parasitic microorganisms.
- After slaughter or harvest, the cells autolyze the food with the effect of the enzymes in them that destroy the cells. In many cases, a limited amount of enzyme activity may be beneficial.
- The ripening of fruits and softening of meat can be given as examples. In most cases, the effects of enzymes can result in harmful results and spoil foods.
- When the cellular structure becomes disordered, foods become vulnerable to attack by microorganisms.
- Bacteria, molds and yeasts transform the complex organic components of foods into simpler components that are the main cause of changes in aroma, texture, consistency, odor and color properties. This change is called microbial degradation.

Food Preservation Methods

- Methods used to preserve food are based on one or more of the following general principles:

(1) prevention of microbial growth and germination of spores in foods,

(2) killing vegetative cells of microorganisms and their spores in foods,

(3) physically removal of microorganisms from foods, and

(4) protection of foods against contamination.

- Agents killing the microbial cells are called **cidal** agents;
- Agents inhibiting the growth of microbial cells (without killing) are referred to as **static** agents.
  - Thus the term **bactericidal** (bactericide) refers to kill bacteria and
  - **bacteriostatic** refers to inhibit the growth of bacteria;
  - **fungicide** kills fungi, and so on.
- **Sterilization** is the complete destruction or elimination of all viable microorganisms including spores, viruses and other agents.
  - Sterilization procedures involve the use of heat, radiation, chemicals, or physical removal of viable microorganisms.

Food Preservation Methods

- The main purpose of food preservation is to prevent autolysis and to kill microorganisms.
- The preservation of foods in the short or long term is provided in various ways.
  - It is a method that enables short-term preservation to prevent the autolysis and deterioration of foods by inactivating the enzymes after exposure to boiling water or water vapor, called blanching.
  - All cooking methods, including the use of microwaves, cause similar effects and ensure the short-term preservation of foods by destroying some microorganisms in foods. In these applications, microbial growth is stopped or delayed.
  - Cooling processes applied with different techniques according to the structure, nature, usage patterns and storage air of the food and controlled atmosphere applications are also included in this group.
  - Sterilization by temperature application, pasteurization, freezing, dehydration (drying), chemical inhibitor addition and irradiation are the methods used for long-term food preservation.
- Filtration and centrifugation (precipitation) techniques, provided that they are suitable for the structure of the food, are also techniques that allow long-term preservation in foods by removing microorganisms from the food.

Food Preservation Methods

1. Prevention of contamination (Asepsis)
2. Removal of microorganisms
   a. Washing
   b. Extraction
   c. Centrifugation
   d. Filtration
3. Inhibition of microbial growth
   a. Preservation of food with chemical preservatives
   b. Low temperature storage (cold and freeze storage)
   c. Reducing water activity (drying, concentration)
   d. Storage in a controlled and modified atmosphere.
   e. Utilizing antagonistic relationships between microorganisms
4. Killing of microorganisms
   a. Heat treatments (pasteurization, sterilization)
   b. Radiation applications (ionizing radiation, microwave rays and UV radiation)
   c. Sterilant gases
   d. High pressure applications
5. Combined methods

Food Preservation Methods

Method	Mechanism of action	Primary recommendation	Major effects and limitations
Moist heat	Denaturation.	Foods, media.	Kills vegetative cells and spores. Restricted to materials that can withstand heat.
Dry heat	Oxidation and burning to ash.	glassware, metal instruments, animal carcasses, dressings, oils.	Kills vegetative cells and spores. Restricted to materials that can withstand higher temperature.
Low Temp.	Slows down the growth.	Preservation of milk, meat, cheeses, poultry, fish, etc.	Low temperature to retard growth of M.o.'s
Drying, curing	Loss of water, plasmolysis	Preservation of fruits, vegetables, sausage	Reduction of a_w to delay or prevent microbial growth.

Food Preservation Methods

Method	Mechanism of action	Primary recommendation	Major effects and limitations
Radiation	DNA destruction, dimmerization	Heat-sensitive plastic materials, packages, air, surface area.	Inactivate target m.o's. Expensive to operate. Require safety precautions.
MAs	Inhibiting or delaying growth.	Preservation of fruits, vegetables, meat, poultry, etc.	Inhibit aerobes and delay growth of facultative anaerobes due to low O_2.
Ferment.	Reduction of pH and production of antimicrobials.	Preservation and increasing the nutritional quality	Inhibiting unwanted m.o's with the production of organic acids, ethanol, bacteriosin, etc.
Emulsion	Nutrient limitation in aqueous droplets	Limitation of water in an area; e.g. in aqueous phase of butter.	Stops or reduces microbial growth.
High pressure	Destruction of cellular structure and constituents.	Application of HP or HCDP on liquid foods.	Inactivation of m.o's by high pressure and/or acid.

Food Preservation Methods

Method	Mechanism of action	Primary recommendation	Major effects and limitations
Removal	Completely remove or reduce m.o.'s.	Applying on liquid foods and air.	Microbial removal depending on the techniques (e.g., pore size of filter), extent of washing. Viruses and *Mycoplasma* may not be eliminated.
Combination methods	Denaturation, growth slows. Plasmolysis.	Cheese production and storage at refrigerator temp.	Inictivation and/or inhibition and/or delay microbial growth.
Food additives	Antimicrobial and plasmolysis	Preservation of different types of foods.	Inhibiting target m.o's.

Food Preservation Principles

a) Prevention or delay of microbial decomposition

- Many preservation methods inactivate or delay microbial growth and
  - prevent decomposition of foods.
- This can be provided by increasing lag and generation time.
- When all microorganisms are killed (or removed),
  - microbial decomposition will be prevented.
- Microbial decomposition of foods can be prevented or delayed by one or more of the following principles.

i) Keeping out microorganisms (asepsis).

- Asepsis is the avoiding the addition of actively growing microorganisms from unclean containers, equipments or utensils.
- Asepsis is the introducing as few spoilage organisms as possible.
- The common example is the application of aseptic techniques during filling of heated fruit juices into packages.

Food Preservation Principles

ii) Removal of microorganisms.

- Removal is an effective method in reducing microorganisms.
- Microorganisms may be removed from foods by filtration, centrifugation, washing or trimming.
- **Filtration** reduces number of microorganisms,
  - used on the liquids; such as fruit juices, soft drinks, wine and water.
- **Sedimentation** is used for the treatment of drinking water,
  - but is insufficient by itself.
- **Washing** raw foods can remove a number of microorganisms from surface area.
  - Washing fresh fruits and vegetables reduce soil microorganisms on surface area.
  - On the other hand, washing foods may be dangerous if water adds microorganisms or increases the moisture.
- **Trimming** away spoiled portions of a food or
  - discarding spoiled part of foods may reduce microbial numbers from food.

Food Preservation Principles

iii. Hindering growth and activity of microorganisms.

- This can be accomplished by one or more unfavorable condition(s), such as moisture, pH, low temperature, dehydration, additives and anaerobiosis,
  - retard the microbial growth.
- Unfavorable condition extend generation time and lag phase,
  - this will increase the keeping time of the food.

iv. Killing the microorganisms.

- Microorganisms can be killed or sublethally injured by heat or radiation.
  - Sublethally injured cells require better culture medium for growth than the untreated cells.
- Microbial inactivation in foods will increase the keeping time of the food.

Food Preservation Principles

b) Prevention or delay of self-decomposition of food

- This can be achieved:

(i) by inactivation of food enzymes, e.g. by blanching,

(ii) by delay of purely chemical reactions,

- e.g., prevention of oxidation using antioxidant.

c) Prevention of damage causing by insects, animals, mechanical causes, etc.

- M microorganisms can damage foods in agricultural area, during transport and storage of foods.
- Mechanical damage can result from processing of foods
  - such as freezing, drying, pressure, streak, etc.

Food Preservation Principles

2) Maintenance of Anaerobic Conditions

- A preservation factor in packaged foods is the creation of anaerobic conditions within the package or container.
- Anaerobic conditions in container or packages can be provided in four ways:
- (i) after filling the products into containers; evacuation of the unfilled space (the head space) and filling by CO_2 or an inert gas such as nitrogen.
  - This will produce anaerobic conditions.
- (ii) After hot filling the products into containers; vapor at headspace condensate during cooling,
  - reduce the amount of O_2 in headspace and
  - dissolving with product, and provide anaerobic conditions.
- (iii) Removal of air from packages; vacuum packed (VP) of foods by removal of air from packages,
  - provides anaerobic condition.
- (iv) During fermentation in a container or package; microorganisms produce anaerobic condition due to reduction of O_2, and production of CO_2 and H_2.

Food Preservation Principles

IV) Heat treatment Employed in Food Processing

- The different degrees of heat commonly used on foods might be classified as

(1) moist heat,

(2) dry heat,

(3) blanching,

(4) boiling and

(5) microwave heating.

Prevention of contamination (Asepsis)

- For the production of healthy and nutritious food, necessary precautions should be taken to prevent microbial contamination and development at every stage of the chain (raw material production, storage, transportation, food processing, wholesale and retail sales, preparation and service) from raw materials to consumers.
- Prevention or control of microbial contamination is achieved by sanitation practices.
- In the food industry, ***sanitation*** is all of the activities carried out to prevent or minimize microbial contamination in the chain from raw material production or harvest to the consumer.

Removal of microorganisms

WASHING

- The washing process is applied to the fruits and vegetables to be consumed fresh and the fruits and vegetables preserved by freezing, drying or processing into canned food.
- The washing process increases the effectiveness of the heat treatments to be applied by removing most of the microorganisms and heat-resistant spores along with the dust and soil on the fresh fruits and vegetables.
- Therefore, the washing water must be of potable quality.
- In some cases, antimicrobial agents can be added to the wash water in varying proportions depending on the purpose.
  - For example, adding 20-50 ppm of free chlorine to the water while washing carcasses is effective in reducing the microbial load on the meat surface.

Removal of microorganisms

SORTING

- With the sorting process applied in the food industry, especially in fruit and vegetable processing, spoiled and moldy fruit and vegetables are removed from the environment.
- This process not only prevents heavy contamination of intact fruit and vegetables, but also reduces the high number of microorganisms that cause spoilage, thus making the preservation method to be applied to the food more effective.

CENTRIFUGAL

- ✓ Centrifugation is not a very effective method for the removal of microorganisms. The main purpose of this method applied to milk is to remove foreign particles suspended in milk from milk.
- ✓ The process also removes some of the bacteria and spores from the environment depending on the centrifugation force applied.
- ✓ For this purpose, 20,000-25,000 x g centrifugation is applied. This process applied to milk is called bactofugation.
- ✓ In this process, 1.5% of the milk is separated with sediment and bacteria.
- ✓ The process removes 90-97% of bacteria found in milk. In the two-stage bactofugation process, it is possible to remove 99% of the bacteria found in the original milk.

Removal of microorganisms

FILTRATION

- Among the methods based on the principle of removing microorganisms, the most effective method is filtration.
- This method, which is an alternative to the thermal pasteurization process, can only be applied in clear liquids and all microorganisms are removed from the liquid.
- Membrane filters with a pore diameter of 0.45 mm used for this purpose retain all microorganisms except viruses.
  - Membrane filters are cellulose esters (acetyl cellulose or nitrocellulose).
- Membrane filtration is used for many purposes other than sterilization of liquid foods. These:
  - Microbiological analysis of clear liquids
  - sterilization of air
  - Sterilization of high temperature sensitive solutions (vitamin solution)
  - Clarification of water used in blending alcoholic beverages

Inhibition of microbial Growth-

1. Preservation of Food with Chemical Preservatives

- Chemical substances that are not included in the basic structure of foods and added to foods during processing, storage and packaging are called additives.
- Additives are often added to foods in very small amounts to improve the appearance, flavor, texture and other storage properties of foods.
- In the classification of food additives, the function of the additive in the food to which it is added is generally taken into consideration.
  - Such as acidifiers, antioxidants, colorants, enzymes, flavors, stabilizers, sweeteners, emulsifiers, anti-caking agents, thickeners and preservatives.
- In the use of these substances in the food industry, it should be aimed to protect the consumer as well as the technological benefit they provide.

1. Preservation of Food with Chemical Preservatives

- As long as the use of food additives for the benefit of the consumer serves the following points, it becomes technologically acceptable and can be used for this purpose:
  1. Preservation of the nutritional value of food.
  2. Increasing the durability of food by improving storage conditions and preventing losses.
  3. Increasing the appeal of food in a way that does not deceive or mislead the consumer.
  4. Having an auxiliary function in the processing of food.

- In some cases, the use of food additives should not be allowed:
  1. In order to cover the defects that arise as a result of improper processing or storage of food under inappropriate conditions,
  2. In order to mislead or deceive the consumer.
  3. In cases where the nutritional value of the food is significantly lost,
  4. Provided that it is economical, it is not allowed to be used in cases where the curative effect obtained by the use of food additives can be achieved by another production technique that can be applied to the food.

1. Preservation of Food with Chemical Preservatives

- Before deciding on the use of food additives, years of toxicological studies are carried out and the following points are taken into account in determining the limits of use:
  - The consumption level of the food for which the use of additives is recommended.
  - The minimum dose that causes adverse effects (toxic and histopathological) in experimental animals.
  - The dose that will not pose a health risk in all consumer groups.
- The points to be considered in the use of chemical preservatives can be listed as follows:
  1. It should be used when another containment method is not applicable or insufficient.
  2. It should be economical and have an antimicrobial effect at low concentrations.
  3. It should extend the storage life of the food.
  4. It should not create an undesirable flavor and odor in the food to which it is added.
  5. It should not have a harmful effect on human health at the levels it is used.
  6. It should be easily identifiable by chemical analysis.
  7. It should not interfere with the activity of digestive system enzymes.
  8. It should have a broad antimicrobial spectrum and preferably be effective on microorganisms that cause food poisoning.

1. Preservation of Food with Chemical Preservatives

- Sodium benzoate
- Parahydroxybenzoic Acid Esters (Parabens)
- Sorbic Acid and Its Salts
- Propionic Acid and Its Salts
- Sulfur Dioxide and Sulfites
- Acetic Acid and Acetates
- Nitrites and Nitrates
- Diethyl Polycarbonate (DEPC)
- Antibiotics
  - Chlortetracycline and Oxytetracycline
  - Nisin and Subtilin
  - Tylosin
  - Nystatin and Pimaricin (Natamycin)
  - Ethylene and Propylene Oxide
- Antioxidants, Sequesterans and Flavoring Agents

Types of antimicrobials used in food		
Traditional preservatives	Synthetic preservatives	Antibiotics
Sugar, salt, smoke, spices. Vinegar, alcohol,	**Organic:** Acetic acid, acetates, sorbic acid, benzoic acid, boric acids, citric acid, formic acid, lactic acid, propionic acid and their salts. **Inorganic:** Carbonic acid, sulphurous acid, sulphites nitrates, nitrites, phosphates, hydrogen peroxide.	Nisin, tylosin, pimaricin

Types of common food additives used in food production			
Type of Ingr.	**What They Do**	**Examples of Uses**	**Names**
Preser-vatives	Prevent food spoilage (antimicrobials); Prevent color changes, delay rancidity (antioxidants); Maintain flavor or texture, freshness	Fruit sauces and jellies, beverages, baked goods, cured meats, snack foods, cereals, margarines, oils, dressings, fruits, vegetables.	Ascorbic acid, citric acid, sodium benzoate, sodium erythorbate, sodium nitrite, calcium sorbate, potassium sorbate, BHA, BHT, tocopherols.
Sweete-ners	Add sweetness with or without the extra calories.	Beverages, baked goods, confections, table-top sugar, substitutes, many processed foods	Sucrose, glucose, fructose, sorbitol, mannitol, saccharin, aspartame, sucralose, acesulfame potassium, corn syrup, high fructose corn syrup.
Color additives	Enhance colors, provide color to colorless and "fun" foods.	Snack foods, soft drinks, margarine, cheese, jams, jellies, gelatins, puding, pie, fillings.	Blue No. 1,2, Green No. 3, Red No. 3,40, Yellow No. 5, 6, Orange B, Citrus Red No. 2,

Types of Ing.	What They Do	Examples of Uses	Names present on Product Labels
Flavors, spices	Natural or synthetic flavors, spices.	Pudding, gelatin, cake mixes, soft drinks, salads, ice cream.	Monosodium glutamate, hydrolyzed soy protein, yeast extract, inosinate, spices.
Emulsifiers	Allow smooth mixing, prevent separation, products stability, more easily dissolving, reduce stickiness, control crystallization.	Salad dressings, peanut butter, chocolate, frozen desserts, margarine,	Soy lecithin, polysorbates, mono- and di-glycerides, egg yolks, sorbitan monostearate.
Stabilizers, thickeners, binders, texturizers	Produce uniform texture, improve "mouth-feel".	Dairy products, cakes, puding, gelatin, jams, jellies, sauces.	Gelatin, pectin, xanthangum, carrageenan,, whey.
Enzyme preparations	Modify proteins, fats, polysaccharides.	Cheese, dairy products, meat.	Lactase, papain, rennet, chymosin.

Inhibition of microbial Growth-
2. Low temperature storage (cold and freeze storage)

Cold storage refers to temperatures above the freezing point. Preservation at temperatures below freezing is understood as freeze preservation.

Cold storage:

- ✓ All chemical reactions slow down at low temperature,
- ✓ The activity of microorganisms and enzymes in the food (in its natural structure) slows down and
- ✓ The formation of adverse changes that may occur in food also slows down.

Freeze preservation:

- ❖ Foods to be stored for a long time are frozen.
- ❖ While the activity of enzymes and chemical reactions naturally found in the food structure slows down with ice cream, microbial development is completely stopped.
- ❖ Freezing and thawing can change the physical structure of some foods.

2. Low temperature storage (cold and freeze storage)

- As the temperature decreases, the delay phase and generation, that is, the time of division into two, of the microorganisms lengthens, while the development slows down and eventually stops completely.
- The increase in the lag phases and generation times of microorganisms and the minimum temperature at which they can develop depend on other factors such as the nutrient content of the environment, pH and water activity. As these factors move away from the optimum, the negative effect of low temperature on the growth rate of microorganisms increases.
- The minimum growth temperature for microorganisms can be defined as the point at which the delay period and generation time are infinite. At this point, the organism cannot reproduce actively, but a slow metabolic activity can continue.
- In addition to the decrease in the growth rate of microorganisms at low temperatures, the slowdown of other chemical and enzymatic reactions that may occur in food also slows down the formation of changes that may occur in food as a result of these reactions.
- Plant and animal foods contain different kinds of bacteria, molds and yeasts. These microorganisms develop under suitable conditions and cause undesirable changes in the physical and chemical structure of the food.
- There is an optimum temperature value at which each microorganism can grow best and a minimum temperature at which it cannot grow. Therefore, low temperatures have different effects on various microorganisms.

2. Low temperature storage (cold and freeze storage)

MECHANISMS FOR MICROBIAL CONTROL

- The enzyme reaction and microbial growth rates are maximum at the optimum growth temperature.
- Food spoilage usually results from biochemical reactions by microbial enzymes.
- Preservative effect of temperatures is used to
  - retard chemical reactions and action of food enzymes,
  - to slow down the growth and activity of microorganisms in foods.
- Shelf life of many foods can be increased by storage at low temperatures.
- As the temperature is lowered, growth of microorganisms slows down.
- The metabolic activity increases with a rise in temperature.

2. Low temperature storage (cold and freeze storage) MECHANISMS FOR MICROBIAL CONTROL

- Each 10°C rise in temperature from minimum,
  - there is a twofold increase in the catalytic activity of enzymes.
- For every 10°C decrease in temperature from optimum,
  - the revere is true.
- Cool storage involves the storage of food at low temperature below the minimal growth temperature of most foodborne microorganisms.
- Water presents in foods as free and bound (with the hydrated molecules, etc.).
- When the temperature of a food is reduced below -2°C,
  - free water in the food and inside the cells starts to freeze and forming ice crystals (while pure water freezes at 0°C).
- As the temperature drops further and more ice crystals form,
  - solute concentration increases in remaining water,
  - which in turn further increase freezing point of water in the solution.

2. Low temperature storage (cold and freeze storage) MECHANISMS FOR MICROBIAL CONTROL

- The water molecules in the food start freezing,
  - water molecules from microbial cells migrate outside,
    - causing dehydration of cells and
    - concentration of solutes and ions.
  - The remaining or unfrozen free water at each temperature therefore becomes more and more concentrated with solutes (salts, proteins, nucleic acids, etc.).
- This can
  - change the pH of cellular matter,
  - concentrate ions,
  - alter colloidal status,
  - denaturate proteins and
  - increase viscosity.
- When the temperature is reduced bellow -20°C,
  - the water in the food and inside the cell is frozen.
- Therefore microbial cells are exposed to
  - low pH (due to concentration of ions) and
  - low a_w (due to concentration of solutes) inside and outside the cells.

2. Low temperature storage (cold and freeze storage) MECHANISMS FOR MICROBIAL CONTROL

- This can cause denaturation and destabilization of the structure and functions of macromolecules in the microbial cells.
- At rapid rate of freezing, the very small ice crystals form rapidly and the cells are not exposed to solution effect as well as slow freezing.
- Psychrotrophs contain increased amounts of unsaturated fatty acids in their membrane lipids when grown at low temperatures.
- This decreases melting point of lipid.
  - Increased synthesis of unsaturated fatty acids at low temperature can minimize the lipid in a liquid and mobile state,
    - thereby allowing membrane proteins to continue to function.
- In addition, the transport permeases of psychrotrophs are apparently more active at low temperatures
  - than mesophiles, and
- a cold-resistant transport system is characteristic of psychrotrophic bacteria.

2. Low temperature storage (cold storage)

- Temperature is an important environmental factor that determines which types of microorganisms can become dominant in foods stored at low temperatures.
- Psychrophilic and psychrotrophic bacteria are the most important bacterial groups in cold-preserved foods. The optimum growth temperature for these bacteria is usually around 15-20°C, but they grow down to -10°C below the freezing point.
- Deterioration of meat, milk and other animal products stored in the cold usually occurs as a result of metabolic activity of aerobic psychrotrophic bacteria such as *Pseudomonas, Moraxella, Acinetobacter, Alcaligenes, Flavobacterium and Alteromonas*.
- On the other hand, lactic acid bacteria, *Brochothrix thermosphacta, psychrotroph Enterabacteriaceae and Aeromonas* species cause spoilage in vacuum-packed cold-stored meats.
- Many types of molds and yeasts can grow, albeit slowly, at temperatures around 0°C and just below.
- In general, food stored at 10°C deteriorates 2 times faster than food stored at 5°C, and 4 times faster than food stored at 0°C.
- Mesophilic bacteria generally cannot grow at temperatures below 4-5°C.

2. Low temperature storage (cold storage)

- The growth and toxin production of bacteria that cause food poisoning are effectively inhibited at temperatures below +4.4°C, except psychrotrophs *Clostridium botulinum* type E, non-proteolytic types B and F, *Yersinia enterocolitica, Aeromonas hydrophila and Listeria monocytogenes*.
- It is known that psychrotroph *Yersinia enterocolitica* can grow to -2°C, psychrotroph *Aeromonas hydrophila* to +4°C and *Listeria monocytogenes* to 0°C under suitable conditions.
- Generally, cold storage is not applied alone in the preservation of foods. For example, psychrophilic bacteria that cause deterioration in animal products such as meat, milk and eggs stored in the cold are aerobic microorganisms. As oxygen is removed from the system in vacuum packaging or packaging in a CO_2 atmosphere together with cold storage, microbial spoilage is significantly delayed.
- Curing, smoking or heat treatments can be applied in order to inhibit microbial growth or reduce the number of microorganisms in foods that are kept cold.
- When it comes to cold storage, temperatures above and below freezing can be understood..

2. Low temperature storage (cold storage)

- 0°C is the freezing point of pure water under standard conditions and no food is frozen at 0°C. The freezing temperature of many foods varies between -0.4 and -2.8°C. Example: beef -1.6 to -2.2°C, chicken -2.8°C, fish -0.6 to -3.3°C, milk -0.5°C, apple -2 It freezes at .0°C, grapes at -2.5°C, lemon at -1.5°C.
- Temperatures between the freezing point of the food and 0°C in cold storage are defined as "***Latent-zone chilling***" and foods can be stored for longer periods in general at these temperatures. However, due to the different characteristics of foods, the temperatures at which they can be best preserved may differ.
- Due to the different characteristics of various foods, the cooling needs also differ. The ideal temperature for the preservation of meat is between 3°C and -1°C. At -2°C, the meat starts to freeze.
- The temperature of the carcasses after slaughter is between 38-40°C. The carcass temperature should be reduced as soon as possible from a microbiological point of view. After cooling the carcasses, keeping them at 16°C for 16-20 hours is recommended in terms of maturation and brittleness of the meat. During maturation, UV lamps can be used to control the microbial growth on the surface.

2. Low temperature storage (cold storage)

- In cured meats, on the other hand, since the curing salts prevent the growth of psychrophilic bacteria, these products can be stored in the cold for a longer period of time compared to fresh meats from a microbiological point of view.
- In chicken carcasses, body temperature should be lowered quickly after slaughter. This is usually done by immersion in cold water.
- The most important factors determining the shelf life of chicken meat in cold storage are the storage temperature and the initial microorganism load on the carcass surface.
- The number of microorganisms at the beginning depends on the slaughter conditions, operating sanitation, and hygiene conditions applied after slaughter and slaughter.
- Chicken meats are more susceptible to spoilage than red meats.
- Fish products are a very sensitive food group to spoilage. Fish should be cooled immediately after fishing. For this purpose, chilled sea water, cold storage or ice storage methods can be applied.
- The quality of fresh fruits and vegetables depends on the growing conditions and the processes applied after picking. After being picked, fruits and vegetables can remain intact for a certain period of time, regardless of the plant from which they were plucked.

2. Low temperature storage (cold storage)

- One of the most important biochemical reactions that continues after harvest in fruits and vegetables is respiration. Many compounds in plant tissues are destroyed by respiration and energy is released as a result of these reactions.
- The respiration rate that continues after harvest in fruit and vegetable tissues is a function of temperature, among other factors mentioned above. As the temperature drops, the respiratory rate in the tissues slows down.
- With the slowing of respiration, other biochemical reactions that may occur in the tissue slow down and maturation is delayed.
- Delayed ripening enables fruits and vegetables to retain their structural properties longer and at the same time to be more resistant to microbial infections.
- In addition to slowing down the chemical and enzymatic reactions occurring in the tissue of the food at low temperature, the microbial growth rate also slows down.
- Minimizing the losses is possible by cooling the product immediately after harvest and keeping it cold.
- The cooling of fruits and vegetables immediately after harvest can be done either by spraying cold water or by vacuum cooling method.
- Hypochlorite can be added to the water in order to increase the efficiency of the method by killing the microorganisms both in the water and on the fruits and vegetables.

2. Low temperature storage (cold storage)

- Vacuum cooling is generally applied to products with high surface/volume ratio such as lettuce and spinach. In this method, water is sprayed on the vegetable first and then evaporated under vacuum. Vacuum cooling level
- evaporation depends on the amount of water.
- As with other foods, temperatures slightly above the freezing point are generally applied when keeping fruits and vegetables fresh.
- However, when some subtropical and tropical fruits and vegetables are stored at low temperatures, a phenomenon called ***chill injury*** occurs, the mechanism of which is not fully explained. For this reason, ideal storage temperatures for such fruits and vegetables are well above the freezing point.
- When cold-damaged foods are exposed to room temperature, first the respiratory rate increases and then the respiration slows down rapidly as a result of the death of the cells. However, cold damage also occurs in some temperate climate products.
- The optimum relative humidity required to be in the atmosphere in cold storage varies according to environmental factors such as temperature and gas composition in the atmosphere and the food stored.
- Too low relative humidity causes moisture and weight loss in fruits and vegetables and ultimately shriveling, while too high encourages microbial growth on the surface of the food.
- Changes in relative humidity or temperature during storage can cause sweating or condensation on the surface of the food.

2. Low temperature storage (freeze storage)

- It is widely used in meat and some fruit and vegetable preservation. Freezers that provide a temperature of (-20°C) are usually the most used. It can be stored for weeks or months at this temperature. However, it should be noted that there may still be growth in the water in the frozen mass. It is required for long-term storage (-80°C/ Dry ice).
- The freezing point of most of the foods is between (-0.5°C) – (-3°C).
- With the freezing event;
  1. Physical damage occurs to both the food and the microorganism.
  2. The water activity of the food decreases. The availability of water for chemical reaction and microbial activity decreases.
  3. Dehydration occurs in tissue and microbial cells. As a result, the intracellular substance concentration increases. Some irreversible changes and protein denaturation occur.
  4. Freezing creates thermal shock on microorganisms. Freezing is not a sterilization method, it is only a method of inactivating microorganisms in the food. However, there is also some microbial death.

2. Low temperature storage (freeze storage)

- ✓ Generally; Gram positive (+) bacteria are more resistant to freezing than gram negative (-) bacteria.
- ✓ In terms of frost resistance; There is no significant difference between mold, yeast and bacteria.

Advantages of Freeze Storage:

1. No need for preservatives or similar chemicals,
2. There is no change in the natural taste,
3. It does not cause a significant loss in nutritional value.

Disadvantages of Freeze Storage:

1. There is a decrease in the number of living micro-organisms, but microorganisms cannot be completely eliminated.
2. Toxins are not affected by the freezing process.

Inhibition of microbial Growth- 3. Reducing water activity

- The basic condition for microbial activity, enzymatic and chemical reactions in foods is the presence of water.
- Water in foods can be reduced to a limiting level for microbial and enzymatic reactions, either by physical removal or by adding water-soluble substances to the food.
- Drying is one of the oldest methods of food preservation.
- The purpose of drying is to reduce the amount of water in foods to a level where microorganisms that cause spoilage and pathogenic microorganisms cannot grow and maintain their enzymatic activities.
  - Drying

Drying

Dryers

- Apart from natural drying under the sun, mechanical drying methods are used for drying many products:
  - I. Spray dryers
  - ii. Lyophilizers
  - iii. Vacuum dryers
  - iv. Foam dryers
  - v. Tunnel dryers
  - vi. Fluidized bed dryers and
  - vii. They are tumble dryers.
- The purpose of drying is to make the food resistant to microbiological and chemical changes by reducing the water activity (aw) value below a certain value.
- The lethal effect of drying on microorganisms depends on the following factors:
- The genus, species, physiological age and number of the microorganism
- Drying conditions (drying method, drying temperature, drying time and dehydration rate)
- Type and composition of the food (pH, inhibitory substances, etc.)

Inhibition of microbial Growth-
4. Storage in Controlled and Modified Atmospheres

- The trend of consuming fresh foods is increasing day by day. The most suitable and effective method for keeping foods fresh by delaying deterioration is the cold storage technique. However, nowadays, in addition to the cold storage application, the application of controlled atmosphere storage or modified atmosphere packaging techniques has found an increasing application area in preserving the freshness of foods for a longer period of time.
- The storage conditions created by adjusting the CO_2 and O_2 ratios in the warehouse atmosphere while keeping the food fresh is called controlled atmosphere.
- In the modified atmosphere, the air in a gas-impermeable or a certain level of gas permeability package is removed by vacuum (vacuum packaging), or after the air in the package is removed by vacuum, the package is filled with N_2, CO_2 or a mixture of these two gases in certain proportions. Modified atmosphere can also be obtained by replacing the air in the package by washing it with N_2 or certain ratios of N_2-CO_2 mixtures.

Inhibition of microbial Growth-
4. Storage in Controlled and Modified Atmospheres

- Four different techniques are used to modify the atmosphere of foods:
  - controlled atmosphere packaging (CAP): concentration of gases controlled during storage,
  - modified atmosphere packaging (MAP),
  - vacuum packaging (VP) and
  - active packaging (AP).
  - Choice of a packaging atmosphere may be due to:
    - effect on aerobic microorganisms,
    - retaining food color,
    - prevention of oxidative deterioration,
    - inhibition of ripening,
    - protecting foods from collapse, etc.

Inhibition of microbial Growth-
4. Storage in Controlled and Modified Atmospheres

- **O_2** reduced or completely removed to
  - Prevent the growth of aerobes.
  - Low level of O_2 in the atmosphere improve color of food.
  - Prevent growth of anaerobic microorganisms.
- **CO_2** presence in MA
  - CO_2 provide anaerobic conditions
    - Inhibit aerobic microorganisms
  - CO_2 solubilize in aqueous phase to form carbonic acid.
  - Carbonic acid reduce internal and external pH.
    - Inhibit microorganisms.
- **N_2** is used as filler gas.
  - Exchange with O_2
    - reduce O_2
    - prevent aerobic growth
  - Prevent collapse of package du to reduction of gas pressure in packaging during storage.

Inhibition of microbial Growth-
5. Antagonistic Relationship Between Microorganisms

- It has been known for a long time that lactic acid bacteria have inhibitory or cidal (lethal) effects on closely related species and different strains within the same species, and on some pathogenic and spoilage microorganisms.
- The antimicrobial effect of lactic acid bacteria on other microorganisms results from the diacetyl, organic acids and hydrogen peroxide they produce, apart from bacteriocins.
- As an alternative to the use of chemical preservatives, there have been intense studies in recent years on the use of the antagonistic effect of lactic acid bacteria on other microorganisms in food preservation.
- Lactic acid bacteria with such antagonistic effects are species belonging to the genera *Lactococcus, Enterococcus, Lactobacillus, Carnobacterium and Pediococcus*.

Inhibition of microbial Growth-
5. Antagonistic Relationship Between Microorganisms

- These compounds, which are produced by lactic acid bacteria and have an inhibitory or lethal effect on bacteria and various pathogens that cause food spoilage, are called bacteriocins as a general name.
- Most bacteriocins are encoded by plasmids and are generally resistant to high temperatures. The vast majority of bacteriocins are proteinaceous and have lethal effects.
- The best known of the bacteriocins is nisin, produced by *Lactococcus lactis*.
- It has been determined that 5% of lactic *Streptococcus* produce bacteriocin or bacteriocin-like substances. Lactosidin, acidolin and acidophilin are bacteriocins produced by *Lactobacillus acidophilus strains, helveticin by Lactobacillus helvelicus, pediocin Pediococcus pentosaceus, lactobrevin and lactobacillin by L.brevis, and bulgarican by L.bulgaricus. Leuconostoc gelidum* produces a bacteriocin-like substance effective against *Enterococcus faecalis and Listeria monocytogenes* species. *Listeria* species are susceptible to lactic antagonism.

Killing of Microorganisms-
1. Heat Treatments-Moist Heat

- While the microorganisms in the environment are killed in the preservation of foods by applying heat treatment, on the other hand, it is a technological problem to preserve the physical quality of this food and to keep the losses in nutritional value to a minimum.
- The meaning and application of sterilization in the preservation of foods by heat treatment is different from sterilization in a microbiological sense.
- Sterilization in Microbiology; While it refers to the killing of all living things in the environment, on the other hand, some aerobic and thermophilic bacterial spores that are resistant to high temperatures can maintain their vitality in sterilized canned foods. For this reason, sterilization applied in the food industry is called "commercial sterilization".
- Some bacterial spores with high thermal resistance can maintain their viability in commercially sterilized and hermetically packaged foods, but they cannot grow due to environmental conditions.
- Although some spores can maintain their vitality, they usually do not develop because they are damaged by heat treatment.

Killing of Microorganisms-
1. Heat Treatments

- Heat treatments applied to foods kill microorganisms and make the food microbiologically stable, while also inactivating the enzymes in the structure of the food.
- Inactivation of enzymes is especially important in HTST (High Temperature Short Time) or flash pasteurization applications. In such applications, enzymes cannot be completely inactivated under conditions often necessary for the killing of microorganisms. Pectolytic enzymes and peroxidase enzymes found in the structures of fruits and vegetables are highly resistant to high temperatures.
- The parameter specifying the time required for the inactivation of enzymes at a certain temperature is called the "***Enzyme inactivation factor***" or "***E-value***". This value varies according to the characteristics of the foods.
- The thermal stability of enzymes also depends on many factors, as in microorganisms. The temperature dependence of enzyme inactivation can be explained by principles applicable to microorganisms.
- In heat treatment calculations based on enzyme inactivation, the enzyme with the highest thermal resistance that will affect the product quality during storage is taken as the target.

Killing of Microorganisms- 1. Heat Treatments

- There are also some changes that affect the nutritional value or sensory properties of foods during heat treatment.
- As a result of these changes, the vitamins in the composition of the food are broken down and the color, taste or structure of the food deteriorates.
- These losses in food items during heat treatment can also be determined. The value representing these losses is called the "***C-value***" (***Cook value***).
- Food components that can be used as indicators for this purpose can be thiamine, ascorbic acid and chlorophyll or Maillard reaction products.
- Thermal resistance of microorganisms.
  1. they are in the form of vegetative cells or spores,
  2. pH and composition of the medium,
  3. age of microorganisms,
  4. applied temperature, time and
  5. factors such as the number of microorganisms intended to be killed.

1. Heat Treatments

Moist Heat

- Moist heat effectively kills cells by denaturating their proteins.
- Moist heat applications in food industry are;
  - pasteurization,
  - tyndallization,
  - blanching,
  - drying/concentration,
  - boiling and
  - autoclaving.

1. Heat Treatments

a) Pasteurization

Effect

- Pasteurization is a mild heat treatment to inactivate enzymes and destroy bacterial cells.
- The main objective of this treatment is to eliminate non-spore-forming pathogenic bacteria.
- A significant proportion of the spoilage microorganisms are also destroyed to extended shelf life.
- For safety and keeping quality,
  - packaging after pasteurization prevents contamination.
- Pasteurized food must be stored at low temperature to prevent the growth of sporeformers.
  - Therefore pasteurization
    - kills pathogenic microorganisms,
    - kills or reduces spoilage microorganisms and
    - kills compiting microorganisms (in fermentation)
      - **but does not sterilize the product.**

1. Heat Treatments

Pasteurization process

- **Flash pasteurization**
  - This involes a continuous system,
  - Foods (e.g. Milk) pass through a heat exchanger with a continuous-flow under high temperature.
  - heated under careful control of product with a flow rate, ane temperature raises.
  - Then product is rapidly cooled.
  - This process is called *flash pasteurisation.*
  - Heated from 72 to 79°C for 15 to 25 sec;
    - to decrease total microbial load and increase the shelf-life of the product.
- At flash pasteurization, nutrient loss is very low when compaired with vat pasteurization.
- When pasteurization is applied together with combination of other methods,
  - this involves pasteurization of product at low temperature and short time exposure compaired with individual pasteruzation.

1. Heat Treatments

- **Vat pasteurization**
  - Milk is heated in a large vat from 63 to 66°C for 30 min.
    - called *vat pasteurization*.
  - Combination methods used to supplement pasteurisation are refrigeration, asepsis (e.g., aseptically packaging), anaerobic conditions (e.g., evacuated, sealed container), high concentration of sugar (e.g., sweetened condensed milk) and presence chemical additives (e.g., organic acids or pickles).

1. Heat Treatments

Pasteurization time-temperature

- is named for Louis Pasteur, first used heat (at 60°C for 30 min) for wine.
- Today, it involves the application of heat treatment below 100°C.
- is applied by heat treatment at 71.7°C for at least 15 sec in high temperature short time (HTST) method (cont. sytem),
- is applied by heat treatment at 62.8°C for 30 min in low temperature long time (LTLT) method (vat past.).
  - Ice cream mix can be pasteurized at 71.1°C for 30 min or at 82.2°C for 16 to 20 sec.
  - Grape wines may be pasteurised at 82 to 85°C for 1 min in bulk.
  - Dried fruits are pasteurized in the package at 65.6 to 85°C for 30 to 90 min.
  - Bottled grape juice is pasteurized at 76.7°C for 30 min or at 80 to 85°C for 15-30 sec,
  - bottled apple juice is pasteurized at 60°C for 30 min or at 85 to 88°C for 30 to 60 sec,
  - vineger at 71.1°C for 15 sec.

1. Heat Treatments

- Time-temperature relationships for pasteurization are determined depending on the inactivation of pathogens:
  - *Mycobacterium tuberculosis* was regarded as the most heat-resistant pathogen likely to occur in milk.
  - The pasteurization temp. is adjusted to 61.6°C for 30 min.
  - Later *Coxiella burnetii* was discovered as a more heat resistant pathogen (causing Q fever) in milk than *M. tuberculosis*.
    - can survive in milk heated at 61.6°C for 30 min.
  - This observation increased the pasteurization temperature to HTST application.
- HTST is sufficient to destroy nonsporeforming pathogenic bacteria;
  - such as *Mycobacterium tuberculosis*, *Coxiella burnetii*, yeasts, molds, and Gram-negative and Gram-positives bacteria.

1. Heat Treatments

To determine whether a particular batch of milk has been **pasteurized or not**:

- preform a phosphatase test.
- Phosphatase is an enzyme present in raw (unheated) milk that is destroyed by adequate pasteurization.
- The relationships between phosphatase enzymes and *M. tuberculosis* for pasteurization are present:
  - Absence of this enzyme indicates proper pasteurization and inactivation of this bacterium.
- In **phosphatase test**,
  - subtrate is added into mil, which is specific for this enzyme.
  - The amount of phenol liberated can be conveniently estimated by the addition of a reagent,
  - which turns blue in the presence of phenol.
  - Color standards are used to interpreter the results of this test.
  - Color change indicate active enzyme and insuffiecient heat treatment.

1. Heat Treatments

b) Tyndallization

- There is a low-technical way to sterilize media on successive 3 days at 80 to 100^{o}C for 30 min.
  - even if there are spores.
  - day 1, most of the vegetative bacteria, yeasts, molds and mold their spores are killed, but some bacterial spores will survive.
    - surviving spores may germinate during overnight incubation into vegetative cells.
  - day 2, the broth is heated after incubation, the vegetative cells from germinated spores are killed.
    - the medium is incubated, if remaining spores present, spores germinate to for vegetative cells
  - day 3, broth is heated as an additional precaution.
  - Tyndallization is used for heat sensitive material which can't withstand autoclaving, have no autoclave.

1. Heat Treatments

c) Blanching

- Blenching fresh vegetables before freezing or drying involves heating briefly at about 90 to 100°C: boiling water or steam.
  - Blanching is a cooking technique involving heating food in water for a very short time.
  - Vegetables are blanched prior to freezing or canning.
- privent enzymatic changes, color, flavor, nutritional value.
- Over-blanching causes loss of flavor, color, vitamins and minerals.

d) Heating at about 100°C

- Home foods are heat treated at about 100°C for varying lengths of time.
- This treatment is sufficient to kill every living forms
  - but not bacterial spores.
  - many acid foods (pH < 4.5).
  - It removes residual sanitizing agents from equipment.

1. Heat Treatments

e) Moist heat (under pressure)

- Lethal temperature depends on
  - the heat resistance of the microorganisms and
  - the amount of water in the environment.
  - requires low temperatures and shorter time.
  - stabilizing bonds (such as C=O….H-N) are more easily broken,
    - when water molecules are available for H_2 bonding.
  - moist heat causes denaturation and coagulation of proteins (such as enzymes).
  - These effects rupture cell membranes, denaturates nucleic acids, destrupts DNA, destrupts cell structure and denturate cellular components.
  - Moist heat is 2500 times more effective than dry heat.
- The use of pure steam under pressure.
- In a closed system (autoclave), temperature of pressurized steam is greater than that of boiling water.
- advantages of rapid heating and greater penetration of heat.
- at a 121°C for 15 to 20 min to achieve sterilization.

1. Heat Treatments

f) Ultrahigh temperature

- Commercial sterility is obtained by heating of food at very high temperatures for a short time.
- can be provided by UHT processing.
- Milk heated to 140-150°C for 1 to 3 sec,
  - packaged product can be stored at room temperature ($\leq$ 30°C),
  - the products generally have a 3-month shelf life.
- The milk is heated by injecting steam at high pressure for a rapid temperature increase.
- Following heat treatment, the milk is aseptically packed.
- Heat-stable toxins may remain active even after heating.
- Microbial heat-stable proteinases or lipases cannot be inactivated at UHT process,
  - they can reduce storage life of product.
- These enzymes are produced by **psychrotrophic** bacteria that grow in raw milk during low temp. storage.

1. Heat Treatments

g) Hot-holding zone

- The minimum temperature for hot-holding zone is 60°C or above.
- The time for a food should not exceed 2 h held at hazardous temperature zone: 7-59ºC.
- This temperature zone prevents bacterial multiplication
  - but would not necessarily kill the contaminants.
- Many prepared menu items are kept for prolonged periods before they are served in the restaurants.
- This temperature zones can support growth of many thermophilic and thermoduric food poisoning and spoilage bacteria.

h) Cooking temperature

- Cooking temperature ranges from 75 to 100°C.
- Heating foods over 80°C will kill the vegetative cells of pathogens but does not kill bacterial spores.
- This temperature activate germination of spore.

1. Heat Treatments

2) Dry sterilization (hot air)

- Dry sterilization or hot air, at sufficient high temperatures will kill microorganisms and spores.
- Dry heat requires much higher temperature as 180°C and 2 h.
- Hot-air ovens are used
- Sterilizing materials such as glassware (bottles, Petri dishes, pipettes, flasks and test tubes), mineral oil, glycerol and metal instruments.
- Dry sterilization is referred for glassware;
  - because there is no condensation of moisture (autoclaving can allow condensation to settle on the glassware).
- Dry heat causes oxidation of the cellular organic constituents (e.g. proteins) of the cell.
    - causes them to “burn” slowly.
  - Due to this approach, it is not effective as moist heat, and much higher temperatures and longer times are necessary for equal treatment.

Killing of Microorganisms- 2. Ionization Radiation

- Radiation has two major divisions: non-ionizing radiation and ionizing radition.
- Non-ionizing radiation (long waves) has enough energy to move atoms in a molecule around or cause them to vibrate,
  - but not enough to remove electrons and not cause any change in atomic structure of molecules,
  - ultraviolet lights and microwaves.
- Ionizing radiations (short waves);
  - are gamma-rays, β-rays, x-rays and cosmetic-rays
  - have enough energy to remove tightly bound electrons from atoms,
    - thus create an ion pair (negative and positive charges).
  - This properties can be used to generate electric power, to kill cancer cells and in many manufacturing processes.
- Ion formation or ionization does not make an atom radioactive.
- For radioactivity, the nucleus of an atom should be disrupted by higher energy.

Killing of Microorganisms- 2. Ionization Radiation

- **1) Nonionizing Radiation**
- Non-ionizing radiation does not carry enough energy to ionize atoms or molecule to completely remove an electron from an atom or molecule.
- Instead of producing charged ions, the non-ionizing radiations have sufficient energy only excitation and the move an electron to a higher energy state.
- Non-ionizing radiations: Ultraviolet, sound waves, visible light, infrared, microwave, radio waves.
- ultraviolet may accelerate radical reactions, such as the breakdown of flavoring compounds.
- Light from sun reaching to earth is largely composed of non-ionizing radiation.
- Non-ionizing radiation has a health risk to peoples if it is not properly controlled.
- Effects of non-ionizing radiation on biological systems are cataract on eye; erythema and pigmentation on skin.
- When non-ionizing radiations are used in food processing, non-ionizing hazard sign must be indicated on the door of processing room.

Killing of Microorganisms- 2. Ionization Radiation

a) Ultraviolet radiation

- Low pressure mercury vapor discharge lamps are used in the generation of ultraviolet (UV) light.
- UV light is a powerful bactericidal agent at the wavelength 240 to 280 nm.
- 80 % of UV emission is at a wavelength 254 nm.
- The great microbiological efficiency of UV is due to damaging nucleic acids.
- UV radiation cause ion shifts on cellular membranes, leading to changes in permeability, functional disturbances and cell rupture.
  - This causes failure of critical metabolic process leading to injury or death.
- The greatest lethality is shown around 260 nm wavelengths.
- This corresponds to a strong absorption of rays by nucleic acid bases and proteins.
- 5 cm of clear water will reduce the intensity of UV radiation by two-thirds; and in milk, 90 % absorbed by a layer only 0.1 mm thick.

Killing of Microorganisms- 2. Ionization Radiation

b) Microwave radiation

- Two frequencies are used in food processing: 2450 and 915 MHz.
- Microwave acts indirectly on microorganisms through the generation of heat.
- When electrically neutral foods are placed in a microwave electromagnetic field,
  - the charged asymmetric molecules are derived.
  - symmetric molecules rapidly change alternating-current field.
  - the molecules oscillate in their axes while through positive and negative poles,
  - create intermolecular friction that produces heat,
    - this is microwave energy.
- Microwaves are used for destruction of microorganisms in bread, beer, wines, potato chips, meat, blanching of fruits, vegetables, bakery products.
- Foods are packed before microwave radiation.

Killing of Microorganisms-
2. Ionization Radiation

2) Ionizing Radiation

- Ionizing radiation has sufficient energy to remove electrons from molecules.
- Ionizing rays have sufficient enegy to convert electrons in food molecules to ions (charged particles) and free radicals (unpaired electrons such as oxygen radicals).
- Ionizing radiation used in food irradiationis limited to high-energy radiation (gamma rays of ^{60}Co or x-rays).
- They are chosen because;
  - (i) they produce desired effects on microorganisms,
  - (ii) they do not induce radioactivity in foods,
  - (iii) they are available in quantities and at have low costs to use commercially,
  - (iv) high penetration of x or gamma-rays into matter.

Killing of Microorganisms- 2. Ionization Radiation

- Types of radiations are permitted for food irradiation:
  - (i) Gamma rays of Cobalt-60 or Cesium-137.
  - (ii) Beta rays.
  - (iii) X-rays.
- The practical usable depth of gamma rays in water is 3.9 cm and x-rays is 23.0 cm.
- High energy gamma-rays have high penetration power and may be considered effective and economical for use in foods.

Killing of Microorganisms- 3. Sterilant Gases

- Some gases are used to kill microorganisms in foods.
- These; Ozone (O_3), CO_2 is ethylene oxide in some countries.
- Ozone is generally used for disinfection of water.
- Recently, studies have been carried out to kill microorganisms with CO_2 extraction in some foods.

Ozon

- Ozone is a gas made up of three oxygen atoms.
- It shows a biocidal effect in aqueous environments.
- It is used for disinfection of sea water in drinking water, swimming pool and mussel farming.
- If 2 ppm is present in the water, 99% of the microorganisms die in a few minutes.
- It is also effective against pathogenic viruses.
- Sterilant effect depends on pH and ozone concentration.
- Its lethal effect on microorganisms is most likely due to direct oxidation of sensitive functional groups in the cell such as SH groups, membrane and cytoplasmic enzymes.

Killing of Microorganisms-
3. Sterilant Gases

Carbon dioxide

- Pasteurization or sterilization of liquid foods can be achieved with pressurized CO_2.
- The same effect is not valid for dry foods.

Ethylene oxide

- 500 ml ethylene oxide/m^3 dose is used for sterilization of spices.
- It is banned in many countries due to its negative effects on humans.

Killing of Microorganisms- 4. High Pressure Application

- It can be used to reduce microorganisms in foods with excess water in their structure.
- Its effect on microorganisms depends on the following factors:
  1. Characteristics of the microorganism, its spore or vegetative form
  2. Applied pressure and application time
  3. Composition and pH value of the medium
  4. The temperature of the environment
- Biological processes are affected by pressure.
- Reactions that release energy can be prevented by the effect of pressure and the vital activities of the cell can be slowed down.
- The denaturation of proteins under the influence of pressure depends on factors such as temperature, pH, and concentration of specific ions.
- Pressure is also effective in reactions catalyzed by enzymes in the cell. High pressure is thought to inactivate some important enzymes.

Killing of Microorganisms-
4. High Pressure Application

- Pressure is also effective on lipids. With increasing pressure, the melting points of triglycerides increase. Liquid lipids crystallize under high pressure.
- Gram (+) bacteria are more resistant to high pressure than Gram (-) bacteria.
- Yeast and molds are sensitive to high pressure. However, bacterial spores are highly resistant.
- Microorganisms are predicted to die due to changes in cell membrane permeability when exposed to a certain level of pressure.
- In general, eukaryotic microorganisms are more sensitive to high pressure than prokaryotes.
- Application Areas of High Pressure
  1. Sterilization of foods (juice, milk)
  2. Structural changes in biological macromolecules (starch)
  3. Denaturation of proteins (meat curing)
  4. Phase modification of lipids (crystallization of fat)
  5. Inactivation of enzymes
  6. Changing freezing and thawing points

Killing of Microorganisms-
4. High Pressure Application

- Although its effect on microorganisms is similar to high hydrostatic pressure, there are additional mechanisms.
- Inactivation of microorganisms is directly proportional to humidity.
- If there is water in the environment, CO_2 dissolves in the water phase of the tissue under the influence of pressure and turns into carbonic acid without dissociation. Carbonic acid lowers the intracellular pH and causes protein-enzyme denaturation.
- CO_2 in this state is called "**supercritical carbon dioxide**", since CO_2 has the characteristic of an organic solution at a certain pressure (7.38 MPa) and at a certain temperature (31.5°C).
- Supercritical CO_2 causes the dissolution and death of important components of the microorganism cell.
- Pressurized CO_2 is also used to kill pests in some foods.
- Valid mechanisms in this regard;

  1. CO_2 (carbonic acid) dissolved by the effect of pressure causes an increase in the acidity of cell fluid and body fluid in insects,
  2. The effect of abrupt release of pressure (highlight)
  3. Removal of oxygen in the environment.

Alternative Food Preservation Methods

1) Ohmic and Inductive heating

- Ohmic heating is defined as electric currents are passed through foods or other materials with heating.
- The heating occurs in the form of internal energy generation within the material.
- Microbial inactivation with ohmic heating can occur as a thermal process such as pasteurization.
- A mild poration on cytoplasmic membrane may occur during ohmic heating.

2) High hydrostatic pressure processing

- HHP processing involves exposing of a packed liquid or solid food in water suspension or an unpacked liquid food in a closed chamber to pressure between 100 and 1000 MPa for a desirable period of time from 1 to 95°C.
- HHP processing inactivates microorganisms and extens shelf life of perishable liquid foods.

Combined Methods

- The use of more than one method in ensuring microbiological stability and safety in foods creates a synergistic effect. This effect is called the "***hurdle effect***".
- This effect is a fundamental element in food preservation and is explained by the "***cumulative effect concept***".
- Hurdle effect is called combined methods, combined processes, combination effect, obstacle technology or hurdle technology.

Physical Factors

- The main of these applications are;
  1. Heat treatments
  2. Low temperature
  3. Electromagnetic energy
  4. Inactivation by UV rays
  5. High pressure
  6. Ultrasound packaging
  7. Packing in modified atmosphere
  8. Storage in modified atmosphere
  9. Storage in controlled atmosphere
  10. Aseptic packaging
  11. These methods can be used together with physicochemical methods.
- Example: By lowering the pH in sterilization, both energy is saved and the product structure and nutritional value are preserved.

Combined Methods

- **Physicochemical factors**
  1. Water activity of the medium
  2. pH
  3. O/R potential
  4. Salt, Nitrite – nitrate
  5. CO_2, O_2, O_3
  6. Phosphates, GDL, Phenols, Chelating agents
  7. Chemicals such as diphenyl applied to the surface
  8. Ethanol, propylene glycol, Maillard reaction products
  9. Spices
  10. Glucose oxidase, Lactoperoxidase, Lysozyme

- **Microbial Factors**
  1. Microorganisms
     1. Competing microflora
     2. Starter cultures
  2. Microorganism metabolites
     1. Organic acids
     2. Bacteriocins
     3. Antibiotics