A study material for M.Sc. Biochemistry (Semester: IV) Students
on the topic (EC-1; Unit IV)

The Microbiology of Food Preservation (Part II)

Dr. Reena Mohanka
Professor & Head
Department of Biochemistry
Patna University
Mob. No.:- +91-9334088879
E. Mail: reenamohanka1@gmail.com

Food Preservation

- Food preservation actually is a continuous fight against microorganisms spoiling food.
- Enzymatic (endogenous) spoilage is also the greatest cause of food deterioration. They are responsible for certain undesirable or desirable changes in fruits, vegetables and other foods. If enzymatic reactions are uncontrolled, the off-odours, and off-colours may develop in foods.
- Chemical spoilage involves oxygen sunlight etc and causes oxidative rancidity of fats and oils.
- An arsenal of preservation techniques are employed by food industry for satisfying consumers choice of maintaining nutritional value, texture and flavour ;these methods singly or in combinations can expand the shelf life of food.

Methods of food Preservation

[a] Physical Methods ---

1. **Blanching**
2. **Pasteurization**
3. **Sterilization**
4. **Dehydration**
5. **Canning**

[b] Non- thermal food preservation

1. **Chilling**
2. **Freezing**
3. **Irradiation**
4. **High pressure processing of food (pascalization)**
5. **Microwave heating**
6. **High intensity white light and UV light food preservation**
7. **Pulsed electrical field technology**
8. **Vacuum packing**

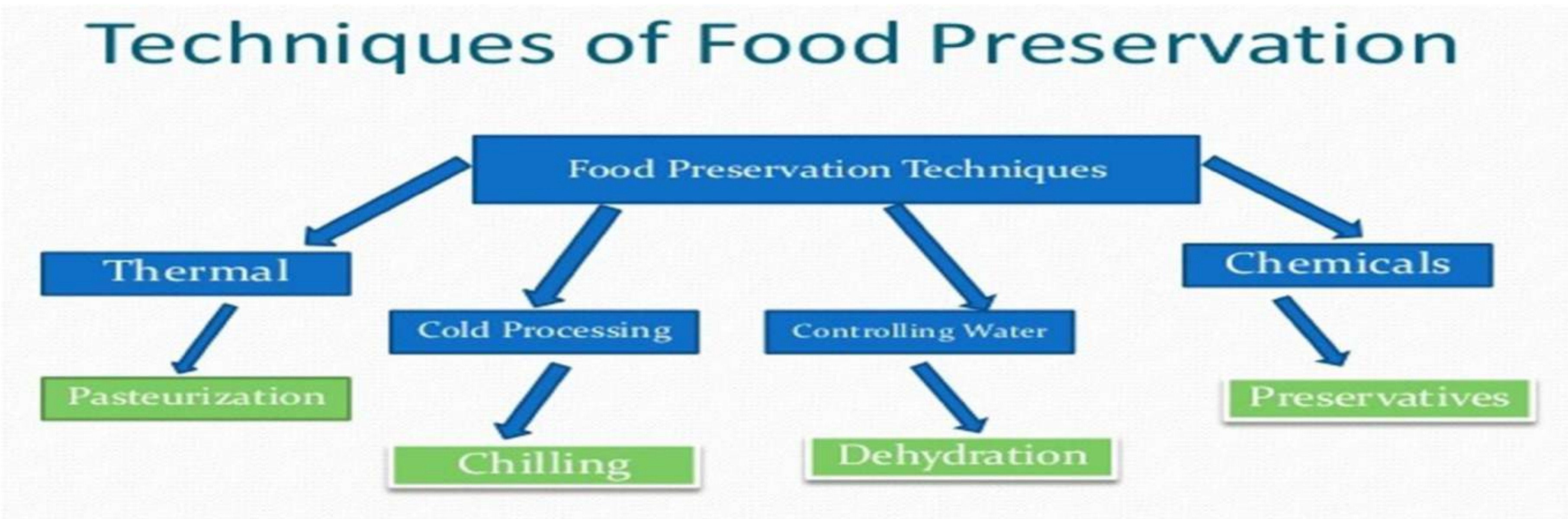
Methods of food Preservation

[c] Chemical Methods: Works as direct microbial poisons or reduces pH to a level that prevents the growth of Microorganisms.

1. Organic acid and its esters -- Propionates , Benzoates , Sorbates, Acetates
2. Nitrites and Nitrates
3. Sulfur Dioxide and Sulphites
4. Ethylene and Propylene Oxides
5. Sugars and Salts
6. Alcohol
7. Formaldehyde
8. Food additives
9. Antibiotics

[d] Biological method:

1. Fermentation



Non thermal food preservation techniques

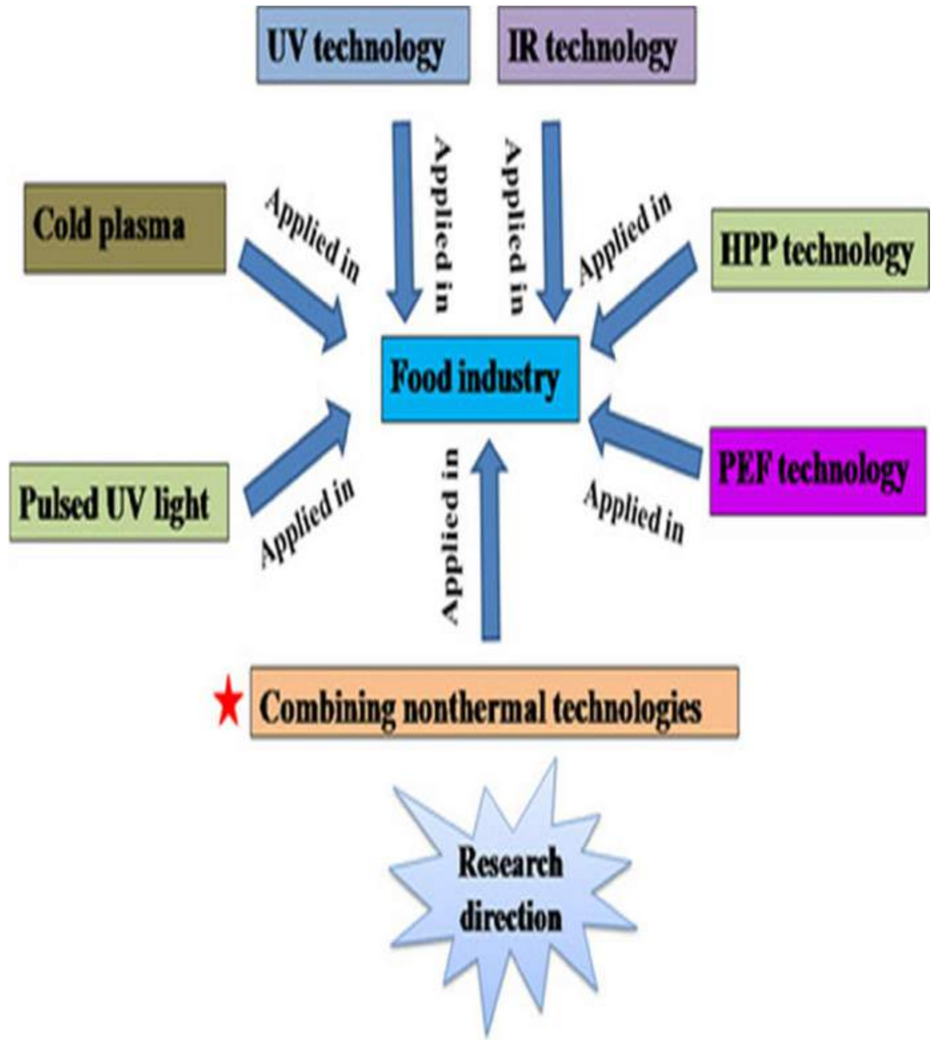
Refers to relatively young technology that uses mechanisms other than conventional heating energy for preservation. In contrast to high temperature, low temperature does not destroy microorganisms or enzymes but just depresses their growth and slows down enzymatic and oxidation process.

Thus it i is not a permanent preservation procedure.

The temperature coefficient (Q10) may be generally defined as follows:

Qio= : (Velocity at a given temp. + 100C Velocity at T

The Qi0 for most biological systems is 1.5-2.5, so that for each 100C rise in temperature within the suitable range, there is a twofold increase in the rate of reaction. For every 100C decrease in temperature, the reverse is true.



Refrigeration or chilling

- Chilling has temperature range of 0°C to 5-8°C,here the activities of food borne microorganisms and enzymes is slowed down and/or stopped.
- Preservation of food by refrigeration is based on a general principle chemistry: molecular mobility is depressed and consequently, chemical reactions and biological processes are slowed down at low temperature.
- Though psychrotrophs (can be molds,yeasts, gram positive and gram negative bacteria) can grow in chilled foods they do so only relatively slowly
- Chilling often used in combination with other unit operations there is greater preservative effect than unit operation (for example fermentation or pasteurization) to extend the shelf life of mildly processed food.

Freezing

Freezing is one of most successful techniques for long term preservation , as nutrient contents are largely retained

Methods of freezing: There are numerous methods, of these ,the common methods are mentioned below.

a). **Standard freezing** : The International Institute of Refrigeration recommended a minimum temperature of -18C for frozen food.Partial freezing inbetween 4-0C.This is mechanical system having re-circulating refrigerant within an air cooler that exchanges heat from air circulating within the freezer to reduce food temperature.

b). Cryogenic freezing Few food require ultrafast freezing. Such materials are subjected to cryogenic freezing which is defined as freezing at very low temperature (−196 °C or −320.8 °F) Cryogenic systems(spraying and immersion) reduce temperature through the direct application of a medium, usually carbon dioxide or liquid nitrogen, within an enclosure that contains the food product.

c). Dehydro-freezing This is a process where freezing is proceded by partial dehydration. In case of some fruits and vegetables about 50% of the moisture is removed by dehydration prior to freezing. This has been found to improve the quality of the food and relatively more stable.

- ❖ **The stronger preserving action** of freezing is due to depression of water activity as a result of conversion of part of water to ice. It is the unit operation in which the temperature of food is reduced below its freezing point. Water undergoes a change in state to form ice crystals. The immobalization of water to ice and the resulting concentration of dissolved solutes in unfrozen water lower the water activity of (aw)of the food. This ‘freeze concentration’effect results indepression of (aw).
- ❖ **Rate of freezing too effects frozen food quality**. At slower rate there is disruption of osmotic equilibrium between cells and their surroundings and may cause irreversible damage to texture of fruits or vegetables. Such damage is minimised in case of rapid freezing
- Preservation is achieved by a combination of low temperature, reduced water activity and, in some foods, pre-treatment by blanching. There are numerous commercially supplied frozen foods.

Mechanism

The ability of an organism to grow at low temperature is associated with the composition and architecture of plasma membrane. As the temperature is lowered, plasma membrane undergoes phase transition from a liquid crystalline state to a rigid gel where solute transport is limited. The temperature of this transition is lower in psychrotrophs and psychrophiles largely as a result of higher levels of unsaturated and short chain fatty acids in their membrane lipid.

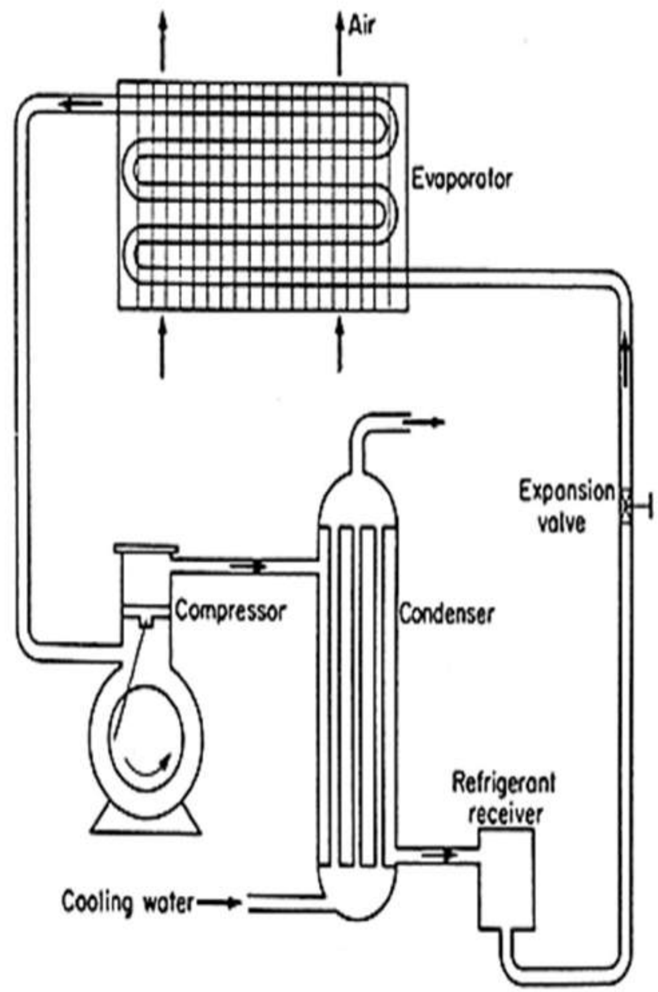
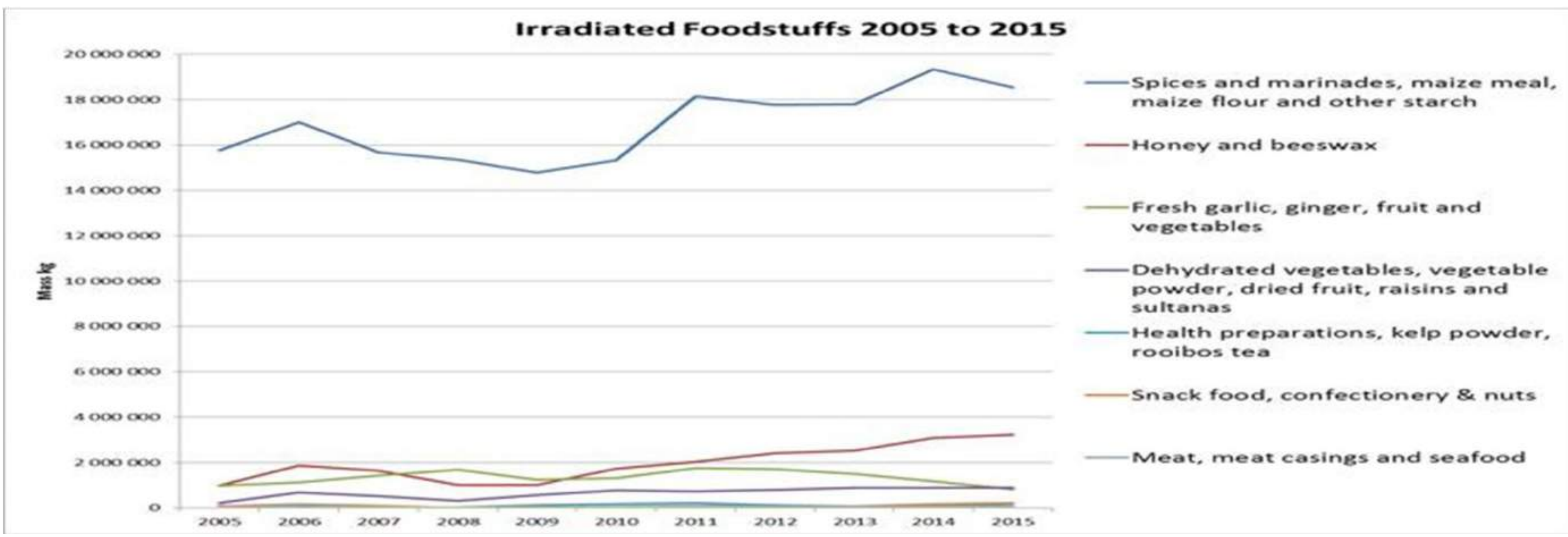


Fig- Mechanical refrigeration circuit

Food Irradiation

Food irradiation is one of recent cold food preservation technologies. It has been established as a safe and effective method of food processing and preservation It is the process of exposing food and food packaging to ionizing radiations such as x-rays,gamma rays or electron beams.It can be transmitted without direct contact to the source of energy(radiation) capable of freeing electrons from their atomic bonds (ionization) in the targeted food. The radiation can be emitted by radioactive substance or generated electrically. Irradiation maintains the safety of food but does not sterilize the food ; eventually food has to be refrigerated.Irradiated food maintain their wholesomeness and nutritive value.

Perception of consumers is negative related to irradiation compared to other means.The food is never in contact with the ionizing source,but still kills the living bacteria in the food.Food and Drug administration (FDA) the world health organization (WHO), have performed studies that confirm irradiation to be safe.Food irradiation is permitted by over 60 countries.



Sources of Irradiation

There are three sources of radiation approved for use on foods.-food irradiation involves uses of either high-speed electron beam or high energy radiations with wavelength smaller than 200 nanometres/2000 angstroms.(e.g.,x-rays and gamma rays).

Gamma rays are emitted from radioactive forms of the element cobalt (Cobalt 60) or of the element cesium (Cesium 137). Gamma radiation is used routinely to sterilize medical, dental, and household products and is also used for the radiation treatment of cancer.Most commonly used in food industry. For the same level of energy gamma rays have greater penetrating power into food.

X-rays are produced by reflecting a high-energy stream of electrons off a target substance (usually one of the heavy metals) into food. X-rays are also widely used in medicine and industry to produce images of internal structures.

Electron beam (or e-beam) is similar to X-rays and is a stream of high-energy electrons propelled from an electron accelerator into food.

The dose of radiation used on food products is divided into three levels. Radappertization is a dose in the range of 20 to 30 kilograys, necessary to sterilize a food product. Radurization is a dose of 1 to 10 kilograys, that, like pasteurization, is useful for targeting specific pathogens. Radicidation involves doses of less than 1 kilogray for extending shelf life and inhibiting sprouting.

Biological effects and mechanisms of irradiation

Irradiation has both direct and indirect effects on biological materials. The direct effects are due to the collision of radiation with atoms, resulting in an ejection of electrons from the atoms. The indirect effects are due to the formation of free radicals (unstable molecules carrying an extra electron) during radiolysis. (radiation-induced splitting) of water molecules. The radiolysis of water molecules produces hydroxyl radicals, highly reactive species that interact with the organic molecules present in foods. The products of these interactions cause many of the characteristics associated with the spoilage of food, such as off-flavours and off-odours.

1.Ionizing radiations act by disrupting progeny cycle of pathogenic microorganisms by destroying DNA/RNA cycles. NON –ionizing radiations have poor penetrating power and bactericidal properties. While these effects may be mutually beneficial to consumers and the food industry, the level of radiation and the alteration of DNA in our food supply are concerns.

2.Proteins are not significantly degraded at the low doses of radiation employed in the food industry. For this reason irradiation does not inactivate enzymes involved in food spoilage, as most enzymes survive doses of up to 10 kilograys. On the other hand, the large carbohydrate molecules that provide structure to foods are depolymerized (broken down) by irradiation. This depolymerization reduces the gelling power of the long chains of structural carbohydrates. However, in most foods some protection against these deleterious effects is provided by other food constituents. Vitamins A, E, and B_1 (thiamine) are also sensitive to irradiation. The nutritional losses of a food product are high if air is not excluded during irradiation.

High intensity white light or Pulsed UV light food preservation

UV rays are non ionizing radiations and is widely used in food industry to control microorganisms by UV lamp Pulsed Light technology is an innovative method of food preservation by using very high power and very short duration of light emitted by inert gas flash lamps.The technique of pulsed light food was developed as a non-thermal food preservation technique, that involves discharge of high voltage electric pulses (upto 70 Kilovolt/cm) into the food product placed between two electrodes for few seconds . It is one of the emerging technologies which are used for the replacement of traditional thermal pasteurization among non thermal processes . It is a decontamination technique which aims at reducing the pests, spoilage microorganisms and pathogens from food without much effect on its quality. This technique has received several names viz.Pulsed UV light,high intensity broad spectrum pulsed light, pulsed light and pulsed white light.

The UV-light occupies a wide wavelength in the non ionizing region of electromagnetic spectrum, between X-rays (100nm) and visible light(400nm).The wavelength of light is divided into three bands –The long(UV-A,400-320nm) and middle (UV-B, 320-280nm) wavelength are present in sunlight having some germicidal value. However, the short wavelength (UV-C,280-100nm) have high germicidal capacity and do not occur naturally.They have to be produced articicially by conversion of electric energy.

This technique uses light energy in concentrated form and exposes the substrate to intense short bursts of light (pulses). Typically for food processing and preservation about one to twenty flashes per second are applied. The pulsed light processing can be described as a sterilization or decontamination technique used mainly to inactivate surface micro-organisms on foods, packaging material and equipments.

The key component of a Pulsed Light unit is a flash lamp filled with an inert gas, such as Xenon, which emits radiation that ranges from UV (200 nm) to NIR (1100 nm). A high-voltage, high-current electrical pulse is applied to the inert gas in the lamp. The strong collision between electrons and gas molecules cause excitation of electrons, which then emit an intense, very short light pulse (1 µs to 0.1 s). but it is generally accepted that UV plays a critical role in microbial inactivation.

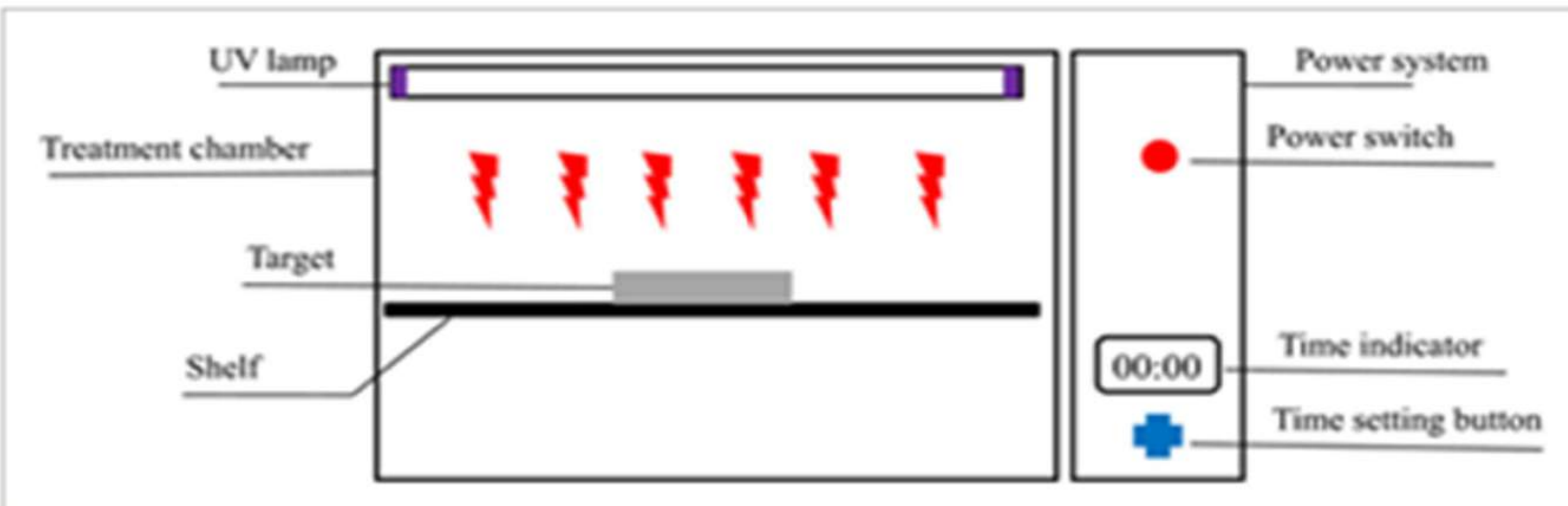


Fig: Diagram of a pulsed UV light system for treating food stuff (From - Google)

Mode of action

- Due to low penetration ability of UV rays, lethal action against microbes is confined to surfaces of organisms. It is absorbed by proteins and nucleic acids in which phytochemial changes are produced that may lead to cell death.
- Poor penetrating power limits its use to surface application. The most effective wavelength against microorganisms is around 260 nm which corresponds to strong absorption by nucleic acid bases. Some applications are as follows.
- Curing and wrapping of cheese
- Tenderization or aging of meat
- Prevention of surface mold growth on food stuffs
- Air purification in food processing industry
- Water treatment
- Disinfection of equipment and glasswares.

UV light as one of the weapons against coronavirus

UV light is well known for its germicidal properties, parallely its exposure can cause skin cancer**.** For disinfection purposes, the optimum wavelength required is in the region of 260 nm to 275 nm, with germicidal efficacy falling exponentially with longer wavelengths. The reason why UV radiation is effective in disinfection is because it has enough energy to break DNA chemical bonds. However that is not the case with narrow band of UV light called far-UV-C light. UV-C radiation, in the range of 260 nm – 275 nm, destroys the genetic information stored in the DNA and hence renders harmful micro-organisms such as bacteria and viruses ineffective. **Dr. Brenner a director of Center of Radiological research at Columbia University has studied UV light ,as potentially life saving weapon against spread of Viruses.** DNA and RNA are the building blocks of life for pathogens such as viruses and bacteria as well and without this genetic material, these pathogens are unable to reproduce which eventually leads to the death of an infectious colony.

Microwave Heating for Food Preservation

Both irradiation and microwave heating employ radiant energies which affect foods when their energies are absorbed. Microwave energy, has been employed to produce rapid and unique effects, one application of which can be food preservation. The UV-light occupies a wide wavelength in the non ionizing region of electromagnetic spectrum, between X-rays (100nm) and visible light(400nm). The wavelength of light is divided into three bands –The long(UV-A,400-320nm) and middle (UV-B, 320-280nm) wavelength are present in sunlight having some germicidal value. However, the short wavelength (UV-C,280-100nm) have high germicidal capacity and do not occur naturally. They have to be produced artificially by conversion of electric energy.

Microwaves cover the broad range of radio frequencies from 300MHz(million cycle per second) to 300GHz(billion cycle per second),corresponding to wavelength between 1 metre and 1 millimetre according to equation:

$$\text{Wavelength(cm)} = \frac{\text{Speed of light(cm/s)}}{\text{Frequency (Hz)}}$$

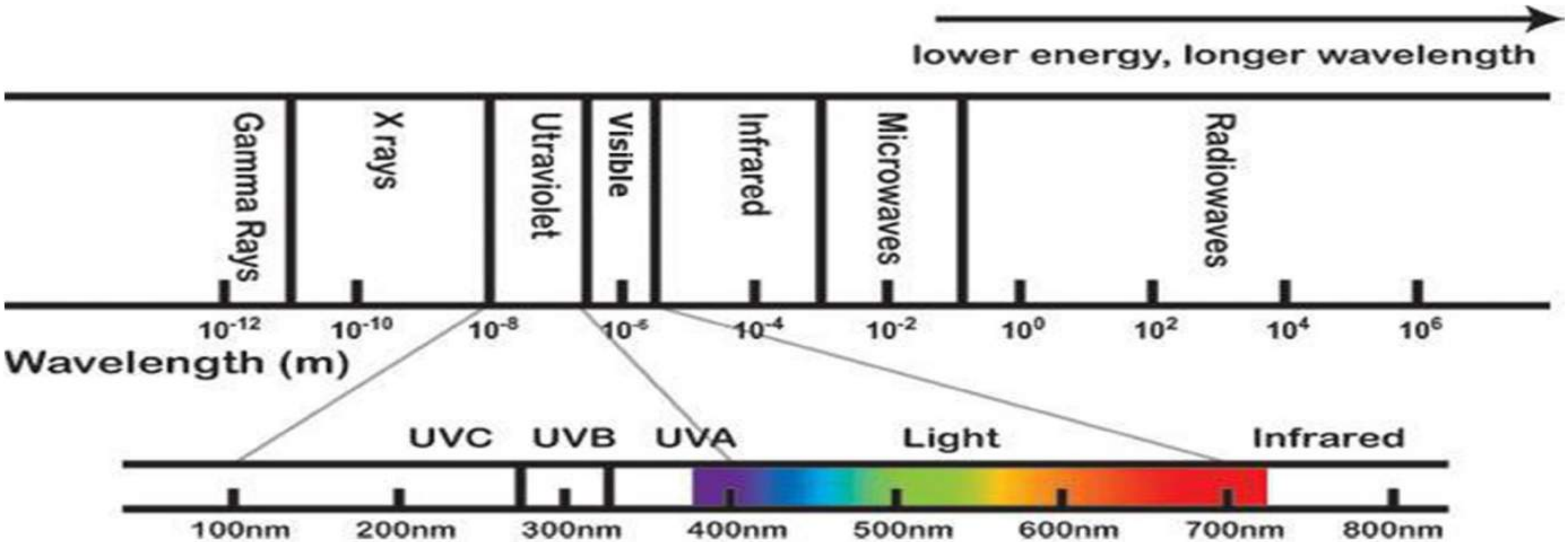
Microwave heating has considerable advantages over conventional heating methods, especially with regard to energy efficiency. Since heat is transferred from the surface of food to the interior by convection and conduction in conventional cooking method, it may result in a temperature gradient between outside and inside food .

Microbial decontamination by microwave heating

Many studies have demonstrated the effectiveness of using MV heating for pasteurization and sterilization of food.For example ,different strains of microorganismslike- *Bacillus cereus, Campylobacter jejuni, Clostridim perfrigens, Escherichia coli, Salmonella, Staphylococcus aureus* etc have been inactivated by MV heating.

Mode of action

Microwave energy has been reported as method for improving microbiological quality and extending shelf life of food. Also said that microbial microbial cell death occurs due to the heat produced by the irradiation and due heat produced by the irradiation and due to the electric field created by the microwaves, which promotes a change in the secondary and tertiary structures of the microorganisms proteins. Presently, microwave energy has been applied in the pharmaceutical industry, in drying and sterilization processes in food industry.



Pulse electrical field technology (PEF)

PEF is a non thermal method of food preservation that uses short pulses of electricity for microbial inactivation.This technology inactivates pathogenic microorganisms and some enzymes, although bacterial spores is not eliminated. Thus to overcome this limitation other methods as irradiation in combination is utilized for bacterial spore elimination.

The principles of pulsed electric field

The basic principle of the PEF technology is the application of short pulses of high electric fields with duration of microseconds micro- to milliseconds and intensity in the order of 10- 80 kV/cm.

The processing time is calculated by multiplying the number of pulses times with effective pulse duration.

The process is based on pulsed electrical currents delivered to a product placed between a set of electrodes; the distance between electrodes is termed as the treatment gap of the PEF chamber.

The applied high voltage results in an electric field that causes microbial inactivation.

After the treatment, the food is packaged aseptically and stored under refrigeration

Food is capable of transferring electricity because of the presence of several ions, giving the product in question a certain degree of electrical conductivity. So, when an electrical field is applied, electrical current flows into the liquid food and is transferred to each point in the liquid because of the charged molecules present.

Mechanism of Microbial inactivation
Two main types:
Electrical breakdown
Electroporation

Electrical breakdown
membrane compression
poreformation with reversible breakdown
large area of membrane subjected to
irreversible breakdown with large pores

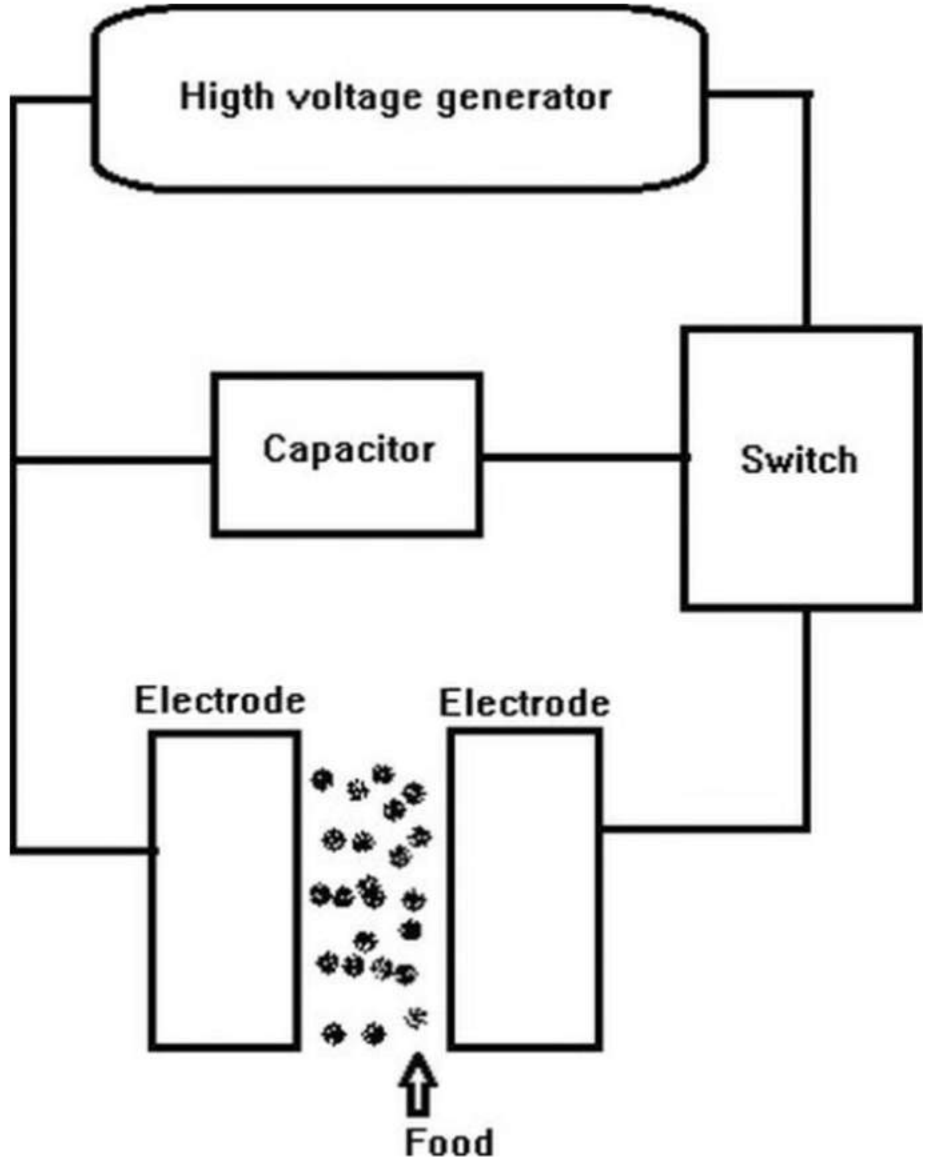


Fig: Pulsed electrical field technology

Electroporation

Cell exposed to high voltage electric field pulses temporarily destabilizes the lipid bi-layer and proteins of cell membranes. Creation of small hydrophilic pores. The reversible or irreversible rupture (or electroporation) of a cell membrane depends on factors such as intensity of the electric field, number of pulses, and duration of pulses. The plasma membranes of cell become permeable to small molecules after being exposed to an electric field; permeation then causes swelling and the eventual rupture of the cell membraneand causes cell death.

Advantages

- For food quality attributes, PEF technology is considered superior to traditional thermal methods as it imparts less changes in the sensory and physical properties of foods.
- Preserves solid, semi-solids and liquid foods
- Considered microbiologically safe and minimally processed foods.
- The method conserves Thermolabile vitamins as, there is no heat treatment.
- Although this technology yet has not been implemented at commercial level. Researchers claim that soon the market will experience the emergence of this technology

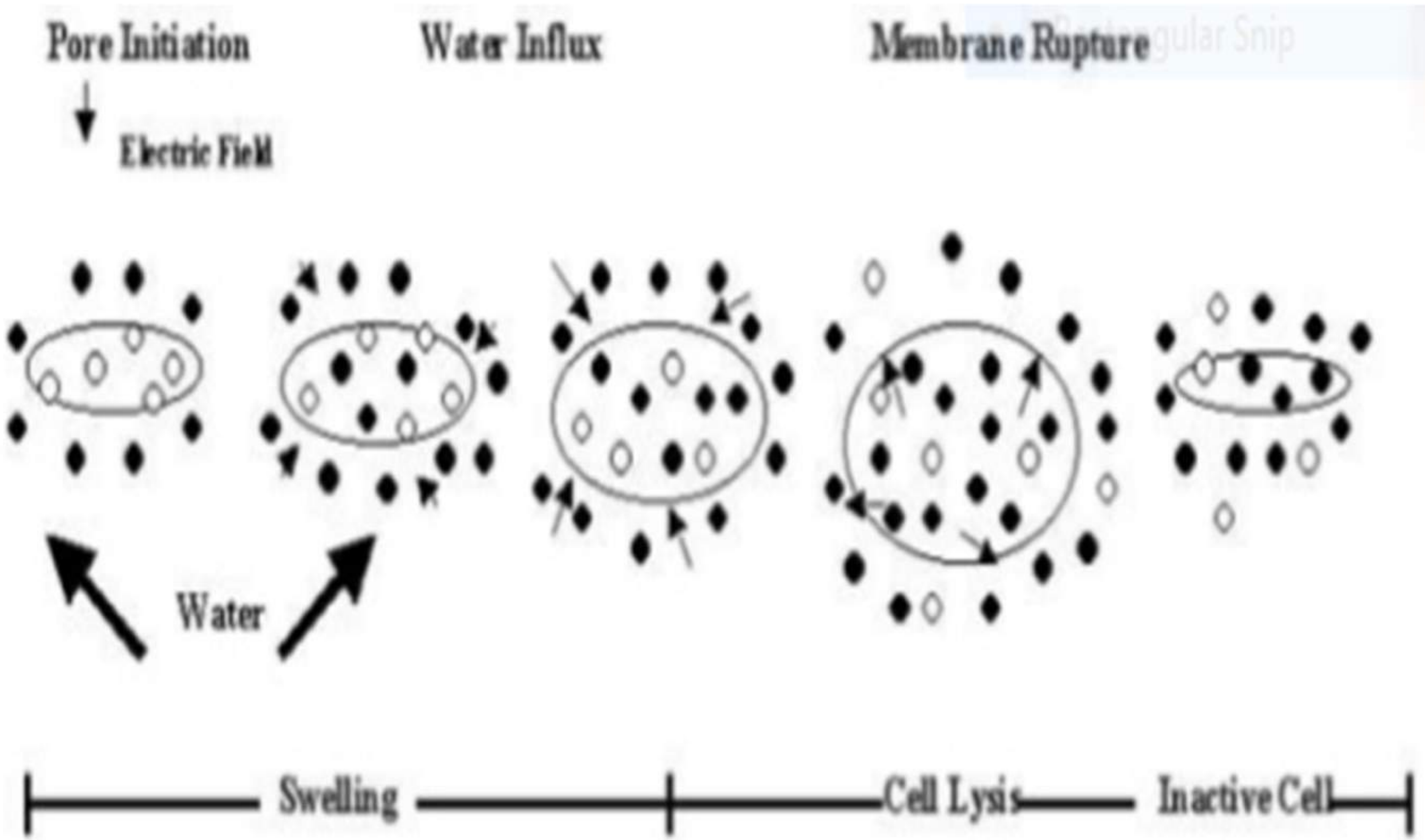
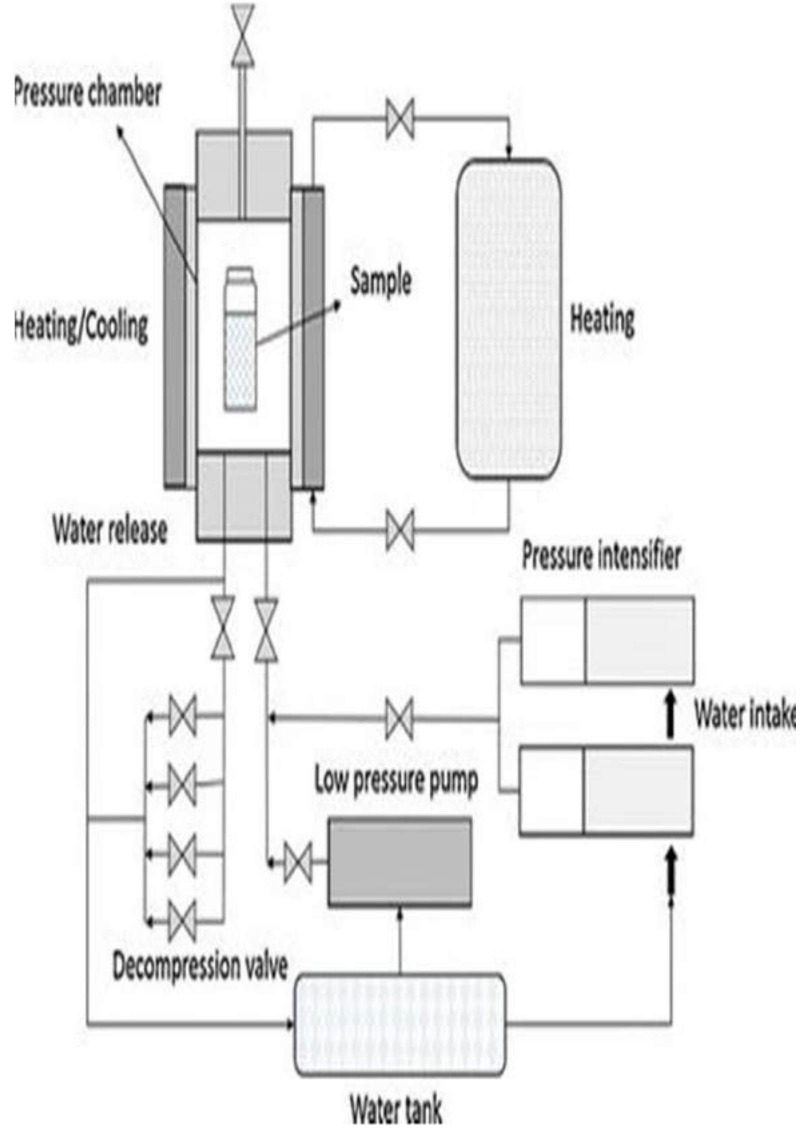


Fig: Mechanism of Electroporation

High pressure processing of food (Pascalization)

- **Pascalization**, bridgmanization, **high pressure processing** (**HPP**)
  or **high** hydrostatic **pressure** (HHP) **processing** is a method of preserving and sterilizing **food**, in which a product is **processed** under very **high pressure**, leading to the inactivation of certain microorganisms and enzymes in the **food**.
- In typical HPP operations, i.e. 400-600 MPa for two minutes or greater, the high pressure applied to foods at room temperature will reduce numbers of most vegetative bacteria by up to 4 log units or greater, and inactivate certain enzymes with only a small change in the organoleptic properties of the food.
- However, the resistance of bacteria and other microorganisms to HPP is highly variable, e.g. some gram positive bacteria such as Listeria monocytogenes can exhibit higher resistance than gram negative bacteria such as Salmonella.



Vacuum packing

Vacuum packing is a method of **packaging** that removes air from the package prior to **sealing**. This method involves (manually or automatically) placing items in a plastic film package, removing air from inside and **sealing** the package. Shrink film is sometimes used to have a tight fit to the contents.

Advantages/Disadvantages of Vacuum Packing

Advantages of Vacuum Packing	Disadvantages of Vacuum Packing
Substantial Increase Shelf Life	External Gases Can Increase Cost
Barrier From External Elements	Proper Gas Levels and Oxygen Levels Must be Known to Increase Shelf Life
Clear and Visible External Packaging	Loss of Preservation Once the Package has been Opened
Minimal Need For Chemical Preserves	Additional Sealer Attachments may be Required Based on Each Product
Quick and Efficient	Additional Labeling Often Needed
Reduced Product Loss	Basic Vacuum Bags can be Difficult to Open
Affordable Packaging Option	
Minimal Up-Front Cost	
Excellent For Freezer Storage	
Professional and Accepted Packaging Option Used Around the World	

Acknowledgement and Suggested Readings:

1. Microbiology, An Introduction; Tortora, Funke and Case; Pearson Publication
2. Microbiology; Prescott, Harley and Klein; The MacGraw-Hill Companies
3. Microbiology: Principles and Explorations; Jacquelyn G Black; John Wiley and Sons Inc.
4. Brock Biology of Microorganisms; Madigan, Martinko, Stahl and Clark; Benjamin Cummings (Pearson Publication)

Thanks