# Food and Dairy Microbiology

**Principles and Methods of Preservation**

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Foods are mainly composed of biochemical compounds which are derived from plants and animals. Carbohydrates, proteins and fats are the major constituents of food. In addition, minor constituents such as minerals, vitamins, enzymes, acids, antioxidants, pigments, flavours are present. Foods are subject to physical, chemical, and biological deterioration. The major factors affecting food spoilage are

1) Growth and activities of microorganisms (bacteria, yeasts, and molds)

2) Activities of food enzymes and other chemical reactions within food itself

3) Infestation by insects, rodents

4) Inappropriate temperatures for a given food

5) Either the gain or loss of moisture

6) Reaction with oxygen

7) Light

The vast majority of instances of food spoilage can be attributed to one of two major causes:

(1) the attack by microorganisms such as bacteria and molds, or

(2) oxidation that causes the destruction of essential biochemical compounds and/or the destruction of plant and animal cells. Chemical and/or biochemical reactions results in decomposition of food- due to microbial growth. There is a adverse effect on appearance, flavour, texture, colour, consistence and/or nutritional quality of food.

**Food Preservation**

Food preservation is the process of treating and handling food to stop or greatly slow down spoilage (loss of quality, edibility or nutritive value) caused or accelerated by micro-organisms. Preservation usually involves preventing the growth of bacteria, fungi, and other micro-organisms, as well as retarding the oxidation of fats which cause rancidity. It also includes processes to inhibit natural ageing and discolouration that can occur during food preparation such as the enzymatic browning reaction in apples after they are cut. Preservative for food may be defined as any chemical compound and/or process, when applied to food, retard alterations caused by the growth of microorganisms or enable the physical properties, chemical composition and nutritive value to remain unaffected by microbial growth.

**Principles of Food Preservation**

A good method of food preservation is one that slows down or prevents altogether the action of the agents of spoilage. Also, during the process of food preservation, the food should not be damaged. In order to achieve this, certain basic methods were applied on different types of foods. For example in earlier days, in very cold weather condition, ice was used to preserve foods. Thus, very low temperature became an efficient method for preventing food spoilage. Let us now list the principles of food preservation.

1. **Removal of micro-organisms or inactivating them**: This is done by removing air, water (moisture), lowering or increasing temperature, increasing the concentration of salt or sugar or acid in foods. If you want to preserve green leafy vegetables, you have to remove the water from the leaves so that microorganisms cannot survive. You do this by drying the green leaves till all the moisture evaporates.

**Control of microorganisms** • Heat • Cold • Drying • Acids • Sugar and salt • Oxygen concentration • Smoke • Radiation • Chemicals (preservatives)

2. **Inactivating enzymes**: Enzymes found in foods can be inactivated by changing their conditions such as temperature and moisture, when you preserve peas, one of the methods of preservations is to put them for a few minutes in boiling water. This method also known as blanching inactivates enzymes and thus, helps in preserving the

food. 3. Removal of insects, worms and rats: By storing foods in dry, air tight containers the insects, worms or rats are prevented from destroying it.

**Control of enzymes** • Heat • Oxygen removal • Acids • Chemicals (antioxidants)

**Control of Other factors•** Protective packaging • Sanitation Preservation methods 1. Thermal processing • Application of heat • Inactivate enzymes • Kill microorganisms. Most bacteria are killed in the range 82-93°c. Spores are not destroyed even by boiling water at 100°c for 30 min. o To ensure sterility (total microbial destruction, including spores), a temperature of 121°c must be maintained for 15 min or longer.

1. **Physical methods**

**I. Preservation by Using High temperature**

The term "thermal" refers to processes involving heat. Heating food is an effective way of preserving it because the great majority of harmful pathogens are killed at temperatures close to the boiling point of water. In this respect, heating foods is a form of food preservation comparable to that of freezing but much superior to it in its effectiveness. A preliminary step in many other forms of food preservation, especially forms that make use of packaging, is to heat the foods to temperatures sufficiently high to destroy pathogens.

The use of high temperatures to preserve food is based on their destructive effects on microorganisms. By high temperatures are meant any and all temperatures above ambient. With respect to food preservation, there are two temperature categories in common use: pasteurization and sterilization. **Pasteurization:** by use of heat implies either the destruction of all disease-producing organisms (for example, pasteurization of milk) or the destruction or reduction in the number of spoilage organisms in certain foods, as in the pasteurization of vinegar. In many cases, foods are actually cooked prior to their being packaged and stored. In other cases, cooking is neither appropriate nor necessary. The most familiar example of the latter situation is pasteurization. Conventional methods of pasteurization called for the heating of milk to a temperature between 145 and 149 °F (63 and 65 °C) for a period of about 30 minutes, and then cooling it to room temperature. In a more recent revision of that process, milk can also be "**flash-pasteurized**" by raising its temperature to about 160 °F (71 °C) for a minimum of 15 seconds, with equally successful results. A process known as ultra high pasteurization uses even higher temperatures of the order of 194 to 266 °F (90 to 130°C) for periods of a second or more.

The pasteurization of milk is achieved by heating as follows:

145°F (63°C) for 30 minutes (low temperature, long time [LTLT])

161ºF (72°C) for 15 seconds (high temperature, short time [HTST] method)

191°F(89°C) for 1.0 second

194°F (90ºC) for 0.5 second

201°F(94°C) for 0.1 second

212°F (100ºC) for 0.01 second.

These treatments are equivalent and are sufficient to destroy the most heat resistant of the nonspore- forming pathogenic organisms—*Mycobacterium tuberculosis* and *Coxiella burnetii*. When six different strains of M. paratuberculosis were added to milk at levels from 40 to 100,000 colony forming units (cfu)/mL followed by pasteurization by LTLT or HTST, no survivors were detected on suitable culture media incubated for 4 months. Milk pasteurization temperatures are sufficient to destroy, in addition, all yeasts, molds, gram negative bacteria, and many gram positives. The two groups of organisms that survive milk pasteurization are placed into one of two groups: **thermoduric** and **thermophiles**. #**Thermoduric organisms** are those that can survive exposure to relatively high temperatures but do not necessarily grow at these temperatures. The non-spore forming organisms that survive milk pasteurization generally belong to the genera *Streptococcus* and *Lactobacillus*, and sometimes to other genera.

**#Thermophilic organisms** are those that not only survive relatively high temperatures but require high temperatures for their growth and metabolic activities. The genera *Bacillus* and *Clostridium* contain the thermophiles of greatest importance in foods.

**Pasteurization** (to destroy spoilage biota) of beers in the brewing industry is carried out usually for 8-15 minutes at **60ºC**.

**Sterilization**: means the destruction of all viable organisms as may be measured by an appropriate plating or enumerating technique. Canned foods are sometimes called "commercially sterile" to indicate that no viable organisms can be detected by the usual cultural methods employed or that the number of survivors is so low as to be of no significance under the conditions of canning and storage. Also, microorganisms may be present in canned foods that cannot grow in the product by reason of undesirable pH, oxidation-reduction potential, or temperature of storage.

**II. Preservation by Using Low temperature:**

The use of low temperatures to preserve foods is based on the fact that the activities of food borne microorganisms can be slowed at temperatures above freezing and generally stopped at subfreezing temperatures. The reason is that all metabolic reactions of microorganisms are enzyme catalyzed and that the rate of enzyme catalyzed reactions is dependent on temperature. With a rise in temperature, there is an increase in reaction rate. The temperature coefficient (Q10) may be generally defined as follows:

**Q10=: (Velocity at a given temp. + 10ºC)/Velocity at T**

The Q10 for most biological systems is **1.5-2.5**, so that for each 10ºC rise in temperature within the suitable range, there is a twofold increase in the rate of reaction. For every 10ºC decrease in temperature, the reverse is true. The lower the temperature, the slower will be chemical reactions, enzyme action, and microbial growth. Each microorganism present has an optimal temperature for growth and a minimal temperature below which it cannot multiply. As the temperature drops from this optimal temperature toward the minimal, the rate of growth of the organism decreases and is slowest at the minimal temperature. Cooler temperatures will prevent growth, but slow metabolic activity may continue. Most bacteria, yeasts, and molds grow best in the temperature range 16-38ºC (except psychrotrophs). At temperatures below 10ºC, growth is slow and becomes slower the colder it gets. The slowing of microbial activity with decreased temperatures is the principal behind refrigeration and freezing preservation.

The term psychrophile was coined by Schmidt- Nielsen in 1902 for microorganisms that grow at 0ºC. This term is now applied to organisms that grow over the range of sub-zero to 20ºC, with an optimum range of 10-15ºC. Around 1960, the term psychrotroph (psychros, cold, and trephine, to nourish or to develop) was suggested for organisms able to grow at 5°C or below. It is now widely accepted among food microbiologists that a psychrotroph is an organism that can grow at temperatures between 0ºC and 7°C and produce visible colonies (or turbidity) within 7-10 days. Because some psychrotrophs can grow at temperatures at least as high as 43ºC, they are, in fact, mesophiles. By these definitions, psychrophiles would be expected to occur only on products from oceanic waters or from extremely cold climes. The organisms that cause the spoilage of meats, poultry, and vegetables in the 0-5ºC range would be expected to be psychrotrophs.

**Methods of freezing**

There are various methods of freezing

1. **Sharp Freezing (Slow freezing)** This technique, first used in 1861, involves freezing by circulation of air, either naturally or with the aid of fans. The temperature may vary from –15 to –29ºC and freezing may take from 3 to 72 hours. The ice crystals formed one large and rupture the cells. The thawed tissue cannot regain its original water

content. The first products to be sharp frozen were meat and butter. Now-a-days freezer rooms are maintained at –23 to –29$^{o}$C or even lower, in contrast to the earlier temperature of –18$^{o}$C.

**2. Quick freezing**: In this process the food attains the temperature of maximum ice crystal formation (0 to – 4$^{o}$C) in 30 min or less. Such a speed results in formation of very small ice crystals and hence minimum disturbance of cell structure. Most foods are quick frozen by one of the following three methods:

**a) By direct immersion:** Since liquids are good heat conductors food can be frozen rapidly by direct immersion in a liquid such as brine or sugar solution at low temperature. Berries in sugar solution packed fruit juices and concentrates are frozen in this manner. The refrigeration medium must be edible and capable of remaining unfrozen at –18oC and slightly below. Direct immersion equipments such as Zarotschenzeff 'Fog' freezer, T.V.A. freezer, Bartlett freezer etc. of commercial importance earlier are not used today.

**Advantages –**

1. There is perfect contact between the refrigerating medium and the product, hence the rate of heat transfer is very high.

2. Fruits are frozen with a coating of syrup which preserves the colour and flavour during storage.

3. The frozen product is not a solid block because each piece is separate.

**Disadvantages-**

1. Brine is a good refrigerating medium but it cannot be used for fruits.

2. It is difficult to make a syrup that will not become viscous at low temperature.

3. The refrigeration temperature must be carefully controlled, as at high temperature the medium will enter the product by osmosis and at low temperature the medium may freeze solid.

4. It is very difficult to maintain the medium at a definite concentration and also to keep it free from dirt and contamination.

**b) By indirect contact with refrigerant:** Indirect freeing may be defined as freezing by contact of the product with a metal surface which is itself cooled by freezing brine or other refrigerating media. This is an old method of freezing in which the food or package is kept in contact with the passage through the refrigerant at –18 to -46oC flows. Knowles Automatic Package feezer, Patterson continuous plate freezer, FMC continuous can freezer and Birds eye freezers are based on this principle.

**c) By air blast**: In this method, refrigerated air at –18 to –34oC is blown across the material to be frozen.

**The advantages claimed for quick freezing over slow freezing (sharp freezing) are**

(1) smaller (size) ice crystals are formed, hence there is less mechanical destruction of intact cells of the food

(2) period for ice formation is shorter, therefore, there is less time for diffusion of soluble material and for separation of ice

(3) more rapid preservation of microbial growth and

(4) more rapid slowing down of enzyme action.

**3) Cryogenic freezing**: Although most foods retain their quality when quick frozen by the above methods, a few require ultrafast freezing. Such materials are subjected to cryogenic freezing which is defined as freezing at very low temperature (below –60oC). The refrigerant used at present in cryogenic freeing are liquid nitrogen and liquid CO2. In the former case, freezing may be achieved by immersion in the liquid, spraying of liquid or circulation of its vapour over the product to be frozen.

**4. Dehydro-freezing:** This is a process where freezing is proceeded 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. Dehydration does not cause deterioration and dehydro frozen foods are relatively more stable.

**5. Freeze drying:** In this process food is first frozen at –18oC on trays in the lower chamber of a freeze drier and the frozen material dried (initially at 30oC for 24 hrs and then at 20oC). Under high vacuum (0.1 mm Hg) in the upper chamber. Direct sublimation of the ice takes place without passing through the intermediate liquid stage. The product is highly hygroscopic, excellent in taste and flavour and can be reconstituted readily. Mango pulp, orange juice concentrate, passion fruit juice and guava pulp are dehydrated by this method.

**III. Dehydration or drying**

Dehydration, or drying, of foods has long been practiced commercially in the production of spaghetti and other starch products. As a result of advances made during World War II, the technique has been applied to a growing list of food products, including fruits, vegetables, skim milk, potatoes, soup mixes, and meats.

Pathogenic (toxin-producing) bacteria occasionally withstand the unfavourable environment of dried foods, causing food poisoning when the product is rehydrated and eaten. Control of bacterial contaminants in dried foods requires high-quality raw materials having low contamination, adequate sanitation in the processing plant, pasteurization before drying, and storage conditions that protect from infection by dust, insects, and rodents or other animals.

Foodstuffs may be dried in air, superheated steam, vacuum, or inert gas or by direct application of heat. Air is the most generally used drying medium, because it is plentiful and convenient and permits gradual drying, allowing sufficient control to avoid overheating that might result in scorching and discoloration. Air may be used both to transport heat to the food being dried and to carry away liberated moisture vapour. The use of other gases requires special moisture recovery systems.

Loss of moisture content produced by drying results in increased concentration of nutrients in the remaining food mass. The proteins, fats, and carbohydrates in dried foods are present in larger amounts per unit weight than in their fresh counterparts, and the nutrient value of most reconstituted or rehydrated foods is comparable to that of fresh items. The biological value of dried protein is dependent, however, on the method of drying. Prolonged exposure to high temperatures can render the protein less useful in the diet. Low-temperature treatment, on the other hand, may increase the digestibility of protein. Some vitamins are sensitive to the dehydration process. For example, in dried meats significant amounts of vitamin C and the B vitamins—riboflavin, thiamine, and niacin—are lost during dehydration.

Dried eggs, meat, milk, and vegetables are ordinarily packaged in tin or aluminum containers. Fibreboard or other types of material may be employed but are less satisfactory than metal, which offers protection against insects and moisture loss or gain and which permits packaging with an inert gas.

Since most disease-causing organisms require a moist environment in which to survive and multiply, drying is a natural technique for preventing spoilage. Indeed, the act of simply leaving foods out in the **sun** and **wind** to dry out is probably one of the earliest forms of food preservation. Evidence for the drying of meats, fish, fruits, and vegetables go back to the earliest recorded human history.At some point, humans also learned that the drying process could be hastened and improved by various mechanical techniques. For example, the Arabs learned early on that apricots could be preserved almost indefinitely by macerating them, boiling them, and then leaving them to dry on broad sheets. The product of this technique, **quamaradeen, homemade food leather** is still made by the same process in modern Muslim countries. Today, a host of dehydrating techniques are known and used. The specific technique adopted depends on the properties of the food being preserved. For example, a traditional method for preserving **rice** is to allow it to dry naturally in the fields or on drying racks in barns for about two weeks. After this period of time, the native rice is threshed and then dried again by allowing it to sit on straw mats in the sun for about three days. Modern drying techniques make use of fans and heaters in controlled environments. Such methods avoid the uncertainties that arise from leaving **crops** in the field to dry under natural conditions. Controlled temperature air drying is especially popular for the preservation of grains such as maize, barley, and bulgur.

**Vacuum** drying is a form of preservation in which a food is placed in a large container from which air is removed. **Water vapor pressure** within the food is greater than that outside of it, and water evaporates more quickly from the food than in a normal atmosphere. Vacuum drying is biologically desirable since some enzymes that cause oxidation of foods become active during normal air drying. These enzymes do not appear to be as active under vacuum drying conditions, however.

**Two of the special advantages of vacuum** drying is that the process is more efficient at removing water from a food product, and it takes place more quickly than air drying. In one study, for example, the drying time of a fish fillet was reduced from about 16 hours by air drying to six hours as a result of vacuum drying. Coffee drinkers are familiar with the process of dehydration known as spray drying. In this process, a concentrated **solution** of coffee in water is sprayed though a disk with many small holes in it. The surface area of the original coffee grounds is increased many times, making dehydration of the dry product much more efficient.

**Freeze-drying** is a method of preservation that makes use of the physical principle known as sublimation. Sublimation is the process by which a solid pass directly to the gaseous phase without first melting. Freeze-drying is a desirable way of preserving food since it takes place at very low temperatures (commonly around 14°F to -13°F [-10°C to -25°C]) at which chemical reactions take place very slowly and pathogens survive only poorly. The food to be preserved by this method is first frozen and then placed into a vacuum chamber. Water in the food first freezes and then sublimes, leaving a moisture content in the final product of as low as 0.5%.

**IV. Canning**

Nicolas Appert, a Parisian confectioner by trade, is credited with establishing the heat processing of foods as an industry. In 1810 he received official recognition for his process of enclosing food in bottles, corking the bottles, and placing the bottles in boiling water for various periods of time. In the same year Peter Durand received a British patent for the use of containers made of glass, pottery, tin, or other metals for the heat preservation of foods. In 1822 Ezra Daggett and Thomas Kensett announced the availability of preserved foods in tin cans in the United States. Tin-coated steel containers, made from 98.5 percent sheet steel with a thin coating of tin, soon became common. These cans had a double seamed top and bottom to provide an airtight seal and could be manufactured at high speeds.

The establishment of the canning process on a more scientific basis did not occur until 1896, when the microorganism Clostridium botulinum, with its lethal toxin causing botulism, was discovered by Émile van Ermengem.

**V. Pulsed Electric Field Technology in Food Preservation**

A wide range of non-thermal processing techniques have gained popularity in the recent times as a potential tool for the substitution or replacement of traditional thermal processing methods of foods. Additionally, non-thermal processes offer several advantages over thermal processes such as low processing temperatures, efficient energy utilization, keeping the quality of food like colour, flavour, taste & nutrient retention and inactivation of quality deteriorative enzymes & spoilage causing microorganisms. Pulsed electric field (PEF) technology is a non-thermal food preservation method that involves the use of short electricity pulses for microbial inactivation while imposing minimal

**Principle of PEF**: electric fields for only a few micro to milliseconds with intensity in the range of 10-80 kV/cm. The process depends on the number of pulses delivered to the product which is held between two electrodes. These electrodes have a specific gap between them which is known as treatment gap of the chamber. During PEF processing, high voltage is applied that results in the inactivation of microorganisms present in the food sample. The electric field is applied in different forms like as exponentially decaying waves, bipolar waves or oscillatory pulses. The process can also be carried at various temperature ranges such as ambient, sub-ambient and above-ambient temperatures. Food is packed after treatment with PEF and then stored under refrigerated conditions. The science involved behind the transfer of electric pulses from food is that food contains several ions that provide a definite level of electrical conductivity to the product. This technique is usually preferred for liquid foods because electrical current flows into the liquid food more efficiently.

**VI. Preservation of Food through Irradiation**

Radiation processing of food involves exposure of food to short wave radiation energy to achieve a specific purpose such as extension of shelf-life, insect disinfestation and elimination of food borne pathogens and parasites. In comparison with heat or chemical treatment, irradiation is considered a more effective and appropriate technology to destroy food borne pathogens. It offers a number of advantages to producers, processors, retailers and consumers. Radiation processing of food involves exposure of food to short wave radiation energy to achieve a specific purpose such as extension of shelf-life, insect disinfestation and elimination of food borne pathogens and parasites.

**Type of Radiation**

The type of radiation used in processing materials is limited to radiations from high energy gamma rays, X-rays and accelerated electrons. These radiations are also referred to as ionizing radiations because their energy is high enough to dislodge electrons from atoms and molecules and to convert them to electrically-charged particles called ions.

Gamma rays and X-rays, like radio waves, microwaves, ultraviolet and visible light rays, form part of the electromagnetic spectrum and occur in the short-wavelength, high-energy region of the spectrum and have the greatest penetrating power. They have the same properties and effects on materials, their origin being the main difference between them. X-rays with varying energies are generated by machines. Gamma rays with specific energies come from the spontaneous disintegration of radionuclides.

Naturally occurring and man-made radionuclides, also called radioactive isotopes or radioisotopes, emit radiation as they spontaneously revert to a stable state. The time taken by a radionuclide to decay to half the level of radioactivity originally present is known as its half-life, and is specific for each radionuclide of a particular element. Only certain radiation sources can be used in food irradiation. These are the radionuclides cobalt-60 or cesium-137; X-ray machines having a maximum energy of five million electron volts (MeV) (an electron volt is the amount of energy gained by an electron when it is accelerated by a potential of one volt in a vacuum); or electron accelerators having a maximum energy of 10 MeV. Energies from these radiation sources are too low to induce radioactivity in any material, including food.

**Unit of Radiation Dose**

Radiation dose is the quantity of radiation energy absorbed by the food as it passes through the radiation field during processing. It is measured using a unit called the Gray (Gy).

In early work the unit was the rad (1 Gy = 100 rads; 1 kGy =1000 Gy).

**Application of Radiation processing of food**

Interest in the practical application of the process is emerging for many reasons. High food losses caused by insect infestation, microbial contamination and spoilage; mounting concern over food borne diseases, harmful residues of chemical fumigants and the impact of these chemicals on the environment, the stiff standards of quality and quarantine restrictions in international trade are some of the reasons. Though irradiation alone can not solve all the problems of food preservation, it can play an important role in reducing post-harvest losses and use of chemical fumigants.

On the basis of radiation dose, applications of radiation can be classified into:

**#Radappertization** is equivalent to radiation sterilization or "commercial sterility," as it is understood in the canning industry. Typical levels of irradiation are 3(MK) kGy.

**#Radicidation** is equivalent to pasteurization— of milk, for example. Specifically, it refers to the reduction of the number of viable specific nonspore- forming pathogens, other than viruses, so that none is detectable by any standard method. Typical levels to achieve this process are 2.5-10 kGy.

**#Radurization** may be considered equivalent to pasteurization. It refers to the enhancement of the keeping quality of a food by causing substantial reduction in the numbers of viable specific spoilage microbes by radiation. Common dose levels are 0.75-2.5 kGy for fresh meats, poultry, seafood, fruits, vegetables, and cereal grains.

**#Radappertization -** Radappertization of any foods may be achieved by application of the proper dose of radiation under the proper

**VII. Pascalization or high hydrostatic pressure (HHP):**

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. High pressure can be used in food processing in a similar way as temperature. For instance, hydrostatic pressure can induce gel formation in egg white and yolk, crude carp actomyosin, and a suspension of soy protein by the application of 1000 – 7000 atm pressure at 25°C for 30 min. The most unique property of HHP is its ability to be transferred instantly and uniformly throughout food system. Thus, the application of HHP is independent of sample mass and geometry. Other important advantages in using this technology in food industry are:

1. Inactivation of microorganisms and enzymes
2. Modification of biopolymers
3. Quality retention, such as colour and flavour
4. Changes in product functionality

In order to implement this new technology in the food industry, we need to understand the mechanism and kinetics of pressure – induced degradation / denaturation / inactivation of several food compounds (e.g. nutrients, proteins, microorganisms, enzymes) and the way in which the degradation / denaturation / inactivation.

In pascalization, food products are sealed and placed into a steel compartment containing a liquid, often water, and pumps are used to create pressure. The pumps may apply pressure constantly or intermittently. The application of high hydrostatic pressures (HHP) on a food product will kill many microorganisms, but the spores are not destroyed. Pascalization works especially well on acidic foods, such as yogurts and fruits, because pressure-tolerant spores are not able to live in environments with low pH levels. The treatment works equally well for both solid and liquid products.

Bacterial spores survive pressure treatment at ambient or chilled conditions. Researchers reported that pressure in combination with heat is effective in the inactivation of bacterial spores. The process is called pressure-assisted thermal sterilization. In 2009 and 2015, Food and Drug Administration (FDA) issued letters of no objection for two industrial petition for pressure-assisted thermal processing. At this time, there are no commercial low-acid products treated by PATP available in the market.

During pascalization, the food's hydrogen bonds are selectively disrupted. Because pascalization is not heat-based, covalent bonds are not affected, causing no change in the food's taste. This means that HPP does not destroy vitamins, maintaining the nutritional value of the food. High hydrostatic pressure can affect muscle tissues by increasing the rate of lipid oxidation, which in turn leads to poor flavor and decreased health benefits. Additionally, there are some compounds present in foods that are subject to change during the treatment process. For example, carbohydrates are gelatinized by an increase in pressure instead of increasing the temperature during the treatment process.

Because hydrostatic pressure is able to act quickly and evenly on food, neither the size of a product's container nor its thickness plays a role in the effectiveness of pascalization. There are several side effects of the process, including a slight increase in a product's sweetness, but pascalization does not greatly affect the nutritional value, taste, texture, and appearance. As a result, high pressure treatment of foods is regarded as a "natural" preservation method, as it does not use chemical preservatives.

**Some advantages of HHP**

1. High pressure is not dependent of size and shape of the food.

2. High pressure is independent of time/mass, that is, it acts instantaneously thus reducing the processing time.

3. It does not break covalent bonds; therefore, the development of flavours alien to the products is prevented, maintaining the natural flavour of the products.

4. It can be applied at room temperature thus reducing the amount of thermal energy needed for food products during conventional processing.

5. Since high pressure processing is isostatic (uniform throughout the food); the food is preserved evenly throughout without any particles escaping the treatment.

6. The process is environment friendly since it requires only electric energy and there are no waste products.

**Some limitation of HHP**

1. Food enzymes and bacterial spores are very resistant to pressure and require very high pressure for their inactivation.

2. The residual enzyme activity and dissolved oxygen results in enzymatic and oxidative degradation of certain food components.

3. Most of the pressure-processed foods need low temperature storage and distribution to retain their sensory and nutritional qualities.

**VIII. Aseptic processing**

The aseptic process involves placing a sterilized product into a sterilized package that is then sealed under sterile conditions. It began in 1914 with the development of sterile filters for use in the wine industry. However, because of unreliable machinery, it remained commercially unsuccessful until 1948 when William McKinley Martin helped develop the Martin system, which later became known as the Dole Aseptic Canning System. This system involved the sterilization of liquid foods by rapidly heating them in tubular heat exchangers, followed by holding and cooling steps. The cans and lids were sterilized with superheated steam, and the sterilized containers were filled with the sterile liquid food. The lids were then sealed in an atmosphere of superheated steam. By the 1980s hydrogen peroxide was being used throughout Europe and the United States for the sterilization of polyethylene surfaces.

**Commercial sterility**: In aseptic processing the thermal process is based on achieving commercial sterility—i.e., no more than 1 nonsterile package for every 10,000 processed packages. The aseptic process uses the high-temperature–short-time (HTST) method in which foods are heated at a high temperature for a short period of time. The time and temperature conditions depend on several factors, such as size, shape, and type of food. The HTST method results in a higher retention of quality characteristics, such as vitamins, odour, flavour, and texture, while achieving the same level of sterility as the traditional canning process in which food is heated at a lower temperature for a longer period of time.

The heating and cooling of liquid foods can be performed using metal plate heat exchangers. These heat exchangers have large surface areas that result in improved heating and cooling rates. Other types of heat exchangers involve surrounding the food with steam or directly injecting steam into the food. Products sterilized with steam are then pumped into a vacuum chamber, where they are cooled rapidly.

Liquid foods that contain large solid particles are heated in scraped-surface heat exchangers. These heat exchangers use blades to continuously scrape the inside surface of the heating chamber. The scraping action protects highly viscous foods from being burned on the heating surface.

An alternate method for heating foods, called ohmic heating, passes a low-frequency electric current of 50 to 60 hertz directly through the food. A liquid food containing solids, such as diced fruit, is pumped through a pipe surrounded by electrodes. The product is heated as long as the electrical conductivity of the food is uniform throughout the entire volume. This uniform rate of heating prevents the over processing of any individual region of the food. Ohmic heating yields a food product of higher quality than those processed using conventional systems.

**Packaging aseptically processed products**: The packaging containers used in aseptic processing are sterilized separately before they are used. The packaging machinery is sterilized using steam, sterile gases, or hydrogen peroxide. The sterilization process is generally monitored by culturing a test organism. For example, the remaining presence of the highly heat-resistant bacterium Bacillus subtilis globigii can be used as a marker to measure the completeness of sterilization.

Packages must be sealed under sterile conditions, usually using high-temperature sealing plates. Foods that are aseptically processed do not require refrigeration for storage.

**Blanching**: Blanching is a thermal process used mostly for vegetable tissues prior to freezing, drying, or canning. Before canning, blanching serves several purposes, including cleaning of the product, reducing the microbial load, removing any entrapped gases, and wilting the tissues of leafy vegetables so that they can be easily put into the containers. Blanching also inactivates enzymes that cause deterioration of foods during frozen storage.

Blanching is carried out at temperatures close to 100 °C (212 °F) for two to five minutes in either a water bath or a steam chamber. Because steam blanchers use a minimal amount of water, extra care must be taken to ensure that the product is uniformly exposed to the steam. Steam blanching leafy vegetables is especially difficult because they tend to clump together. The effectiveness of the blanching treatment is usually determined by measuring the residual activity of an enzyme called peroxidase.

**IX. Microwave Heating for Food Preservation**

Since food is generally of low thermal conductivity, heating by conventional methods remains relatively slow. Thanks to its volumetric and rapid heating, microwave (MW) technology is successfully used in many applications of food processing. In this chapter, fundamental principles of MW heating are briefly presented. MW drying and MW microbial decontamination are extensively reviewed as innovative methods for food preservation. However, the complex interactions between microwaves and materials to be heated are not yet sufficiently controlled. Moreover, MW heating heterogeneity and thermal runaway are the main drawbacks of this technology. Several methods have been proposed and investigated in the literature to overcome these problems in order to assure the microbiological safety and quality of food products. Microwaves (MWs) are electromagnetic (EM) waves, which are synchronized perpendicularly oscillations of electric and magnetic fields that propagate at the speed of light in a free space. MWs are characterized by the frequency (between 300 MHz and 300 GHz) and the wavelength (ranging from 1 m to 1 mm). According to the countries and regions, five frequencies (433, 896, 915, 2375, and 2450 MHz) are authorized for MW heating operations. The 2450 MHZ is the exclusive frequency for home appliances.

**Mechanisms of microwave heating**

The interaction of a wave with the material depends on its own characteristics (frequencies, wavelength) and the nature of the material, particularly its absolute permittivity $\varepsilon^*$, a complex number that determines how the material stores the electrical energy of the EF and its dissipation into heat. The readers can consult more specialized references for detailed information. We can define rapidly here the real permittivity, or dielectric constant, of a material which denotes the capacity of the material to store electrical energy and the effective loss factor which expresses the ability of the material to absorb energy of the wave and dissipate it into the heat by dielectric relaxation and ionic conduction. If a material contains free charges (ions) and polar molecules (e.g., water molecule) when this material is subjected to an EF, the ions will move at an accelerated rate according to their charge, which will cause collisions between them and, by the result, a conversion of the kinetic energy into heat (ohmic heating). In the same way, the polar molecules of this material, which was initially randomly oriented, will be oriented according to the polarity of the field. If the EF is an alternative, these molecules will rotate to remain aligned on it. This dipolar rotation will generate frictions between the molecules which will lead to an internal generation of heat (dielectric heating).

**Penetration and absorption of a wave in a material**

When an EM wave is directed toward a material, a part of the wave is reflected on the surface, while the other part penetrates it to be absorbed. The absorption of the wave during its crossing results in a decrease of the amplitude of the internal EF and so of its power. For small or thin materials, the accurate calculation of the internal electric field is recommended by using the Maxwell equations. Lambert's law (exponential EF decayed) may be used for larger objects. The penetration depth is defined as the penetration distance in the material for which the 63% of incident power of the incident wave has been absorbed. This depth depends on the dielectric properties of the material as well as the wavelength. The penetration depth at 915 MHz is larger than the penetration depth at 2450 MHz at the same conditions. More power will be absorbed when the loss factor is high.

**Microwave pasteurization and sterilization of foods**

Pasteurization and sterilization are widely used to extend the shelf life of most foods. The main goals of pasteurization are to destroy vegetative pathogenic microorganisms and to deactivate some enzymes in foods. Pasteurization temperatures and treatment time vary, primarily, depending on the nature, the pH of the product, and the target microorganism. In most pasteurization processes, the food is heated up to 60–85°C for a time varying from a few seconds to an hour. Pasteurization requires refrigerated storage conditions (3–4°C) for a storage life of 2–6 weeks. Sterilization, which can be seen as further pasteurization, destroys bacterial spores. In solid or semisolid products, the heat transfer takes place mainly by conduction from the surface to the centre often considered as the "cold" point. This leads to apply more severe conditions to reach the target temperature at the cold point, which results in an overcooking of the surface and a degradation of the quality of products. Optimizing thermal treatments (i.e., maximizing inactivation of bacteria while minimizing nutrient degradation) is therefore an important issue. However, this is not an easy task and it is always a technical and scientific challenge. Thanks to the direct and volumetric interaction between MWs and food, MW heating has the advantage of overcoming the limitation imposed by slow thermal diffusion of conventional heating.

2. **PRESERVATION BY USING CHEMICALS:**

A preservative is defined as only substance which is capable of inhibiting, retarding or arresting the growth of microorganisms. Microbial spoilage of food products is also controlled by using chemical preservatives. The inhibitory action of preservatives is due to their interfering with the mechanism of cell division, permeability of cell membrane and activity of enzymes.

Pasteurized squashes, cordials and crushes have a cooked flavour. After the container is opened, they ferment and spoil within a short period, particularly in a tropical climate. To avoid this, it is necessary to use chemical preservatives. Chemically preserved squashes and crushes can be kept for a fairly long time even after opening the seal of the bottle. It is however, essential that the use of chemicals is properly controlled, as their indiscriminate use is likely to be harmful. The preservative used should not be injurious to health and should be non-irritant. It should be easy to detect and estimate.

**I. SULPHUR DIOXIDE**

It is widely used throughout the world in the preservation of juice, pulp, nectar, squash, crush, cordial and other products. It has good preserving action against bacteria and moulds and inhibits enzymes, etc. In addition, it acts as an antioxidant and bleaching agent. These properties help in the retention of ascorbic acid, carotene and other oxidizable compounds. It also retards the development of nonenzymatic browning or discolouration of the product. It is generally used in the form of its salts such as sulphite, bisulphate and metabisulphite. Potassium metabisulphite ($K_2O$ $_2So_2$ (or) $K_2S_2O_5$) is commonly used as a stable source of $So_2$. Being a solid, it is easier to use than liquid (or) gaseous So2.It is fairly stable in neutral (or) alkaline media but decomposed by weak acids like carbonic, citric, tartaric acid and malic acids. When added to fruit juice (or) squash it reacts with the acid in the juice forming the potassium salt and $So_2$, which is liberated and forms sulphurous acid with the water of the juice. The reactions involved are as follows

Potassium Meta bisulphate + Citric acid → Potassium Citrate + Sulphur dioxide + $H_2O$

$SO_2 + H_2O \rightarrow H_2SO_3$ (Sulphurous acid)

$SO_2$ has a better preservative action than sodium benzoate against bacteria and moulds. It also retards the development of yeasts in juice, but cannot arrest their multiplication, once their number has reached a high value.

It is well known that fruit juices with high acidity do not undergo fermentation readily. The preservative action of the fruit acid its due to is hydrogen ion concentration. The pH for the growth of moulds ranges from 1.5 to 8.5, that of yeasts from 2.5-8.0, and of bacteria from 4.0 to 7.5. As fruit beverage like citrus squashes and cordials have generally a pH of 2.5 to 3.5, the growth of moulds and yeasts in them cannot be prevented by acidity alone. Bacteria, however, cannot grow. The pH is therefore, of great importance in the preservation of food product and by regulating it, one or more kinds of microorganisms in the beverage can be eliminated. The concentration of $So_2$ required preventing the growth of microorganism at different pH levels are as under.

**Table 1**

| pH | S.ellipsoideus (yeasts) | Mucor (mold) | Penicillium (mold) | Mixed bacteria |
|---|---|---|---|---|
| 2.5 | 200 | 200 | 300 | 100 |
| 3.5 | 800 | 600 | 600 | 300 |
| 7.0 | Above 5000 | Above 5000 | Above 5000 | Above 1000 |

The toxicity of $So_2$ increases at high temperature. Hence its effectiveness depends on the acidity, pH, temperature and substances present in fruit juice. According to FPO (fruit products order), the maximum amount of $So_2$ allowed in fruit juice is 700 ppm, in squash, crush and cordial 350 ppm and in nectar 100 ppm.

**The advantages of using $So_2$ are**

(a) It has a better preserving action than sodium benzoate against bacterial fermentation.

(b) It helps to retain the colour of the beverage for a longer time than sodium benzoate.

(c) Being a gas, it helps in preserving the surface layer of juices also.

(d) Being highly soluble in juices and squashes, it ensures better mixing and hence their preservation.

(e) Any excess of $so_2$ present can be removed either by heating the juice to about 71$^o$c or by passing air through it or by subjecting the juice to vacuum.

**Disadvantages (or) limitations**

(a) It cannot be used in the case of some naturally coloured juices like those of jamun, pomegranate, strawberry, coloured grapes, plum etc. on account of its bleaching action.

(b) It cannot also be used for juices which are to be packed in tin containers because it not only corrodes the tin causing pinholes, but also forms $H_2S$ which has a disagreeable smell and reacts with the iron of the tin container to form a black compound, both of which are highly undesirable.

(c) $So_2$ gives a slight taste and colour to freshly prepared beverages but these are not serious defects if the beverage is diluted before drinking.

**II. Benzoic acid**

It is only partially soluble in $H_2O$ hence its salt, sodium benzoate is used. One part of sodium benzoate is soluble in 1.8 parts of water at ordinary temperature, whereas only 0.34 parts of benzoic acid is soluble in 100 parts of water. Sodium benzoate is thus nearly 170 times as soluble as benzoic acid, pure sodium benzoate is tasteless and odourless. The antibacterial action of benzoic acid is increased in the presence of $Co_2$ and acid e.g. Bacillus subtilis cannot survive in benzoic acid solution in the presence of $Co_2$. Benzoic acid is more effective against yeasts than against moulds. It does not stop lactic acid and acetic acid fermentation. The quantity of benzoic acid required depends on the nature of the product to be preserved, particularly its acidity. In case of juices having a pH of 3.5-4.0, which is the range of a majority of fruit juices, addition of 0.06 to 0.10% of sodium benzoate has been found to be sufficient. In case of less acid juices such as grape juice at least 0.3% is necessary. The action of benzoic acid is reduced considerably at pH 5.0. Sodium benzoate is excess of 0.1% may produce a disagreeable burning taste. According to FPO its permitted level in RTS and nectar is 100 ppm and in squash, crush and cordial 600 ppm. In the long run benzoic acid may darken the product. It is, therefore, mostly used in coloured products of tomato, jamun, pomegranate, plum, watermelon, strawberry, coloured grapes etc.

**III. Salting**

Salting is the addition of salt (sodium chloride or NaCl) to food for the purpose of preservation. The growth of microorganisms is inhibited by the salt, which has the effect of drawing water out of the bacterial cells so they become dehydrated and die. In this manner, salt, in combination with other measures, acts as a preservative in many foods such as butter, cabbage, cheese, cucumber, meat and fish. It also gives a desired flavour to the food. Salting can be done by rubbing adequate quantities of dry salt into foods, or by immersion, where the food item is soaked in a concentrated salt solution (i.e. brine). For effective preservation, the concentration of the brine solution has to be maintained above 18%. This is approximately one cupful of salt to five cups of water. The precise mechanism by which salting preserves food is not entirely understood. It is known that salt binds with water molecules and thus acts as a dehydrating agent in foods. A high level of salinity may also impair the conditions under which pathogens can survive. In any case, the value of adding salt to foods for preservation has been well known for centuries. At one time, **sodium chloride** brine solutions were widely used for this purpose. A 10% brine solution, for example, has a freezing point of about 21°F (-6°C), well within the desired freezing range for many foods.

**IV. Sugaring**

Sugar appears to have effects similar to those of salt in preventing spoilage of food. The use of either compound (and of certain other natural materials) is known as curing. A desirable side effect of using salt or sugar as a food preservative is, of course, the pleasant flavor each compound adds to the final product. Sugaring refers to the action of sugar in food preservation. It is similar to the action of salt in that it depends on the removal of water. In concentrations of at least 65%, sugar solution is widely used as a sweetening and preserving agent. However, care is needed because at low concentrations, sugar solution can support the growth of microorganisms. It has been found that microorganisms rarely survive in solutions above 20–25% sugar concentration.

**V. Smoking**

**Smoking** is one of the oldest methods used to improve the quality of food and is commonly used to preserve meat and fish. The smoking process involves exposing food to smoke from burning or smouldering wood or other plant material. It partially preserves the food by surface drying, i.e. removing moisture from the surface of the food, but it is not a reliable method of preservation unless combined with some other method such as salting or drying.

### VI. Organic Acids (OAs) Groups and Their Application in Food Preservation

OAs are grouped into the monocarboxylic, dicarboxylic, alpha hydroxyl and sugar acids. Monocarboxylic acids include formic, acetic, propionic and sorbic acid. According to literature, acetic acid is used as emulsifiers, stabilizers, preservatives, flavour enhancers and firming. Formic acid which is the simplest carboxylic acid with one carbon atom, is used as a preservative, acidifier in animal feed as well as a flavouring agent at low concentrations. Propionic acid can be used as a pH control agent, preservative and flavour enhancer while sorbic acid has found application as preservatives. Dicarboxylic acid includes adipic, fumaric and succinic acid and have found application in beverage, feed and food preservation. Salts of adipic acid such as calcium and magnesium adipate are used in food processes as sequestrants, acidity regulators, baking additives, preservatives and flavour enhancers. Fumaric acid can be used as pH control agents, flavour enhancers, firming agents and as emulsifiers and dough conditioner during esterification process. Succinic acid is also used as flavour enhancers, preservatives, pH control agents and in baking. Alpha hydroxyl acids which include citric, lactic and malic acid have found application in beverage, food and animal nutrition. Citric acid and its salts are used as sequestrants, pH regulators, preservatives, flavour enhancers, and firming agents. Esters of citric acid can also be used as emulsifiers and solvents. Lactic acid has been used for a long time as acidity regulators, preservatives, baking additives, and flavor enhancers. Lactic acid can also be used as a humectant due to its hygroscopic activity. Malic acid on its part can be used as synergists, acidity regulators, preservatives, and flavour enhancers. Other OAs are called the sugar acid and they include ascorbic, gluconic, lactobionic and tartaric acid. Ascorbic acid and its isomer erythorbic acid are used as antioxidants, synergist, sequestrants and reducing agents. Gluconic acid and its salt are used as processing aids in the prevention of **milkstone** in dairy industry and also in animal nutrition. Lactobionic acid is used as gelling agent, flavour enhancer, antioxidant, acidity regulators, baking additives and firming agents

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**Table 2. Position of acetic acid among straight-chain, saturated carboxylic acids**

| Carbon atoms | Common name | IUPAC name | Chemical formula | Common location or use |
|---|---|---|---|---|
| 1 | Formic acid | Methanoic acid | HCOOH | Insect stings |
| 2 | Acetic acid | Ethanoic acid | $CH_3COOH$ | Vinegar |
| 3 | Propionic acid | Propanoic acid | $CH_3CH_2COOH$ | Preservative for stored grains |
| 4 | Butyric acid | Butanoic acid | $CH_3(CH_2)_2COOH$ | Butter |
| 5 | Valeric acid | Pentanoic acid | $CH_3(CH_2)_3COOH$ | Valerian |
| 6 | Caproic acid | Hexanoic acid | $CH_3(CH_2)_4COOH$ | Goat fat |
| 7 | Enanthic acid | Heptanoic acid | $CH_3(CH_2)_5COOH$ | |
| 8 | Caprylic acid | Octanoic acid | $CH_3(CH_2)_6COOH$ | Coconuts and breast milk |
| 9 | Pelargonic acid | Nonanoic acid | $CH_3(CH_2)_7COOH$ | Pelargonium |
| 10 | Capric acid | Decanoic acid | $CH_3(CH_2)_8COOH$ | Coconut and Palm kernel oil |
| 11 | Undecylic acid | Undecanoic acid | $CH_3(CH_2)_9COOH$ | |
| 12 | Lauric acid | Dodecanoic acid | $CH_3(CH_2)_{10}COOH$ | Coconut oil and hand wash soaps |
| 13 | Tridecylic acid | Tridecanoic acid | $CH_3(CH_2)_{11}COOH$ | |
| 14 | Myristic acid | Tetradecanoic acid | $CH_3(CH_2)_{12}COOH$ | Nutmeg |
| 15 | Pentadecanoic acid | | $CH_3(CH_2)_{13}COOH$ | |
| 16 | Palmitic acid | Hexadecanoic acid | $CH_3(CH_2)_{14}COOH$ | Palm oil |
| 17 | Margaric acid | Heptadecanoic acid | $CH_3(CH_2)_{15}COOH$ | |
| 18 | Stearic acid | Octadecanoic acid | $CH_3(CH_2)_{16}COOH$ | Chocolate, waxes, soaps, and oils |
| 19 | Nonadecylic acid | | $CH_3(CH_2)_{17}COOH$ | Fats, vegetable oils, pheromone |
| 20 | Arachidic acid | Icosanoic acid | $CH_3(CH_2)_{18}COOH$ | Peanut oil |

Source: https://en.wikipedia.org/wiki/Carboxylic_acid.

**Table 3. Properties of some acids, arranged in order of decreasing acid taste and with tartaric acid as reference**

| Acid | Properties of 0.05N solutions | | | Ionisation constant | Taste sensation | Found in |
|---|---|---|---|---|---|---|
| | Taste | Total acid g/L | pH | | | |
| Hydrochloric | +1.43 | 1.85 | 1.70 | - | - | - |
| Tartaric | 0 | 3.75 | 2.45 | $1.04 \times 10^{-3}$ | Hard | Grape |
| Malic | -0.43 | 3.35 | 2.65 | $3.9 \times 10^{-4}$ | Green | Apple, pear, prune, grape, cherry, apricot |
| Phosphoric | -1.14 | 1.65 | 2.25 | $7.52 \times 10^{-3}$ | Intense | Orange, grapefruit |
| Acetic | -1.14 | 3.00 | 2.95 | $1.75 \times 10^{-5}$ | Vinegar | - |
| Lactic | -1.14 | 4.50 | 2.60 | $1.26 \times 10^{-4}$ | Sour, tart | - |
| Citric | -1.28 | 3.50 | 2.60 | $8.4 \times 10^{-4}$ | Fresh | Berries, citrus, pineapple |
| Propionic | -1.85 | 3.70 | 2.90 | $1.34 \times 10^{-5}$ | Sour, cheesy | - |

Source: deMan (1999).

**Monocarboxilic Groups:** The chemistry and antimicrobial activity of the saturated straight-chain monocarboxylic acids have been documented as well as derivatives of this group— for example, unsaturated (cinnamic, sorbic), hydroxylic (citric, lactic), phenolic (benzoic, cinnamic, salicylic) and multicarboxylic (azelaic, citric, succinic) acids. OAs are distinguished from other acids by the carboxylic functional group -COOH to which an OA group or a hydrogen atom are attached. Common names used to describe this group of organic compounds include fatty, volatile fatty, lipophilic, weak, or carboxylic acids. The acetic acid of vinegar, the formic acid of red ants, and the citric acid of fruits all belong to the same family of compounds - carboxylic acids. Table 2 shows the position of acetic acid among straight-chain, saturated carboxylic acids. Aspects of the use of OAs include but not limited to animal husbandry as animal feed additives and in abattoirs and food-processing plants where they may be used in controlling microbial contamination of carcass meat. Citric acid is a hydroxy tricarboxylic acid produced naturally by various plants.

**Citric acid** is water soluble, approved for direct addition to multiple foods, is affirmed as **generally regarded as safe** (GRAS) and is approved for use in the manufacture of fresh and processed meats and poultry at concentrations specific to its purpose (USDA-FSIS, 2010). Citric acid and its salts have demonstrated efficacy for pathogen control in both fresh and processed meat

and poultry, but their usage is potentially limited by possible negative sensory impact and the need for low pH maintenance for optimum antimicrobial activity. However, citric acid does not fit under the description of classic weak organic acids, that is, the lipophilic, undissociated acids. Therefore, citric acid acts more as a chelator, exerting its antibacterial activity by sequestering metal ions such as $Ca^{2+}$, $Mg^{2+}$, and $Fe^{3+}$ from the external medium required for bacterial homeostasis. Similar to lactic acid, citric acid can also act as a permeabilizing agent of the outer membrane of Gram-negative bacteria, as well as a potentiator of the effect of other antibacterial agents.

**Dicarboxylic Acids:** Dicarboxylic acids ($HO_2C$-R-$CO_2H$) is an organic compound which contains two carboxyl functional groups (-COOH). They have similar chemical properties to monocarboxylic acids. However, they have two dissociation constants; one for the dissociation into a mono anion and the second into a di anion. The first ionization constant of dicarboxylic acids is usually larger than their monocarboxylic analogues because there are two potential sites for ionization making the effective concentration of the carboxyl group twice as large. Dicarboxylic acids are widely used in industries for the production of polymers (adipic acid), as food preservatives (oxalic acid) and as amino acids in human body (glutamic acid). Dicarboxylic acids can take various configurations depending on whether they are linear, branched chain, unsaturated or aromatic. Dicarboxylic acids are found naturally in plants and animals sources but in very little amount. Most dicarboxylic acids used industrially are synthetically produced from the oxidations of fatty acids.

**Mechanisms of Acetic Acid Antimicrobial Action:** The mechanism of inactivation by weak OAs lays down in the ability of undissociated form of OA to penetrate through the cell membrane, and to dissociate inside the cell, resulting in decreased intracellular pH value, which is essential for the control of ATP synthesis, RNA and protein synthesis, DNA replication and cell growth. Beside the decrease in intracellular pH, the perturbation of the membrane functions by organic acid molecule might be also responsible for the microbial inactivation. The high concentration of anions (due to dissociation) inside the cells might result in an increased osmolarity and consequently to the metabolic perturbation. As for other non-thermal inactivation treatments, the microbial sub-lethal injury might occur when the decontamination with organic acids is applied Traditionally, the principal use of acetic acid is in food and food-related applications, while other uses are for non-food industrial applications. As a weak acid, is frequently used as an inexpensive and effective intervention to reduce number and prevalence of bacterial pathogens on food products. Of all OAs evaluated in literature, acetic and lactic acid are found to be the most acceptable. At the same pH, OAs have a greater taste effect than inorganic acids (such as hydrochloric acid). The most common acids found in foods and compared with hydrochloric acid are presented in Table 3. The application of 2% lactic acid spray solution on beef carcasses and chicken breasts has been effective in reducing population of E. coli O157:H7 for more than 1.5 log CFU/cm2. OAs such as acetic, lactic and citric acids at concentration of 1.5–2.5% have been approved as acceptable innervations for reduction of microbial pathogens on meat carcasses in the United States. European Union provided the legal bases to permit the use of substances other than potable, clean water to decontaminate products of animal origin (EU, 2004).

**V: Nitrite and nitrates:**
Nitrites are preservatives added to processed meats (sodium nitrite 250 and sodium nitrate 251). They're not bad in and of themselves, but they do turn into harmful chemicals called nitrosamines. **Nitrosamines are**

**carcinogens found in cigarette smoke**. Nitrites form nitrosamines when they're cooked at high heat, and sometimes even when exposed to the high acid environment of the stomach. Nitrites are added to meats to keep the pink-red colour and prevent "browning." Mostly in bacon, ham, sausages and lunch meats. Since nitrites can change into nitrosamines, nitrites are one-step away from being the "bad guys." Another interesting thing is that processed meats have been linked with colon cancer. Because of the nitrites? Perhaps, but either way, nitrosamines are a confirmed health-buster. Since nitrosamines (from nitrites) are the bad guys and are formed by cooking nitrites at high heat, what are nitrates? Nitrates are naturally found in many healthy foods like vegetables. They're especially high in beets. Sometimes our enzymes or gut bacteria change these healthy nitrates into nitrites. However, they rarely form nitrosamines because they're two steps away from becoming these "bad guys."
Nitrate and nitrite have been used for centuries in curing and preserving meats and fish, and in the manufacture of certain cheeses. For commercial purposes, salt mixtures were found to be more effective in curing processes if they contained saltpetre (potassium nitrate). During preparation, nitrate is reduced to nitrite which is the major active ingredient in these salt mixtures. Nitrate is reduced to nitrite by bacteria under anaerobic conditions, using the molybdopterin-containing nitrate reductase. Dietary nitrate may be reduced to nitrite by bacteria present in the mouth and sometimes in the stomach. When added to foods such as cured meats, nitrite has at least three functions. Firstly, it contributes to the flavour; this may be due to the inhibition of development of rancid off favours. Secondly, it reacts with myoglobin to give mono-nitrosyl-haemo-chrome, which gives the characteristic pink colour of cured meat. Thirdly, it inhibits the growth of food spoilage bacteria, and most importantly, Clostridium botulinum. C. botulinum thrives under anaerobic conditions, and produces a neurotoxin which is one of the most lethal natural products known. Nitrite, together with cooking and the addition of salt, is a protection against food poisoning by this microorganism. Although the preservatives which are permitted in foods are considered to be without potential adverse effects there have been concerns about the safety of nitrites. Nitrite, in high concentrations, is undoubtedly toxic to humans. Acute effects have been observed from accidental ingestion, for example in contaminated drinking water, sausages and medicines. The principal toxic effect is oxidation of oxyhemoglobin to ferrihemoglobin, leading to methemoglobinaemia. This can be fatal, particularly in newborn infants in which the methemoglobin-reducing capacity is low, leading to so-called `blue baby syndrome'.

**VI. Bacteriocin:**

All organisms produce organic compounds that are not directly involved in their normal growth, development, or reproduction and are termed as secondary metabolites. They are generally low-molecular-mass products that include antibiotics, pigments, toxins, effectors of ecological competition and symbiosis, pheromones, enzyme inhibitors, immunomodulating agents, receptor antagonists, agonists and so on. Likewise, bacteriocins are produced by bacterial ribosomes as secondary metabolites. Antibiotics are also produced by some bacteria and, hence, along with bacteriocins they should be considered as different antimicrobial compounds. A large group of functionally diverse antimicrobial compounds found in all major lineages of bacteria and archaea constitute the bacteriocin family. While some possess a narrow bactericidal spectrum, many others exhibit a broader activity against some of the distantly related species. Over-all, specific immunity proteins responsible for this are encoded in the bacteriocin operon. Majority of them exert their action through the formation of transitory poration complexes or ionic channels which cause reduction or dissipation of the proton motive force leading to the formation of pores in the cytoplasmic membrane of sensitive cells. They also employ other killing mechanisms like cell wall interference and nuclease activity. Considering the antibacterial property of bacteriocins, some researchers speculate on considering them and antibiotics under the same class but since bacteriocins are bactericidal peptides, while antibiotics are produced by multi-enzyme complexes, there remains a demarcation between the two bactericidal agents. Most bacteriocins kill a narrow spectrum of bacteria, as compared to the traditional antibiotics. Some bacteriocins produced by lactic acid bacteria (LAB) are called **lantibiotics** and they have potential applications in the food industry. Currently existing synthetic food preservatives raise some concern with regard to the quality of food and are associated to several health hazards. Mode of action of bacteriocins Bacteriocins affect different essential functions of the living cell viz., transcription, translation, replication, and cell wall biosynthesis due to great variety of chemical structures, but most of them destroy the energy potential of sensitive cells by forming membrane channels or pores. The best-described mechanism is pore formation. The presence of molecular receptors in the membrane of the target cell is suggested by the relatively small action spectrum of some bacteriocins, although this has not been demonstrated. A model demonstrated using nisin suggests that, using C-terminal, nisin primarily binds to phosphatidylglycerol, a universal receptor, present on the cytoplasmic membrane of target bacteria. Hence a complete loss of activity could be observed if the C-terminal is removed. Binding of nisin is followed by insertion and penetration of a part of peptide into the cytoplasmic membrane. Fluorescence studies indicate a parallel orientation of nisin molecule with respect to the membrane surface having N-terminal inserted slightly deeper than C-terminal. This initiates the process of membrane insertion and pore formation, leading to rapid cell death. In general, the helical amphiphilic structure of class II peptides allows them to get inserted into the membrane of the target cell, leading to depolarization and death. At the hydrophilic N-terminus of the peptides, the initial interaction takes place with the heads of the anionic membrane phospholipids. The C-terminus of the peptide is thought to be involved in hydrophobic interactions with the membrane as it is more hydrophobic than the N-terminal. **Class II bacteriocins** allow the insertion of the peptide into the membrane of sensitive microorganism, using its amphiphilic structure, and causes depolarization and death.

On the contrary, the large bacteriolytic proteins, such as lysostaphin (**Class III bacteriocins**), lead to death and cell lysis by directly acting on the cell wall of Gram-positive target cells. Under the influence of various mechanisms, bacteriocins cause microbial cell killing either in an isolated or most consorted manner. Unbalanced cytoplasmic membrane function, inhibition of nucleic acid synthesis, interference with protein synthesis and changing the cell translator mechanism lead to bacteriocins killing a microbial cell. Due to the development of specific immunity mediated by a protein, bacteriocin-producing cells are not affected by the action of their own bacteriocin. Bacteriocins have been noteworthy in detections and causing fatality to competitor bacteria, with little or no damage to their producing cell. Microorganisms have competitive superiority when they dispute ecological niches in the environment where they are developing their metabolic activities. Based on this narrow action, bacteriocins play a role as intra-specific mediators or promoters of interactions among microbial populations. When 2 or more microorganisms are present in an environment and adversely interfere with growth and survival of other ones, antibiosis occurs. It is the antagonistic action exerted by bacteriocin-producing bacteria on the other bacteria in the same environment.

**VII. Antibiotics as Food Preservatives:**

The first of the modern antibiotics, penicillin, was discovered in 1929 by Fleming. However, the "era of antibiotics" in medicine and therapeutics may be considered to have actually begun in 1941 with the clinical use of penicillin. Similarly, the "antibiotic era" of food preservation may be considered to have had its potential inception in 1948 with the discovery by Duggar of Aureomycin or chlortetracycline. The commercial application of an antibiotic for perishable food preservation became possible on November 30, 1955, when the Food and Drug Administration cleared the use of chlortetracycline and established a tolerance of 7 p.p.m. for residues of chlortetracycline in or on uncooked poultry. The Dominion of Canada cleared the use of this substance for the preservation of poultry and fish on September 26, 1956 and set tolerances of 7 and 5 p.p.m., respectively, in the uncooked products. Later, the United States and Canada extended their approval for the similar use of oxytetracycline with the same tolerances.

**Fish-**The ineffectiveness of such narrow-spectrum antibiotics as penicillic acid, penicillin, and streptomycin for the preservation of fish, compared to the activity of sulfa drugs and sodium nitrite, was found out soon after their introduction (17, 18). Following the discovery of the so-called broadspectrum antibiotics, a number of reports appeared which indicated their potential usefulness for fish preservation. Chlortetracycline, oxytetracycline, and chloramphenicol at 10 to 25 p.p.m. inhibited bacterial growth in minced halibut, salmon, and brill up to 10 days at 33° and 37°C. Spoilage of whole eviscerated fish was markedly retarded by ices containing 1 to 4 p.p.m. of chlortetracycline, by holding in sea water containing 2 p.p.m. at -1°C. for six days, and by a I-min. dip in a 50 or 100 p.p.m. chlortetracycline solution prior to icing. The general observation that can be made from the studies on fish preservation by antibiotics is the almost unanimous finding that the tetracyclines, and particularly chlortetracycline which has been tested to the greatest extent, will appreciably prolong the storage life and delay spoilage of fish and fillets, the actual increase in keeping time depending among other factors on the storage temperature and on the freshness of the starting material.

**Shellfish-**The preservative effect of antibiotics on shellfish is somewhat complicated by the fact that some shellfish are handled in both a raw and a cooked state. Furthermore, the presence of the shell or "peel" on shrimp adds another complicating factor. Nevertheless, there are a number of reports which are unequivocal and show the potential beneficial effect of some antibiotics for shellfish. In one of the early studies on the effect of antibiotics on shrimp freshness, Farber found that immersion of raw beheaded unpeeled shrimp in a 2 p.p.m. chlortetracycline in 5 per cent salt solution did not retard spoilage (34). Later studies from this laboratory have shown that a 5-min. dip in a 15 p.p.m. solution definitely prolonged the freshness of both peeled and unpeeled raw beheaded shrimp and that the actual extension of the keeping quality depended upon the storage temperature.

**Poultry-**A number of investigators in this and other countries have reported that the addition of 10 p.p.m. chlortetracycline to the chill tanks resulted in prolonging the shelf life of whole eviscerated or cut-up chickens and of turkeys by 3 to 14 or more days, depending upon the storage temperature. The superiority of chlortetracycline over other antibiotics for prolonging the storage life of poultry has been brought out in various studies. When chloroxy-, and tetracycline, or a mixture of bacitracin and neomycin were given in the drinking water within 24 hr. before slaughter the resulting carcasses had a longer shelf life than the controls receiving only water. A mixture of 10 p.p.m. chlortetracycline with 5 to 10 p.p.m. of nystatin prevented the growth of yeasts and molds and the development of resulting spoilage odors on chicken parts during storage.

**Meat and meat products**-. Of six antibiotics tested in ground beef, only chlortetracycline, oxytetracycline, and chloramphenicol at 0.5 to 2 p.p.m. extended the storage life beyond that of the untreated controls and inhibited the growth of a number of organisms isolated from spoiled meat. Rounds and whole animals were infused with chlortetracycline to put about 2 p.p.m. into the meat. The storage life of the treated rounds and of the cut-up meat from the infused animals at room temperature and in a chill room was prolonged beyond that of untreated meat. The tenderization of steaks could be speeded up by a 48-hr. room temperature storage folIowed by a five-day chiIIing to equal that found at two weeks postmortem in untreated controls. Curing solutions for hams containing 15 to 45 p.p.m. oxytetracycline and 4 per cent salt gave hams a longer storage life at 37°F. A dip in a 1 per cent chloramphenicol solution was less effective in preventing the growth of enterotoxigenic Staphylococci or Salmonellae on the surface of beef and pork than a 1 per cent acetic acid solution.

**Canned foods-** 5 to 20 p.p.m. of subtilin in conjunction with a 5-to-1O min. heating at 212°F. prevented food spoilage and the growth of toxic clostridia started an extensive series of studies on the possible use of subtilin and other antibiotics in canning. The goal was the reduction of the rather long heat treatments necessary to sterilize low acid foods safely and to prevent thermophilic spoilage. Even though subtilin with a mild heat treatment was found to have a sporostatic action on spores of Clostridium botulinum and of thermophilic flat-sour organisms, and to lower their thermal resistance, Subtilin and nisin along with mild heating have been found to inhibit flat-sour thermophilic spoilage of tomato juice.

**Dairy products-**The penicillin in milk is sporocidal against aerobic sporeformers and flat-sour spoilage organisms, and penicillin inhibited the natural milk flora, were later shown not to be of practical use, since neither penicillin, nor streptomycin, nor subtilin with a mild heating prevented the spoilage of normal and concentrated milk. For short-term preservation chlortetracycline and streptomycin arefound effective for human milk. Cow's milk was preserved for periods to 8 to 10 days at 30°C. by mixtures of 100 p.p.m. chlortetracycline with 100 p.p.m. of patulin and penicillin, and for three days by 20 p.p.m. of these antibiotics. Raw cow's milk was preserved for one, two, three, and four days at 30°C. by 200 p.p.m. penicillin and streptomycin, 100 p.p.m. chloramphenicol, 200 p.p.m. patulin, and 100 p.p.m. chlortetracycline and oxytetracycline, respectively. Pasteurized milk was preserved for 12 to 15 days at 30°C. by the same antibiotics. the pasteurized milk is protected longer than raw cow's milk by penicillin, streptomycin, chlor- and oxytetracycline at 25 p.p.m. Nisin, the antibiotic produced by certain strains of Streptococcus lactis, has been reported to control clostridial spoilage in cheese.

**Fruits and vegetables-** The shelf life of packaged spinach which has been inoculated with soft-rotproducing bacteria was extended by a dip or spray of 0.5 to 0.1 per cent streptomycin or oxytetracycline for one day at 21.1°C. Streptomycin at 0. 1 per cent concentration also controlled bacterial soft-rot of packaged cole slaw for 3 days. Potato slices were protected from blackleg rot by streptomycin and dihydrostreptomycin and from Pseudomonas fiuorescens rot by chlortetracycline. Bacterial spoilage of vegetables was delayed up to 48 hr. by 25 and 50 p.p.m. oxytetracycline. Salad vegetables, singly and in mixtures, were preserved for 1, 7, and 12 days at 30, 10, and 5ºC., respectively, by 25 to 50 p.p.m. oxytetracycline. Strawberries were protected from mixed Rhizopus stolonifer and Botrytiscinerea rot for 48 to 72 hr. at 21°C. by 100 p.p.m. dips in the antifungal antibiotics cycloheximide and oligomycin.

**Public health aspects**

A number of factors of possible public health significance have to be taken into account before the use of antibiotics for perishable food preservation can become widespread. Among these are oral toxicity, the development of sensitivity which may influence later therapeutic use, the development of a resistant flora which may produce an enhanced spoilage, and the development of a resistant pathogenic flora which could affect later therapy. The tetracycline group of antibiotics is characterized by a step-by-step or progressive type of resistance development by bacteria, in contrast to what may be called the "one-step" development of a high resistance to such agents as streptomycin. Since "the microbial contaminants on each incoming consignment of untreated foods will normally have had no previous contact with the antibiotic, repetitive exposure to a particular antibiotic will not occur, and, in the instance of the tetracyclines, a high degree of resistance would not be expected to develop," and "a single exposure in foods, at the low levels recommended, to a member of the tetracycline group ... is unlikely to induce resistance to the much higher concentrations therapeutically used"

**VIII. Ethylene and Propylene Oxide:**

 Ethylene oxide exists as gas, kills all microorganisms. Propylene oxide can also kills many

microorganism, is not as effective as that of ethylene oxide.

 They are thought to acts as an alkylating agent and employed as fumigant in warehouses and

applied to dried fruits, eggs, dried yeast, cereals, spices etc.

 Hydroxyl ethyl group blocks reactive groups within microbial proteins and inhibits them.

 Ethylene oxide is used as gaseous sterilant (500 to 700 mg/L) and used for flexible and semi

rigid containers.

**IX. Fermentation and pickling**

Not all microorganisms are bad. Certain microorganisms are necessary in the preparation and preservation of many foods and beverages. Essentially, fermentation (a controlled microbial action) is a process of anaerobic or partially anaerobic oxidation of carbohydrates that produces acids and alcohol. It is one of the oldest methods of food preservation. In fermentation, food preservation is achieved by the presence of acid or alcohol, which creates unfavourable environmental conditions for decomposing and other undesirable bacteria.

Foods commonly processed and preserved by fermentation methods are milk and milk products, beef, vinegar, drinks like beer and wine, and pickled fruits and vegetables. Pickling is the process of preserving food by anaerobic fermentation either in brine (salt solution) or in an acid solution, usually vinegar. The concentrations of the pickling agents and the time needed for pickling are determined by the type of food. Fermented and/or pickled food products are semi-perishable and must be protected from moulds, which are able to attack the acids and permit the invasion of spoilage organisms.