# Preservation of food by Radiation (UV , Gamma and microwave ),

The possible utilization of radiations of various frequencies, ranging from low-frequency electrical current to high-frequency gamma rays is focused for the improved methods of food preservation.

The entire spectrum of radiation is grouped into 2 categories, one on each side of visible light.

A) **Low frequency, long-wavelength, low-quantum energy radiations: -** These range from radio waves to infrared. The effect of these radiations on microorganisms is related to their thermal agitation of the food.
B) **High-frequency, short-wavelength, high-quantum energy radiations: -** These actually excite or destroy organic compounds and microorganisms without heating the product.

**# Cold sterilization: -** Microbial destruction by using radiations without the generation of high temperatures is called as **" Cold sterilization".**

**# Ionizing radiations: -** Radiations of higher frequencies have high energy contents and are capable of actually breaking individual molecules into ions, hence are called as ionizing radiations.

In food industry, following radiations are used—

**I) Ultra-violet radiation**
**II) Ionizing radiation**
**III) Microwave heating**

**I) Ultra-violet irradiation: -**

Of the various electromagnetic radiations, UV irradiation has been the most widely used in the food industry. Radiation with wavelengths near 160 nm is absorbed strongly by purines and pyrimydines and is therefore the most germicidal.

*** Germicidal lamps: -** The usual source of UV radiation in the food industry is from quartz-mercury vapour lamps or low pressure mercury lamps, which emit radiation at 254 nm. The lamps are available in various sizes, shapes, and power.

*** Factors influencing effectiveness of UV treatment: -**

**1) Time: -** The longer the time of exposure to a given concentration, the more effective the treatment.

**2) Intensity: -** The intensity of the rays reaching an object will depend on the power of the lamp, the distance from the lamp to the object, and kind and amount of interfering material in the path of the rays. The intensity increases with the power of the lamp. It is measured in *microwatts per square centimetre* ($\mu W / cm^2$ ). A lamp is about 100 times as effective in killing microorganisms at 5 in, than at 8 ft from the irradiated object.

**3) Penetration: -** The nature of the object or material being irradiated has an important influence on the effectiveness of the process. Penetration is reduced by clear water, dissolved mineral salts, fatty or greasy material etc.

*** Effects on human and animals: -** Gazing at ultraviolet lamps produces irritation of the eyes within a few seconds, longer exposure of the skin results in erythema, or reddening.

*** Action on microorganisms: -** The germicidal effect of UV is determined by the intensity of the rays, time, the location of the organism and kind of the organism. Each microorganism has a characteristic resistance to UV. This can vary with the phase of growth and physiological state of the cell.

The amount of ultraviolet radiation needed to destroy several different microorganisms is summarized in the following table—

| Sr.No. | Microorganism | Dose needed for 1 log cycle reduction or 1 D value ($\mu$ W sec X $10^3$) |
|---|---|---|
| **1** | **Gram negative bacteria** | |
| | Facultative anaerobes | 0.8 – 6.4 |
| | Aerobes | 3.0 – 5.5 |
| | Phototrophic | 5.0 – 6.0 |
| **2** | **Gram positive bacteria** | |
| | Bacillus | 5.0 – 8.0 |
| | Bacillus spores | 8.0 – 10.0 |
| | Micrococcus | 6.0 – 20.0 |
| | Staphylococcus | 2.2 – 5.0 |
| **3** | **Molds** | 10.0 – 200.0 |
| **4** | **Yeasts** | 3.0 – 10.0 |

The location of the organism during the tests has a marked influence. E.G. 97 to 99 % of *Escherichia coli* in air were killed in 10 sec at 24 in with a 15-watt lamp, but 20 sec at 11 in was necessary for bacteria on the surface of an agar plate. Capsulation or clumping of bacteria increases their resistance. Bacterial spores usually take from 2 to 5 times as much exposure as the corresponding vegetative cells. Yeasts are 2 to 5 times and molds are 10 to 50 times as resistant as bacteria.

*** Applications in the food industry: -**

Examples of the successful use of UV rays include—

- Treatment of water used for drinking purposes and in beverages
- Aging of meats
- Treatment of knives for slicing bread
- Treatment of bread and cakes
- Packaging of the sliced bacon
- Sanitizing of eating utensils
- Prevention of growth of slim yeast on pickles, vinegar, sauerkraut vats
- Killing of spores on sugar crystals and in syrups
- Storage and packaging of cheese
- Prevention of mold growth on walls and shelves
- Treatment of air used for or in storage and processing rooms.

## II) Ionizing irradiations

### * Kinds of ionizing radiations

Radiation classified as ionizing includes X-rays or Gamma rays, Cathode or Beta rays, Protons, Neutrons and Alpha particles. Neutrons result in residual radioactivity in foods, and protons and alpha particles have little penetration. Therefore these rays are not used in food preservation and will not be discussed.

**X-rays: -** These are penetrating electromagnetic waves which are produced by bombardment of a heavy-metal target with cathode rays within an evacuated tube. They are not currently considered economical for use in the food industry.

**Gamma rays: -** These are like X-rays but are emitted from by- products of atomic fission or from imitations of such by-products. Cobalt 60 and Cesium 137 have been used as source of these rays.

**Beta rays: -** These are streams of electrons (beta particles) emitted from radioactive material.

**Electrons: -** These are small, negatively charged particles of uniform mass that form part of the atom. They are deflected by magnetic and electric fields. Their penetration depends on the speed with which they hit the target. The higher the charge of the electron, the deeper its penetration.

**Cathode rays: -** These are streams of electrons (beta particles) form the cathode of an evacuated tube.

### * Definition of terms: -

1. **roentgen (r): -** It is the quantity of gamma or x-radiation which produces one electrostatic unit of electric charge of either sign in a cubic centimetre of air under standard conditions.
2. **roentgen-equivalent-physical (rep): -** It is the quantity of ionizing energy which produces, per gram of tissue, an amount of ionisation equivalent to a roentgen. It is a measure of the absorbed energy that is effective within the food.
3. **megarep: -** It is 1 million rep. One r or 1 rep is equivalent to the absorption of 83 to 90 erg per gram of tissue.
4. **rad: -** It is the unit of radiation dose, equivalent to the absorption of 100 erg per gram of irradiated material.
5. **megarad (Mrad): -** 1 million rad.
6. **kilorad (Krad): -** 1,000 rad.
7. **electronvolt (eV): -** It is the energy gained by an electron in moving through a potential difference of 1 volt.
8. **me V: -** 1 million electronvolts. It is a measure of the intensity of the irradiation.
9. **Gray (Gy): -** It is equal to 100 rads.
10. **Radappertization: -** It is the term used to define 'radiation sterilization' which would imply high dose treatments, with the resulting product being shelf-stable.
11. **Radurization: -** It refers to 'radiation pasteurization' low-dose treatments, where the intent is to extend a product's shelf life.
12. **Picowaved: -** It is the term used to label foods treated with low-level ionizing radiation.

**A) Use of X-rays: -**

X-rays have good penetration power but the greatest drawback is the low efficiency and high cost of the production. Only 3 to 5 % of the electron energy applied is used in the production of x-rays.

**B) Gamma rays and Cathode rays: -**

**Source: -** Chief source of gamma rays are i) radioactive fission products of uranium and cobalt, ii) the cobalt circulated in nuclear reactors, and iii) other fuel elements used to operate a nuclear reactor.

**Penetration: -** Gamma rays have good penetration, are effective up to 20 cm in most foods. Cathode rays have poor penetration and are effective at only about 0.5 cm per meV.

**Efficiency: -** Because cathode rays are directional, they can be made to hit the food and therefore are used with greater efficiency than are gamma rays.

**Safety: -** the use of cathode rays presents fewer health problems than the use of gamma rays.

**Effects on microorganisms: -** The bactericidal efficacy of a given dose of irradiation depends on—

i) The kind and species of organisms.
ii) The number of organisms (or spores) originally present.
iii) The composition of the food.
iv) The presence or absence of oxygen.
v) The physical state of the food during irradiation.
vi) The condition of the organisms.

**Applications: -**

Currently food irradiation has been approved only in a very limited way in the United States. Low-level irradiation ( 1 kiloGray) can be used on fresh fruits and vegetables to kill insects and to inhibit spoilage. Dry or dehydrated vegetables (herbs and spices) can be irradiated at up to 30 kiloGray to kill insects and bacteria.

- **Microwave processing**

Microwave heating and processing of foods is becoming increasingly popular. Microwaves are electromagnetic waves between **infrared and radio** waves. Specific frequencies are usually at either 915 megacycles or 2450 megacycles. The energy or heat produced by microwaves as they pass through a food is a result of the extremely rapid **oscillation** of the food molecules in an attempt to align them with the electromagnetic field being produced.

This rapid oscillation, or intermolecular friction generates heat. The preservative effect of microwaves or the bactericidal effect produced is really a function of the heat that is generated.

- **Preservation of food by use of high temperature**

The killing of microorganisms by heat is supposed to be caused by the denaturation of the proteins and especially by the inactivation of enzymes required for metabolism. The heat treatment necessary to kill organisms or their spores varies with the kind of organism, its state, and the environment during heating. Depending on the heat treatment employed, only some of the vegetative cells, most or all of the cells, part of the bacterial spores, or all of them may be killed. The heat treatment selected will depend upon the kinds of organisms to be killed, other preservative methods to be employed, and the effect of heat on the food.

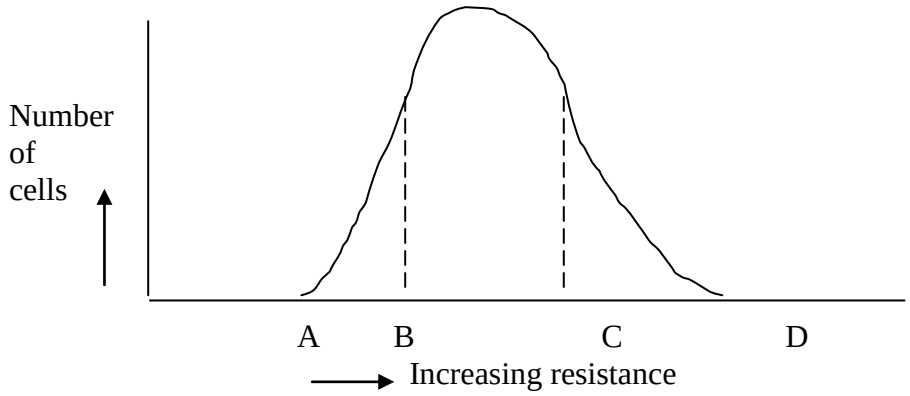
**Heat resistance (Thermal Death Time, TDT): -**

The heat resistance of microorganisms is usually expressed in terms of their **Thermal Death Time (TDT).**

**Definition of TDT: -** It is defined as the time it takes at a certain temperature to kill a stated number of organisms (or spores) under specified conditions.

**Definition of Thermal Death Point (TDP): -** It is the temperature necessary to kill all the organisms in 10 minutes.

Cells and spores of microorganisms differ widely in their resistance to high temperatures. There are differences in heat resistance within a population of cells or spores, as illustrated by the frequency distribution curve in the following graph.




***Frequency distribution curve showing heat resistance in a culture***

Points A to B: - Small numbers of cells have low resistance
Points B to C: - Most of the cells have a medium resistance
Points C to D: - Small numbers of cells have high resistance

*** Factors affecting heat resistance of the cells: -**

1. The temperature time relationship
2. Initial concentration of cells or spores
3. Previous history of the vegetative cells or spores
    a) Culture medium
    b) Temperature of incubation
    c) Phase of growth or age
    d) Desiccation

4. Composition of the substrate in which cells or spores are heated

a) Moisture
b) pH (Hydrogen ion concentration)
c) Other constituents of the substrates

**1. The temperature time relationship: -**

The time for killing cells or spores under a given set of conditions decreases as the temperature is increased. This is illustrated in the following table by the results of 115,000 spores of bacteria / ml in corn juice at pH 6.1

| Temperature Degree Celsius | Thermal Death Time, or time to destroy all spores in minutes |
|---|---|
| 100 | 1200 |
| 105 | 600 |
| 110 | 190 |
| 115 | 70 |
| 120 | 19 |
| 125 | 7 |
| 130 | 3 |
| 135 | 1 |

**2. Initial concentration of cells or spores: -**

The more cells or spores present, the greater the heat treatment necessary to kill all of them. Following is the table showing TDT of spores in corn juice at pH 6.0 at 120 $^{0}$ C.

| Initial concentration of spores. Number / ml | Thermal Death Time or time required to kill all spores, minutes at 120 $^{0}$C |
|---|---|
| 50,000 | 14 |
| 5,000 | 10 |
| 500 | 9 |
| 50 | 8 |

**3. Previous history of the vegetative cells or spores: -**

The conditions under which the cells have been grown and spores have been produced and their treatment thereafter will influence their resistance to heat.

Following are the conditions affecting the resistance in they are grown

a) Culture medium
b) Temperature of incubation
c) Phase of growth or age
d) Desiccation

**a) Culture medium: -**

The medium in which growth takes place is especially important. In general the better the medium for growth, the more resistant the cells or spores. The presence of an adequate supply of accessory growth factors usually favours the production of heat resistant cells or spores.

**b) Temperature of incubation: -**

In general, resistance increases as the incubation temperature is raised towards the optimum for the organism and for many organisms increases further as the temperature approaches the maximum for growth. E.g. *Escherichia coli* is considerably more heat resistant when grown at 38.5 $^0$ C, which is near its optimal temperature, than at 28 $^0$ C.

**c) Phase of growth or age: -**

The heat resistance of vegetative cells varies with the stage of growth and spores with their age. Bacterial cells show their greatest resistance during the late lag phase but almost as great resistance during their maximum stationary phase, followed by decline in resistance. The cells are least resistant during their phase of logarithmic growth. Very young (immature) spores are less resistant than are mature ones.

**d) Desiccation: -**

Dried spores of some bacteria are harder to kill by heat than are those kept moist, but this apparently does not hold for all bacterial spores.

**4. Composition of the substrate in which cells or spores are heated: -**

The material in which the spores or cells are heated is so important that it must be stated if a thermal death time is to have meaning. It is dependent on following factors.

a) Moisture
b) pH (Hydrogen ion concentration
c) Other constituents of the substrates

**a) Moisture: -**

Moist heat is much more effective killing agent than dry heat. For sterilization of liquid material 121 $^0$C for 15 minutes are required and for solid material 160 to 180 $^0$C for 3 to 4 hours are required.

**b) Hydrogen ion concentration (pH): -**

In general, cells or spores are most heat resistant in a substrate that is at or near neutrality. An increase in acidity or alkalinity hastens killing by heat.

**c) Other constituents of the substrate: -**

Sodium chloride present in food has a protective effect on some spores.

Sugars seem to protect some organisms or spores but not others. The optimal concentration for protection varies with the organism. It is high for some osmophilic organisms and low for others, high for spores and low for non-osmophilic cells. The protective effect of sugar may be related to a resulting decrease in $a_w$. A reduced $a_w$ does result in an increase in observed heat resistance.

Solutes differ in their effect on bacteria. Glucose e.g. protects *E. coli* and *Pseudomonas fluorescens* against heat better than sodium chloride. On the other hand, glucose affords no protection or is even harmful to *staphylococcus aureus*, where as sodium

chloride is very protective. Concentration of solutes may affect the heat process necessary for sterilization.

Colloidal materials, especially proteins and fats are protective against heat. This is well illustrated in the following table by the data of Brown and Peiser (1916) who used *Thermal death points.*

| Sr.No. | Substance | *S. lactis* $^0C$ | *E. coli* $^0C$ | *L.bulgaricus* $^0C$ |
|---|---|---|---|---|
| 1 | Cream | 69-71 | 73 | 95 |
| 2 | Whole milk | 63-65 | 69 | 91 |
| 3 | Skim milk | 59-63 | 65 | 89 |
| 4 | Whey | 57-61 | 63 | 83 |
| 5 | Broth | 55-57 | 61 | 81 |

*** Effect of protective substances an heat resistance of bacteria**

**Heat resistance (TDT) of yeasts and yeast spores: -**

In general the ascospores of yeasts need only 5 to 10 C more heat for their destruction than the vegetative cells from which they are formed. Most ascospores are killed by 60 C for 10 to 15 min; a few are more resistant, but none can survive even a brief heating at 100 C. Vegetative yeasts usually are killed by 50 to 58 C for 10 to 15 minutes. Both yeasts and their spores are killed pasteurization treatments given milk (62.8 C for 30 min or 71.7 C for 15 sec), and yeasts are readily killed in the baking of bread, where the temperature of the interior reaches about 97 C.

**Heat resistance (TDT) of molds and mold spores: -**

Most molds and their spores are killed by moist heat at 60 in 5 to 10 min, but some species are considerably more heat-resistant. The asexual spores are more resistant than mycelium. Many species of *Aspergillus, Penicillium and Mucor* are more resistant to heat than are other molds. Sclerotia are especially difficult to kill by heat. Some can survive a heat treatment of 90 to 100 C for a brief period and have been known to cause spoilage in canned fruits. It was found that 1,000 min at 82.2 C or 300 min at 85 C was necessary to destroy Sclerotia from a species of *Penicillium.*

Molds spores are fairly resistant to dry heat. Dry heat at 120 C for 30 min will not kill some of the more resistant spores.

**Heat resistance (TDT) of Bacteria and Bacterial spores: -**

The heat resistance of vegetative cells of bacteria varies widely with the species. Cocci are usually more resistant than rods. The higher the optimal and maximal temperatures for growth, the greater the resistance to heat. Bacteria that clump or form capsules are more difficult to kill than those which do not. Bacteria high in lipid content are harder to kill than are other cells.

A few examples of TDT of bacterial cells are shown in following table: -

| Sr. No. | Bacterium | Time in Min | Temperature C |
|---|---|---|---|
| 1 | *Neisseria gonorrhoeae* | 2-3 | 50 |
| 2 | *Salmonella typhi* | 4.3 | 60 |
| 3 | *Staphylococcus aureus* | 18.8 | 60 |
| 4 | *Escherichia coli* | 20-30 | 57.3 |
| 5 | *Streptococcus thermophilus* | 15 | 70-75 |
| 6 | *Lactobacillus bulgaricus* | 30 | 71 |

The heat resistance of bacterial spores varies greatly with the species of bacterium and conditions during sporulation. Resistance at 100 C may vary from less than 1 min to over 20 hours.

A few examples of TDT of bacterial spores are shown in following table: -

| Sr. No. | Spores of Bacterium | Time to kill at 100 C in Min |
|---|---|---|
| 1 | *Bacillus anthracis* | 1.7 |
| 2 | *Bacillus subtilis* | 15-20 |
| 3 | *Clostridium botulinum* | 100-330 |
| 4 | *Clostridium calidotolerans* | 520 |
| 5 | *Flat sour bacteria* | Over 1030 |

**Heat treatments employed in processing foods: -**

The temperature and time used in heat processing a food will depend on what effect heat has on the food and what other preservative methods are to be employed. Some foods, such as milk can be heated to only a limited extent without undesirable changes in appearance or loss in palatability, where others like corn or pumpkin can undergo a more rigorous heat treatment without marked changes. The greater the heat treatment, the more organisms will be killed. The heating must destroy all potential spoilage organisms. In canning, an attempt is made to kill organisms that could spoil the food during later handling. In pasteurization, most of the spoilage organisms are killed but others survive and must be inhibited by low temperatures or some other preservative methods. Following are some methods in which various degrees of heating is used on foods—

**1) Pasteurization**
**2) Heating at about 100**
**3) Heating above 100 C**

**1)Pasteurization: -**

It is a heat treatment that kills part but not all of the microorganisms present and usually involves the application of temperatures below 100 C.

The heating may be by means of steam, hot water, dry heat, or electric currents, and products are cooled promptly after the heat treatment.

Pasteurization is used when—

--> More rigorous heat treatments might harm the quality of the product, as with market milk.
--> Aim is to kill pathogens, as with market milk
--> The main spoilage organisms are not very heat resistant, such as the yeasts in fruit juices.

--> Any surviving spoilage organisms will be taken care of by additional preservative methods, as in the chilling of market milk

Preservative methods used to supplement pasteurization include --> Refrigeration e.g. milk
--> Keeping out microorganisms, usually by packaging the product in a sealed container
--> Maintenance of anaerobic conditions, as in sealed containers
--> Addition of high concentrations of sugar, as in sweetened condensed milk
--> Presence or addition of chemical preservatives e.g. the organic acids in pickles.

Time and temperatures used in the pasteurization process depend on the method employed and the product treated. There are 2 methods commonly used in pasteurization—

**I) High Temperature Short Time (HTST) method: -**

For milk 71.7 C for 15 seconds, for Ice-cream 82.2 C for 20 seconds, for beer 80 C for 20 seconds, for wines 82 to 85 C for 1 minute, for fruit juices 85 to 87 for 30 to 60 seconds heat treatments are used.

**II) Low Temperature Holding (LTH) method: -**

For milk 62.8 C for 30 minutes, for Ice-cream 71.1 C for 30 min, for beer 60 C for 30 minutes, for wines 65 to 70 C for 20 minutes, for fruit juices 65 to 75 C for 30 minutes heat treatments are used.

**2) Heating at about 100 C: -**

Formerly, home canners processed all foods for varying lengths of time at 100 C or less. But nowadays they use pressure cookers. During **baking** the internal temperature of bread, cake etc approaches but never reaches 100 C due to which bacterial spores that survive the baking of bread may cause ropiness. **Simmering** is gentle boiling with temperature about 100 C. In **roasting** meat the internal temperature reaches about 80 C. **Frying** gets the outside of the food very hot, but the centre does not reach 100 C. **Cooking** is a indefinite term.

**3) Heating above 100 C: -**

Temperatures above 100 C usually are obtained by means of steam under pressure in steam-pressure sterilizers or retorts. The temperature in the retorts increases with rising stem pressures.

| **Pressure lb** | **Temperature C** |
|---|---|
| 0 | 100 |
| 5 | 109 |
| 10 | 115.5 |
| 15 | 121.5 |

When liquid foods are to be sterilized before their introduction into sterile cans, high stem pressures are used to apply a high temperature for a few seconds.

**Ultrapasteurization or Ultra High Temperature: -**

Heating of milk at 137.8 C for 2 seconds by use of steam injection or steam infusion followed by **"flash evaporation'** of the condensed steam and rapid cooling is called as ultrapasteurization.

## Canning: -

**Definition:** -It is defined as the preservation of foods in sealed containers and usually implies heat treatment as the principal factor in the preservation of spoilage.

Most canning is in **'tin cans',** which are made of tin-coated steel, but glass containers, aluminium, or composite material may be used.

**The canning procedure: -**

Raw food for canning should be freshly harvested, properly prepared, inspected, graded if desired, and thoroughly washed before introduction into the can.

Many vegetable foods are **blanched** or **scaled** briefly by hot water or steam before packaging. The blanching washes the food further, sets the colour, softens the tissues to aid packing, helps to form vacuum, and kills some microorganisms.

**A brine,** consisting of salt solution or salt plus sugar, is added to some canned vegetables; and sugar syrups may be added to fruits.

The container is evacuated before sealing, usually by heating the headspace, or unfilled part of the container, but often by mechanical means.

**The heat process in canning: -**

The canner aims for complete sterilization of most foods but does not always attain it. The canner may kill all that could spoil the food under normal conditions of storage and may leave some that are unable to grow.

The heat process necessary for the canning depends on the factors that influence the heat resistance of the most resistant spoilage organisms and those which affect heat penetration.

The heating is ordinarily done in retorts, with or without steam pressure as food demands.

**I) HTST heat process: -** It is now used for some liquid foods' requiring special equipment for sterilizing the food in bulk, sterilizing the containers and lids, and filling and sealing the sterile containers under aseptic conditions. Examples of HTST process are as follows—

**# The Dole process: -** In which Heat-Cool-Fill (HCF) system is used. **# Martin HTST System: -** Mixed liquid and solid pieces are heated directly by contact with high temperature steam before aseptic canning.

**# Sterilizing and Closing (SC) method: -** sterilization of the food is accomplished before the can is sealed.

**# Pressure Filler Cooker method: -** The food is sterilized by high-pressure steam and filled into the can' then the can is sealed and the heat processing is continued as long as necessary before cooling.

**# Dehydrocanning: -** The food e.g. the apple slices are dried to about half its original weight before canning.

**II) By direct gas flame**

**III) By steam injection**

**IV) By heating in a fluidised bed of granular solids**

**V) Hydrostatic sterilizers: -** It consists of a vertical tank with conveyors that carry cans down through a water leg up into live steam, and then up and out through a second water leg.

**VI) Flash 18 method: -** Canning is done in a high-pressure (18-psi) chamber. The product is given an HTST treatment to bring it to processing temperature, and the cans are filled, closed and partially cooled in the chamber.

**VII) Heat plus other combination methods: -** Heat plus antibiotics, irradiation or chemicals are used.

**Pressurized packaging of foods in canning: -**

Pressurized packaged liquids or pastes, called aerosols, are packed under pressure of a propellant gas, usually carbon dioxide, nitrogen, or nitrous oxide, so as to dispense the food as a foam, spray, or liquid. E.g. whipped cream and other toppings, beverage concentrates, salad dressings, condiments, oils, jellies, and flavouring substances.

The pressurized foods are subject to microbial spoilage unless adequate preservatives methods are employed. Acids foods may be heated, canned, and then gassed.

The gas used as a propellant may have an influence on the kinds of organisms likely to grow. E.g. Nitrogen would not inhibit aerobes if a little oxygen was present, but carbon dioxide would be inhibitory under the same condition. Carbon dioxide inhibits many microorganisms, but does not inhibit lactic acid bacteria, *Bacillus coagulans, Streptococcus faecalis or yeasts.*

**The cooling process in canning: -**

Following the application of heat, the containers of food are cooled as rapidly as is practicable. The cans may be cooled by immersion in cold water or by a spray of water.

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- # Preservation of food by use of low temperature

Low temperatures are used to retard chemical reactions and the action of food enzyme and to slow down or stop the growth and activity of Microorganisms in food. The lower the temperature, the slower will be chemical reactions, enzyme reaction, and microbial growth.

Each microorganism has an optimal or best temperature for growth and a minimal temperature below, which it cannot multiply. Cooler temperatures will prevent growth, but slow metabolic activity may continue. Low temperature storage can therefore act as a significant environmental factor influencing the type of spoilage flora to predominate as illustrated in the following table.

| Sr. No. | Bacterium | Spoilage flora at each temperature %<br>1 C 10 C 15 C | | |
|---|---|---|---|---|
| 1 | *Pseudomonas* | 90 | 37 | 15 |
| 2 | *Acinetobacter* | 7 | 26 | 34 |
| 3 | *Enterobacteriacae* | 3 | 15 | 27 |
| 4 | *Streptococcus* | | 6 | 8 |
| 5 | *Aeromonas* | | 4 | 6 |
| 6 | *Others* | | 12 | 10 |

The growth and metabolic reactions of microorganisms depend on enzymes, and the rate of enzyme reactions is directly affected by temperature. When the temperature is lowered, the rate of growth of a microorganism decreases.

| Sr. No. | Temperature C | Average exponential generation time, in minutes |
|---|---|---|
| 1 | 0 | 667 |
| 2 | 2.5 | 462 |
| 3 | 5.0 | 300 |
| 4 | 7.5 | 207 |
| 5 | 10.0 | 158 |
| 6 | 20.0 | 65 |

Growth rate of *Pseudomonas fragi* at various temperatures

**Temperatures employed in low temperature storage**

**1) Common or Cellar storage**
**2) Chilling or Cold storage**
**3) Freezing or Frozen storage**

**1) Common or Cellar Storage: -**

The temperature in common or cellar storage usually is not much below than that of the room temperature. It is near 15 C. Root crops, potatoes, cabbage, apples etc can be stored for limited periods. The deterioration of such fruits and vegetables by their own enzymes and by microorganisms is not prevented but is slower than at atmospheric temperatures.

Too low a humidity in the storage cellar results in losses of moisture from the stored food, and too high a humidity favours spoilage by microorganisms. In locations where no refrigeration is available common storage of all foods is used.

**2) Chilling or Cold storage: -**

Chilling storage is at temperatures not far above freezing and usually involves cooling by ice or by mechanical refrigeration. Most perishable foods, including eggs, dairy products, meats, seafood, vegetables, and fruits may be held in chilling storage for a limited time. Enzymatic and microbial changes in the foods are not prevented but are slowed considerably.

**Factors to be considered in chilling storage**

a) Temperature
b) Relative humidity

**c)** Ventilation
**d)** Composition of storage atmosphere
**e)** Irradiation

**a) Temperature: -**

Most foods will keep best at a temperature just above their freezing point. The chilling temperature is selected on the basis of the kind of food and the time and conditions of storage. Certain foods have an optimal storage temperature well above the freezing point and may be damaged by lower temperatures. A well-known example is the banana, which should not be kept in the refrigerator; it keeps best at about 13 to 16.7 C. Some varieties of apples undergo 'low temperature breakdown' at temperatures near freezing, and sweet potatoes keep best at 10 to 12.8 C.

The temperature of a refrigerator is mechanically controlled but varies in different parts, usually between 0 and 10 C.

**b) Relative humidity: -**

The optimal relative humidity of the atmosphere in chilling storage varies with the food stored and with environmental factors such as temperature, composition of the atmosphere, and ray treatments. Too low a relative humidity results in loss of moisture and hence of weight, the wilting and softening of vegetables, and the shrinkage of fruits. Too high a relative humidity favours the growth of spoilage microorganisms.

The highest humidity is required for most bacterial growth, less moisture is needed by yeasts (90 to 92 %), and still less by molds (85 to 90 %). A moist surface favours microbial spoilage e.g. slime on the moist surface of sausage.

**c) Ventilation: -**

Ventilation or control of air velocities of the storage room is important in maintaining a uniform relative humidity throughout the room, removing odours, and preventing the development of stale odours and flavours. The rate of air circulation affects the rate of drying of foods. If adequate ventilation is not provided, food in local areas of high humidity may undergo microbial decomposition.

**d) Composition of storage atmosphere: -**

The amount and proportions of gases in the storage atmosphere influence preservation by chilling. It has been found that in the presence of optimal concentrations of $CO_2$ or $O_3$ ---

--> a food will remain unspoiled for a longer period
--> a higher relative humidity can be maintained
--> a higher storage temperature can be used

**d) Irradiation: -**

The combination of ultraviolet irradiation with chilling storage helps to preserve some foods and may permit the use of a higher humidity or storage temperature than is practicable with chilling alone. Ultraviolet lamps have been installed in rooms for the storage of meat and cheese.

**3) Freezing or Frozen storage**

The storage of foods in the frozen condition has been an important preservative method for centuries where outdoor freezing temperatures were available. With the development of mechanical refrigeration and the quick freezing process, the frozen food industry has expanded rapidly. Even in the home, the freezing of the foods has become extensive. In this method, microbial growth is prevented entirely and the action of food enzymes is greatly retarded.

**Freezing of foods: -**

The rate of freezing of foods depends on number of factors, such as the method employed, the temperature, circulation of air or refrigerant, size and shape of package, and kind of food.

**Sharp freezing: -** Freezing in air with only natural air circulation at temperature – 23.3 C or lower and freezing may take form 3 to 72 hours.

**Quick-freezing: -** Food is frozen in short time at temperatures – 17 to –45 C within 30 minutes.

**Nitrogen freezing: -** For the overseas shipment of frozen, packaged foods involves nitrogen freezing of the cartooned foods in a special aluminium case. Certain fruits and vegetables, fish, and mushrooms now are being frozen by means of liquid nitrogen.

**Dehydrofreezing: -** Fruits and vegetables have about their moisture removed before freezing.

**Advantages of quick freezing over slow freezing: -**

i) Smaller ice crystals are formed; hence there is less mechanical destruction of intact cells of food.
ii) There is shorter period of solidification and therefore less time for diffusion of soluble materials and separation of ice.
iii) There is more prompt prevention of microbial growth
iv) There is more rapid slowing of enzyme action

**# Response of microorganisms for freezing (Factors affecting)**

i) The kind of microorganism and its state
ii) The freezing rate
iii) The freezing temperature
iv) The time of frozen storage
v) The kind of food
vi) Influence of defrosting
vii) Alternate freezing and thawing
viii) Possible events during freezing of the cell

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## *Preservation of food by Use of Food additives & chemical preservatives

**Food additive: -** According to **WHO**, A food additive is a substance or mixture of substances, other than the basic food stuff, which is present in the food as a result of any aspect of production, processing, storage or packaging.

**Chemical preservatives: -** According to **The Federal Food, Drug, and Cosmetic Act,** as amended by the Food Additives Amendment of 1958, Chemical preservative is defined as any chemical which, when added to food, tends to prevent or retard deterioration thereof; but does not include common salt, sugars, vinegars, spices, or oils extracted from spices, or substances added by wood smoke.

Most of the more common antimicrobial additives used in foods are as follows—

i) Organic acids and their salts
ii) Nitrites and nitrates
iii) Sulfur dioxide and Sulphites
iv) Ethylene and Propylene oxide
v) Sugar and Salt
vi) Alcohol
vii) Formaldehyde
viii) Wood smoke
ix) Spices and other condiments
x) Antibiotics
xi) Others

**i) Organic acids and their salts: -**

Citric, lactic, propionic, benzoic, sorbic, acetic acids or their salts may be added to or developed in foods.

*** Citric acid: -** It is used in syrups, drinks, jams and jellies as a substitute for fruit flavours and for preservation.

*** Lactic acid: -** It is developed in curd, whey, cheese, butter or added in brines, green olives etc.

*** Propionates: -** Sodium or Calcium propionate is used most extensively in the prevention of mold growth and rope development in baked food products, cheese. In limited scale, it is used in butter, jams, jellies, figs, apple slices, and malt extract.

**Mechanism of action: -** It affects cell-membrane permeability. Its action on molds is not known.

*** Benzoates: -** Sodium benzoate is used extensively as an antimicrobial in jams, jellies, margarine, carbonated beverages, fruit salads, pickles, fruit juices etc. It is effective at pH 2.5 to 4.0. It is more effective on bacteria and less effective on yeasts and molds.

Two esters of *p*-hydroxybenzoic acid, methylparaben and propylparaben are also used.

**Mechanism of action: -** Not known

***Sorbates: -** Calcium or Sodium or Potassium sorbate is used as a direct antimicrobial additive in foods and as a spray, dip, or coating on packaging materials. It is widely used in cheeses, baked products, beverages, syrups, fruit juices, jellies, jams, fruit cocktails, dried fruits, pickles etc.

It is more effective against yeasts and molds but is less effective against bacteria.

**Mechanism of action: -** Not known

*** Acetates: -** Derivatives of acetic acid e.g. monochloroacetic acid, peracetic acid, dehydroacetic acid and sodium diacetate have been recommended as preservatives but not all are approved by the FDA. Sodium diacetate is used in cheese spreads and malt syrups.

Acetic acid in the form of vinegar is used in pickles, sausages and pigs' feet. It is more effective against yeasts and bacteria than molds.

**ii) Nitrites and Nitrates: -**

Combinations of these various salts have been used in curing solutions and curing mixtures of meats. Nitrites decompose to nitric acid, which forms nitrosomyoglobin when it reacts with haeme pigments in meats and thereby forms a stable red colour. Nitrites can react with secondary and tertiary amines to form nitrosamines, which are known to be carcinogenic.

Nitrates only act as a reservoir for nitrites and have limited effect on microorganisms.

**iii) Sulfur dioxide and Sulfites: -**

These are used in the wine industry to sanitize equipment and to reduce the normal flora of the grape must. In aqueous solutions, sulfur dioxide and various sulfites, including sodium sulfite, potassium sulfite, sodium bisulfite, potassium bisulfite, sodium metabisulfite and potassium metabisulfite, form **sulfurous acid**, the active antimicrobial compound.

The fumes of burning sulfur are used to treat most light coloured dehydrated fruits, while dehydrated vegetables are exposed to a spray of neutral bisulfites and sulfites before drying. Sulfur dioxide has also been used in syrups and fruit juices and wine making.

**Mechanism of action: -** Many mechanisms for the action of sulfurous acid on microbial cells have been suggested, including the reduction of disulfide linkages, formation of carbonyl compounds, reaction with ketone groups, and inhibition of respiratory mechanisms.

**iv) Ethylene and propylene oxide: -**

These two gases are sterilants. Ethylene oxide kills all microorganisms while propylene oxide kills many microorganisms but not as effective. These are used as sterilants for packaging materials, fumigation of warehouses, and 'cold sterilization' of plastics, chemicals, pharmaceuticals, syringes, and hospital supplies. Also these are used in dried fruits, dried eggs, gelatin, cereals, dried yeasts and spices.

**Mechanism of action: -** These act as strong alkylating agents attacking labile hydrogens.

**v) Sugar and salt: -**

These compounds lower the $a_w$ and thus have an adverse effect on microorganisms. NaCL is used in brines and curing solutions or is applied directly to the food. It is used in pickles.

Sugars such as glucose or sucrose make water unavailable to microorganisms. These are used in high concentrations in sweetened condensed milk, fruit syrups, jellies and candies.

**Mechanism of action: -** i) These cause high osmotic pressure and hence plasmolysis of cells. ii) These dehydrate foods by drawing out and tying up moisture as they dehydrate microbial cells. iii) NaCl ionizes to yield chlorine ions which are harmful to microorganisms.

**vi) Alcohol: -**

Ethanol is added to vanilla and lemon extracts. The alcoholic content of beer and wine is not great enough to prevent their spoilage by microorganisms. Liqueurs and distilled liquors usually contain enough alcohol to prevent spoilage by microorganisms.

Methanol is poisonous and should not be added to foods.

Glycerol is antiseptic but not important in food preservation.

Propylene glycol has been used as a mold inhibitor and as a spray to kill airborne microorganisms.

**Mechanism of action: -** Ethanol is a coagulant and denaturizer of cell proteins and is most germicidal in in concentrations between 70 to 95 %.

**vii) Formaldehyde: -**

It is not added in foods. It is used in the treatment of walls, shelves, floors etc to eliminate molds and their spores. It is effective against molds, bacteria, and viruses.

**Mechanism of action: -** It combines with free amino groups of the proteins of cell protoplasm, injures nuclei and coagulates proteins.

**viii) Wood smoke: -**

The smoking of foods has two main purposes, first is to add desire flavours, colours and finishing; second is preservation.

Smoke is obtained form the burning of the wood such as hickory, apple, oak, maple, beech, birch, walnut and mahogany. Sawdust is added to the fire to give a heavy smudge. Temperature and humidity are controlled at levels favourable to the product being smoked and the duration of the smoking depends on the kind of the food. Smoking temperatures for meat vary from 43 to 71 C and the smoking period lasts from a few hours to several days.

Wood smoke contains a large number of volatile compounds that may have bacteriostatic and bactericidal effect. Formaldehyde is the main compound of wood smoke; others are phenols, cresols, aliphatic acids from formic through caproic, primary and secondary alcohols, ketones, acetaldehyde, other aldehydes, waxes, resins, methyl & propyl isomers, catechols, pyrogallol and methyl ester.

**Mechanism of action: -** The smoking process helps preservation by adding the chemical preservatives developed from smoke and by drying the surface due to heat. Due to chemicals and heat coagulation of proteins of the microorganisms takes place.

**ix) Spices and other condiments: -**

These do not have any marked bacteriostatic effect in the concentrations customarily used. The inhibitory effect of spices differs with the kind of spice and the microorganism being tested.

- ➔ ***Mustard oil & flour*** are very effective against *Saccharomyces cerevisiae.*
- ➔ ***Cinnamon and cloves:*** **-** These contain aldehyde and eugenol respectively. These are bacteriostatic.
- ➔ *Ground pepper corn, mace, nutmeg, ginger, thyme, bay leaves, marjoram, rosemary* have weak inhibitory power against most organisms.
- ➔ *Garlic, onion, horseradish,* may be bacteriostatic. Acrolein is the active principle in onions and garlic.

**X) Antibiotics: -**

Most of the better-known antibiotics have been tested on raw foods like meats, fish, and poultry. **Chlortetracycline** has been found superior to other antibiotics due to its broad spectrum of activity. **Oxytetracycline** is also used. **Chloramphenicol** is also successful.

Penicillin, Streptomycin, neomycin, polymyxin, nisin, bacitracin etc are not satisfactory.

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# Biosensors in food industry

**Introduction of biosensors**

Biosensors can be defined as an analytical device containing biological or biologically derived sensing elements. They can also be described as the offspring of biology and electronics. Multidisciplinary skills of biologists, physicists, chemists and engineers have been combined to produce biosensors. In a biosensor the analyte or the sensing element could be a bio catalyst such as an enzyme, organism, tissue or an affinity system such as an antibody, or a nucleic acid.

**Technology**

As a result of the development of the microprocessor applied technology and rapid growth of biotechnology, production and application of biosensors expanded dramatically. Quality assessment and ensuring compliance with legislation are the two basic needs of food analysis. Although human sense organs are sensitive, instruments provide better quantitative results than them. However conventional instrumental methods are incompatible in cases where quick results are needed. In addition to this, biosensors have various advantages as compared to conventional analytical methods. They are relatively cheap, easy to handle, portable and the user does not require special skills. The term biosensor is used for a whole class of sensors that utilize a biochemical reaction to determine a specific compound.

In a biosensor a bio-receptor molecule is immobilized in a suitable matrix to form a bio-layer which is then placed in the immediate vicinity of a transducer. Depending on the nature of the transducers and the transduced parameter, there are different types such as Electrochemical, Piezoelectric, and Thermometric and Optical biosensors in the analytical field.

**Electrochemical Biosensors**

An electrochemical biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is retained in direct spatial contact with an electrochemical transduction element.

**Piezoelectric biosensor**

The development of a piezoelectric biosensor based on nucleic acids interaction is presented focusing on the methodology for probe immobilization. This is a key step in any DNA biosensor development. Often, the detection limits and, in general, the analytical performances of the biosensor can be improved by optimizing the immobilization of the receptor on the transducer surface.

**Thermometric biosensors**

Thermometric biosensors are constructed by combining enzymes with temperature sensors. When the analyte is exposed to the enzyme, the heat of reaction of the enzyme is measured and is calibrated against the analyte concentration.

**Optical biosensors**

Optical Biosensors provides the most comprehensive analysis of optical biosensors and relevant technologies to date. According to the optical configuration, optical sensors have classified into two modes. When light is reflected at an optical interface where there is a change of refractive index, there is a decay of energy from the point of reflection into the surrounding medium. This energy field which extends into the medium depends upon the medium in which the wave guide is dipped. The resultant changes of luminescence, absorption or fluorescence can hence be determined. When the glass surface of the biosensor is coated with a thin layer of metal (silver, gold), the intensity of the resonance angle changes depending on the concentration of the medium in which electrode is immersed. This phenomenon is called the surface plasma resonance (SPR).

**Food analysis and application of biosensors**

Dietary habits of people throughout the world are different depending on the availability, ethnicity, cultural influences and the preparation and preferences for food. The range of food analytes comprises of liquid and gases as ionic radical or neutral species. The form of analyte may range from macromolecule to a microelement and heterogeneous distribution of analyte in the food has made the situation worse for the analyst. In most cases, the analyst needs to separate the analyte from the food before detection.

In food industry optic coated with antibodies are commonly used to detect pathogens and food toxins. The light system in these biosensors has been fluorescence, since this type of optical measurement can greatly amplify the signal. The exact wave length of this resonance depends on the amount of antibody, immobilized in the coupling matrix. The antibody-antigen interaction causes a shift in the resonance to longer wavelengths. The amount of the shift can be related to the concentration of antigens. The speed of detection is critical in preventing and diagnosing food related illnesses. It plays an important role in food processing plants by minimizing time gap between two unit operations. In conclusion, biosensors form an interesting part of food analysis, and they have achieved a notable success.

# Utilization and disposal of whey

The whey constitutes a major ecological burden to be disposed of as a waste material because the biological oxygen demand (BOD) of whey is very high at 40,000 mg per kg. Utilization of this nutrient rich byproduct, which would otherwise go as a waste is predominantly favored due not only to the economic opportunities because of the nutrients but also from the disposal point of view.

How effectively the whey can be processed into useful products?

1. By simple removal of water by spray or roller drying to yield whey powder.
2. By increasing the ratio of protein in the end product through ultrafiltration in the manufacture of whey protein concentrate, processes involving fractionation in the manufacture of whey protein isolates and heat treatment to produce lactalbumin.
3. The processes that target the conservation of lactose in the whey by treating it with lactase, heat or acid and fermentation process to yield lactic acid, citric acid and single cell protein.
4. Electrolysis and ion exchange mechanisms to alter the mineral composition of the product.

If the whey is produced in factories in smaller quantities and the production is scattered, it is ideal to condense the whey using vacuum condensers and this method of reducing bulk is found to be economical and stored under deep freezing temperatures till use. The condensed whey can be easily transported to the central whey processing plant.

**Manufacture of lactalbumin and whey protein isolates**

The whey proteins can be easily denatured or destroyed and the aim is to separate the proteins without any detrimental effect so that they retain the functional properties. They are rich in protein content and very useful to the food industry. The manufacturing process involves use of non specific absorbent to bind the proteins in the whey, followed by elution of the proteins by treatment of the absorbent with a specific elutent. Carboxyl methyl cellulose and different variants of mineral oxides serve as absorbents.

Though the absorbents are relatively non specific, they show specificity to bind to certain proteins under set conditions of pH, ionic strength and temperature. These processes can be effectively utilized to produce protein isolates with a higher ratio of alpha lactalbumin to beta lactoglobulin than that present in whey. These two components hold big promise in the preparation of non allergic infant feeds.

**Fermentation of whey**

Biomass of yeasts can be successfully produced commercially through whey fermentation process. Commonly used yeast strains are *Kluyveromyces marxianus* var. *lactis* and *Kluyveromyces marxianus* var. *marxianus*. The famous “Bet fermentation process” in France is an example of this kind of fermentation.

Sweet whey is the choice for fermentation. It is first deproteinated and then diluted to a lactose concentration of 20-25 kgm-3. Since whey is limiting in nitrogen, ammonium salts are added to compensate the deficit along with certain trace metals like iron, copper, manganese and zinc to stimulate the yeast growth. The fermentation is continuous and it is operated at a dilution rate of 0.33 per hour and temperature of fermentation is set at 39°C. The mixing and aeration of the culture is continuous and the residual sugar level is 1

kgm-3. The biomass yield works out to 0.55 to 0.6 kg yeast on dry matter basis per kilogram of lactose metabolized and the biomass production is about 4.5 Kgm-3 per hour.

The yeast is then separated and concentrated in a two stage washing cum centrifugation process and later plasmolyzed to make the yeast protein more accessible and finally dried and packed. The crude protein content of the biomass is 50% on dry matter basis and the only significant limitation is the sulfur containing amino acids. The value of protein is almost similar to casein and because of their functional properties, they are more sought after in baking industries, as a substitute for milk solids in the manufacture of yogurt, ice cream and other dairy products

**Whey Powder**

Whey is the by-product in the manufacturing of cheese and casein. Disposing of this whey has long been a problem. For environmental reasons it cannot be discharged into lakes and rivers; for economical reasons it is not desirable to simply dump it to waste treatment facilities. Converting whey into powder has led to number products that it can be incorporated into. It is most desirable, if and where possible, to use it for human food, as it contains a small but valuable protein component. It is also feasible to use it as animal feed. Between the pet food industry and animal feed mixers, hundred's of millions of pounds are sold every year. The feed industry may be the largest consumer of dried whey and whey products.

Whey powder is essentially produced by the same method as other milk powders. Reverse osmosis can be used to partially concentrate the whey prior to vacuum evaporation. Before the whey concentrate is spray dried, lactose crystallization is induced to decrease the hygroscopicity. This is accomplished by quick cooling in flash coolers after evaporation. Crystallization continues in agitated tanks for 4 to 24 h.

A fluidized bed may be used to produce large agglomerated particles with free-flowing, non-hygroscopic, no caking characteristics.

**Whey Protein Concentrates**

Both whey disposal problems and high-quality animal protein shortages have increased world-wide interest in whey protein concentrates. After clarification and pasteurization, the whey is cooled and held to stabilize the calcium phosphate complex, which later decreases membrane fouling. The whey is commonly processed using ultrafiltration, although reverse osmosis, microfiltration, and demineralization methods can be used. During ultrafiltration, the low molecular weight compounds such as lactose, minerals, vitamins and nonprotein nitrogen are removed in the permeate while the proteins become concentrated in the retentate. After ultrafiltration, the retentate is pasteurized, may be evaporated, then dried. Drying, usually spray drying, is done at lower temperatures than for milk in order that large amounts of protein denaturation may be avoided.