

FOOD AND INDUSTRIAL MICROBIOLOGY

Food as substrate for microorganisms

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Microorganisms and its growth in food

Microorganisms are associated with plants and animals in nature. They play important role for survival of plants and animals. Our food consists of materials from plants and animals and it is obvious that food can contain microorganisms. Some microorganisms serve us as food, some present in food are helpful and some others are harmful to our health. Microorganisms present in food use them as source of nutrients or their growth media and grow. Growth of microorganisms in food can result in improving the quality of food and in some cases can deteriorate their quality depending on the type of microorganism. Growth of harmful microorganisms in food can result in spoilage and some times cause several diseases on consumption of such food. Food spoilage by microorganisms is due to increase in their numbers, utilizing nutrients, causing enzymatic changes resulting in bad flavors due to breakdown of some food materials or synthesis of new compounds. Due to such microbial activities, food become unfit for human consumption. Microorganisms bring about oxidation of reduced carbon, nitrogen, sulfur compounds present in dead plants and animals in nature and become important part in cycling of these elements.

Food acts as good medium for transmission of many diseases. If the food is contaminated by pathogenic microorganisms, they can grow and increase their population and cause diseases on consumption of such food. Some time microorganisms may not grow in food but they are transported through food. Therefore, food act as good medium for spread of diseases. Several food born diseases are the result of microorganism present in food or their growth in them.

Growth of microorganisms in nature is dependent on various factors. The factors influencing the growth of microorganisms are physical, chemical and biological in nature. The important factors which contribute to the growth of microorganisms in food are temperature, pH, moisture content, redox potential, nutrient content, inhibiting substances and other microorganisms present in food.

Temperature

Growth of microorganisms is influenced by the temperature which has effect on biocatalytic activity of enzymes present in them. Every organisms has its minimum, optimum and maximum limits of temperature for its survival, growth and biocatalytic activity. Microorganisms present in food are influenced by the temperature at which they are processed and preserved. Temperature also influences food for their chemical composition and oxidation status. Food at room temperature are influenced by the growth of mesophilic microorganisms. Most of the food spoilage is caused by microbial growth at mesophilic temperature. Therefore, the practice of preserving food at low temperature in refrigerators is to prevent spoilage by mesophilic microorganisms. However, continuous practice of storing food at low temperature may result in natural enrichment of psychrophilic and psychrotrophic microorganisms which become cause of food spoilage in refrigerators or cold storages.

Hydrogen ion concentration (pH)

Biocatalytic activities are influenced by pH. Growth of microorganisms is affected by the pH of growth environments in food (growth medium) which is the result of large number of enzymes responsible for metabolism and growth. Every organism or enzyme has a minimum, optimum and maximum pH requirements for their survival, growth or biocatalytic activity. Influence of pH of food not only has effect on growth of microorganisms but also on processing conditions. Food having acidic contents promote growth of acid loving microorganisms such as yeasts,

molds and some bacteria. Most of the spoilage bacteria grow in food at neutral pH. Food are preserved with the addition of preservatives such as citric acid, acetic acid etc. which create acidic environment and protect food to some extent from the growth of bacteria and other microorganisms requiring neutral or alkaline environment. However there may be chances of contamination and growth of acidophilic molds or yeasts. Therefore, growth of molds is frequently observed in fruits, fruit juices and other processed acidic food. Food acidification by fermentation in home food preparations is the oldest practice man has been doing. It is due to production of organic acids in food by growth and fermentation of microorganisms such as lactic and acetic acid bacteria. Lactic acid and acetic acid are considered as antibiotic in earlier scientific observations.

Moisture Content and Water Activity

Life exists only with water. Every living organism has the demand for water, without which no growth can occur. Similarly, microorganisms require water for their growth. Water is an excellent solvent for all life processes in every living organism for biocatalytic activity. The amount of water required varies for different organisms. Water requirement of microorganisms is expressed as available water or water activity (a_w). Water activity is the vapor pressure of the solution (of solutes in water in most food) divided by the vapor pressure of the solvent (usually water). Available water for microorganisms varies depending on microorganism and the type of food. In general, bacteria require more water activity than molds and yeasts. Most bacteria grow well in a medium with a water activity approaching 1.0. They grow well in low concentrations of sugar or salt, although there are exceptions. Gram negative bacteria have higher water requirements than gram positive bacteria. Most of the food spoilage bacteria do not grow below a_w 0.91, while spoilage molds can grow even at a_w 0.80. The aerobic food poisoning bacterium, *Staphylococcus aureus* is found to grow at a_w as low as 0.86 while anaerobic *Clostridium botulinum* does not grow below a_w 0.94. Different molds differ considerably in optimal a_w for vegetative growth and spore germination. Each mold also has an optimal a_w and range of a_w for growth. The lowest water activity values permitting growth of spoilage microorganisms is given in the Table below.

Group of Microorganism	Minimal (a_w) value
Bacteria	0.91
Yeasts	0.88
Molds	0.80
Halophilic bacteria	0.75
Xerophilic fungi	0.65
Osmophilic yeasts	0.60

Table 1: Lowest water activity (a_w) values for most of the different groups of microorganisms spoiling food

The effect of lowering a_w below optimum is to increase the length of the lag phase of growth and to decrease the growth rate and size of final population of microorganisms. This is due to

adverse influences of lowered water on all metabolic activities in microorganisms since all chemical reactions in cells require an aqueous environment. The a_w is influenced by other environmental parameters such as pH, Eh (redox potential) and growth temperature required for microorganisms. The strategy employed by microorganisms as protection against osmotic stress is the intracellular accumulation of compatible solutes.

Generally, the effect of lowered a_w on the nutrition of microorganisms is of a general nature where cell requirements that must be mediated through an aqueous milieu are progressively shut off. In addition to the effect on nutrients, lowered a_w undoubtedly has adverse effects on the functioning the cell membrane, which must be kept in a fluid state.

Redox potential (Eh)

Prevailing oxygen concentration of food in their environment, chemical composition and type of microorganisms associated, contribute to the oxidation-reduction (O-R) potential of food and affect growth of microorganisms in them. The O-R potential of food is determined by characters such as (a) oxygen tension of atmosphere above the food, (b) access of atmosphere to the food, (c) resistance of food to the changes occurring and (d) O-R state of materials present in food. Oxygen content in head space of the food contributes to the growth of microorganisms as microorganisms are aerobic, micro-aerophilic, facultative and anaerobic for oxygen requirement to grow. Aerobic and micro-aerophilic organisms require free oxygen in air but in different concentrations, facultatives grow in presence or absence of oxygen and anaerobes grow only in the absence of oxygen. Most of the fungi, bacteria and yeasts grow in food under aerobic conditions and anaerobic bacteria such as *Clostridium botulinum* require anaerobic conditions. Oxygen concentration of head space on food depends on its access to air. Food stored under evacuation or canned promote growth of anaerobic bacteria.

Redox potential of a system is expressed as *Eh* and expressed in millivolts (mV). Highly oxidized substrates have positive *Eh* and reduced substrates have negative *Eh*. Therefore aerobic organisms require positive *Eh* and anaerobic ones require negative *Eh* values for their growth. Most of the fresh plant and animal food have low redox potential because of reducing substances present in them. Fresh vegetables and fruits contain reducing substances such as ascorbic acid, reducing sugars and animal tissues have sulfhydryl (-SH) and other reducing group compounds considered as anti-oxidants. Fresh vegetables, fruits and meat support growth of aerobic microorganisms at their surfaces because of positive redox potential. However, the anaerobic microorganisms grow in inner parts of vegetables, fruits and meat because of negative redox potential. Most of processed plant and animal food gain positive redox potential therefore promote growth of aerobic organisms. Food subjected to heat and/or packed under negative pressure promote growth of anaerobic organisms. Microorganisms growing in food may create different redox potential states depending on their oxygen requirement and redox changes they bring about in food contents. Quality of processed food in terms of redox status depends on the changes brought about by processing methods, type of microorganisms and chemical changes.

Nutrient content

Nutritional quality of food in terms of their chemical composition, nutritive value or nutrients, their proportion and growth promoting ability are important for growth of microorganisms. Food for energy have more carbohydrates and other carbon compounds like esters, alcohols,

peptides, amino acids, organic acids and their salts. Carbohydrates may include polysaccharides like cellulose, starch and different sugars. Depending on the type of carbohydrate and other energy compounds present, there will be variation in terms of microorganisms growing in food. Most of the sugary food have growth of yeasts and bacteria and starchy food promote growth of molds which are able to hydrolyze starch by producing amylases. Cellulolytic and pectinolytic molds grow on food containing these materials. Carbon for growth usually comes from carbohydrates and other organic compounds in most of the heterotrophic microorganisms.

The total nitrogen content and different nitrogenous nutrients in food may vary. Microorganisms differ in their ability to use various nitrogenous compounds as source of nitrogen for growth. Many organisms are unable to hydrolyze proteins due to lack of proteolytic enzymes. Proteolytic organisms produce proteases and hydrolyze proteins to peptides and amino acids which are used as source of nitrogen for growth by them and others. Proteolytic property is different among microorganisms depending on the type of protease enzyme produced. Bacteria are more proteolytic than fungi in general. Protein rich food promote more growth of bacteria than molds and yeasts. Some of the lactic acid bacteria grow best with polypeptides as nitrogen source, cannot attack casein, and do not grow well with only a limited number and kinds of amino acids present. Presence of fermentable carbohydrates in a food results in an acid fermentation and suppression of proteolytic bacteria preventing production of obnoxious nitrogenous products. Many proteolytic bacteria grow best at pH values near neutrality and are inhibited by acidity.

Some microorganisms are not able to prepare vitamins and other growth factors. They need to be provided in growth medium. Food contain different vitamins, minerals and other growth factors and their composition and content may vary. Fresh plant and animal food contain B complex group of vitamins but stale food lack some of these vitamins. Meats are high in B vitamins and fruits are low, but fruits are high in ascorbic acid. Processing of food often reduces the vitamin content. Thiamine, pantothenic acid, folic acid and ascorbic acid are heat-labile and drying causes loss in vitamins such as thiamine and ascorbic acid. Storage of food for long may also result in decrease in vitamins and other growth factors. Some microorganisms produce vitamins and other growth factors which support growth of others organisms present in food. Each kind of microorganisms has a range of food requirements. Some have wide range and growth takes place in variety of substrates, such as coliform bacteria. Some pathogenic bacteria have very narrow range of substrates and require presence of specific kinds of substrates for growth. Generally molds can utilize many kinds of food ranging from simple to complex substrates by producing variety of hydrolytic enzymes. Depending on the composition, complexity and nutritive value, different food support growth of different microorganisms which are responsible for food contamination and spoilage.

Microorganisms important in food microbiology

Food serves as growth medium for different kinds of microorganisms. Microorganisms enter into food and grow as contaminants or intended additions. Growth of microorganisms in food may spoil food quality or improve depending on the types of microorganisms and the changes they bring about. Food are assessed for their quality in terms of physical, chemical, sensory and microbiological characteristics. Microbiological characteristics are assessed in terms of the

microorganisms present in food, their characters, ability to change the quality, their influence on health of consumer. It is necessary for food microbiologists to become acquainted with the microorganisms important in food at least to the extent that will enable them to identify the main types with their characteristics. Important microorganisms associated with food are molds, yeasts, bacteria, viruses and some protozoa parasites. Knowledge of general characters and primary identification methods is necessary for the people working with food microbiology.

Molds

Growth of molds on food as fuzzy or cottony appearance is the common observation for every one and moldy or 'mildewed' food are considered as unfit to eat. Molds are concerned in spoilage of many kinds of food, but some special molds are useful in manufacture of certain food or ingredients of food. Molds are used in making Oriental food, such as soy sauce, tempeh, and ripening of some kinds of cheese.

Molds are multicellular, cell wall containing organisms with filamentous structures when observed under low power magnification. Under high magnification, molds appear as complex morphological structures. The body or 'thallus' of a mold consists of mycelium and dormant spores. Mycelium is a mass of filaments called 'hyphae' (singular, 'hypha'). Each hypha may have width in the range of 5-10 μm and length in several μm formed by joining together of many cells. Rigid walls of hyphae are made of chitins, celluloses and glucans.

Hyphae may have different morphological structures for different functions. Hyphae may be classified as either coenocytic or septate. Coenocytic hyphae do not have septa (singular, septum), which are cross walls between the cells that make up a long filament. Each coenocytic hypha is essentially a long cell containing many nuclei. Septate hyphae have septa that divide the filament into distinct cells containing nuclei. However, there is a pore in each septum that allows cytoplasm and nuclei to migrate between cells. Some septa are complete without any pores. A hypha grows by elongation at its tip, called apical growth, and each filament that contains nuclei is capable of growing into a new organism. Some hyphae are embedded into solid media such as bread or soil (or agar in laboratory growth medium) to give the thallus support and nourishment. These specialized hyphae are called 'rhizoids', because they are root like. Reproductive hyphae may grow upward into the air to disseminate the spores they produce. Each spore on germination puts out a 'germ tube', a short, hyphalike extension that soon grows into a thallus. Hyphae with no specialized division of labour may simply grow along the surface of a substrate and are referred to as vegetative hyphae. Other hyphae can become organized into large structures to form the so called fleshy fungi, such as the mushrooms, puffballs. Some hyphae, several of them come together and become compact tissue like structures called fungal tissues to make specialized fruiting body structures like rhizomorph, sclerotium etc.

Molds are observed under microscope by making a slide of moldy material in lactophenol-cotton blue solution under cover glass using 10x and 40x magnifications of objective lenses. Morphological observation is an important characteristic for identification of molds. Important criteria for identification of molds are morphology of hyphae, spores, fruiting bodies. Nature of growth medium, colour of fungal colony, environmental conditions required for cultivating molds are additional identification characteristics. Molds generally grow under aerobic or microaerophilic conditions and therefore are important food spoilage organisms. Many

pathogenic fungi exhibit dimorphism, existing either in a unicellular, yeast like form or in a filamentous form depending on the growth environment. Molds reproduce by fragmentation, asexual sporulation and sexual spore formation in specialized structures called fruiting bodies.

Yeasts

Yeasts are normally unicellular fungi. Single yeast cells are generally larger than most bacteria, ranging widely in size from 1-5 μm in width and 5-30 μm or more in length. They are commonly oval, but some are elongated or spherical in shape. Each species of yeast has characteristic shape, but even in a pure culture there is considerable variation in size and shape of individual cells. Yeasts lack flagella and other means of locomotion. On an agar medium, they form smooth, glistening colonies that resemble those of bacteria. These colonies are quite different from the spreading, furry or filamentous colonies formed by molds. Most yeasts reproduce by budding, a projection of cell developing into an independent cell after separation. An yeast cell may have one or several budding structures. These are characteristically identified under microscopic observation.

Most yeasts are useful in food microbiology as fermenting organisms. Art of making breads, cakes, wines, beers and other fermented food making use of yeasts is oldest process of food preparation. Metabolic characters of yeasts to utilize sugars and produce variety of products in fermented food make different food products with difference in quality and nutritive value. Generally yeasts are more osmo-tolerant than molds and bacteria and therefore used in food fermentations.

Variety of yeast genera are usually found on fruits and bring about spoilage of fruit products. Many yeasts are capable of attacking the sugars found in fruits and bring about fermentation with production of alcohol and carbon dioxide. Contamination and unwanted growth of yeasts in food leads to spoilage and reduction in shelf-life.

Bacteria

Bacteria are unicellular microorganisms with small cell size ranging from 0.5-1 μm width and 1-5 μm length. Several bacteria are spherical in shape called cocci (singular, coccus) with a diameter of 0.5-1 μm . Several other bacteria are with different morphological structures like, oval, rod, comma, spiral and spring like shapes. Being very small, bacteria require high magnification to observe under light microscope with upto 100x objective lenses resulting in total magnification of 1000 – 2000 times.

Bacteria are prokaryotic in cellular structure with no defined nucleus and other cell organelles except 70S ribosomes. They have unique cell wall structure consisting of mureine or peptidoglycan containing D-amino acids. Peptidoglycan content in cell wall is more in Gram positive bacteria than Gram negative bacteria which have lipopolysaccharide as major cell wall constituent. Being tiny organisms, bacteria have more surface area volume ratio and grow very fast compared to other organism. This is an important character for their growth in food spoilage. They require more water content in growth environment and different groups of bacteria are capable of growing in psychrophilic, mesophilic and thermophilic conditions and grow at wide range of pH. Some bacteria produce endospores that make them resistant to adverse conditions including high temperature.

Bacteria grow on wide range of nutrients starting from simple elemental nutrients to complex animal fluids like foetal calf serum. Photoautotrophic bacteria are important in producing biomass carbon in aquatic environment. Nutritionally, bacteria have the simplest nutrient requiring organisms to very complex undefined nutrient requiring fastidious organisms and some of them are obligate parasitic pathogens like *Treponema pallidum*, *Mycobacterium leprae*, Rickettsiae and Chlamydiae. Heterotrophic bacteria are important in degradation of different organic carbon compounds and recycling of several elements in nature. They are important microorganisms in protecting our environment by detoxifying several toxic materials and recycling organic compounds. Majority bacteria are useful to human welfare and some are harmful causing different infectious diseases.

Bacteria have spectrum of metabolic reactions and produce variety of products using a wide range of substrates. This character is very useful in preparing several food of microbial fermentation and fermented milk products. They produce several enzymes like amylases, proteases, lipases, etc. which are important in food industry. Bacteria have become part of every household food preparations and maintaining good health. Lactic acid bacteria like *Lactobacillus* sp are good probiotic candidates for prevention of several infectious gastroenteritis diseases and are part of natural flora of human body. Several bacteria are the causative agents of human diseases. Some of the important human pathogens are *Staphylococcus aureus*, *Streptococcus pyogenes*, *Clostridium botulinum*, *Salmonella typhi*, *Shigella dysenteriae*. These are also important food contaminants and agents of food borne diseases and food poisoning. It is necessary to know about characters of such bacteria for their growth, metabolic products and the diseases they cause.

Bacteria are cultivated in laboratory using different laboratory growth media depending on nutritional requirements. It is relatively easy to grow bacteria under laboratory conditions compared to other organisms and understand their characteristics. Important bacteria of food fermentations like *Lactobacillus acidophilus*, *L. lactis*, *Acetobacter* sp are grown as starter cultures and used for large scale production of fermented food products.

Protozoa

These are microorganisms with animal like characteristics, lack of rigid cells wall, locomotion, and ingestion of food. Many protozoans absorb dissolved nutrients but some are predators and eat bacteria and other protozoa. These organisms may be free living in marine and fresh water and in soil or they can be symbionts in or on living hosts. Some protozoa are pathogenic to higher animals, like *Entamoeba histolytica*, *Plasmodium* sp. *Trepanosoma* sp., *Trichomonas* sp. Some of these are obligate parasites like Entamoeba and cause food or waterborne diseases frequently. Under developed countries face the challenge of diseases like amoebiasis, malaria, sleeping sickness caused by Entamoeba, Plasmodium, Trepanosoma. Diverse forms of protozoa are classified based on the structural features of cells as seen under high magnification microscopes. The general groups of protozoa are flagellates, amoebas, sporozoa and ciliates. Protozoa are observed under light compound microscopes and identified based on the morphology and ecological characteristics.

Viruses

Viruses are minute infectious agents that can be seen only with the help of electron microscope. They are 10 – 100 times smaller than bacterial cell with size range of 20 – 300 nm (0.02 – 0.3 μm). Viruses pass through the filter pores that retain bacteria and therefore called as filterable agents. Viruses are acellular entities, structurally very simple without complex organization. They consist of either DNA or RNA wrapped in a protein coat. Viruses are incapable of independent growth in non living laboratory media and always require living host cell for their growth, therefore they are obligate intracellular parasites. The host range for viruses include, bacteria, plant or animal cells.

Viruses are considered as borderline between living and non-living. They behave as non-living in the absence of suitable host cell and as living in suitable host cell.

Much is not known about the incidence of viruses in food than about bacteria and fungi. Being obligate parasites, viruses do not grow on culture media as do bacteria and fungi and therefore do not grow in food. Since they do not replicate in food, their numbers may be expected to be low relative to bacteria and therefore extraction and concentration methods are necessary for their study in food. Laboratory identification of viruses in food is not much practiced. Most of the food borne viral diseases are caused by the presence of viruses as contaminants in food and their consumption. Viral gastroenteritis is the common disease caused in people consuming food and water contaminated by viruses. Some of the common food borne viral pathogens are picornaviruses (polio viruses), reoviruses (rotaviruses), hepatitis A virus, parvoviruses (human gastrointestinal viruses), papovaviruses (human BK and JC viruses) and adenoviruses (human adenoviruses). It is necessary to detect and eliminate viruses contaminated in food for safety, particularly in meat and meat products.

Principles of Food Preservation

We get food from different sources like plant, animal and microbial. The nutritive quality of food depends on quality and quantity of nutrients they possess. Food are prepared for consumption by taking materials of plant, animal or microbial origin. Some food of plant origin are directly consumed as fresh raw food such as vegetables, fruits etc and some are eaten after cooking. Most of the animal based food are consumed after cooking. Microorganisms serve as food such as single cells proteins or makers of food such as bread, cake and other fermented food. Due to activity of microorganisms, the nutritive value of food becomes enriched in terms of more digestibility, increased mineral and vitamin content, taste, flavour and keeping quality. Most of the plant based food such as grains are available in dried form for long term preservation and consumption. The preservation and keeping quality of food and food materials depend mainly on their composition and water content. Long term preservation or storage of food or food materials brings changes in their chemical composition and nutritive value. These changes could be due to physical, chemical or microbiological activities occurring in food or food materials. Microbiological changes occurring in food or food materials mainly depend on water content, water activity (a_w) and temperature of storage.

Food preservation is the process of physical, chemical or microbiological treatments of food and beverages to prevent food spoilage and food borne diseases. Food have to be preserved to increase their shelf life to avoid spoilage by microorganisms and to curtail pollution of land and

water with spoilt food. Food preservation should also take the type of food into consideration before applying the preservation method. There could be perishable food, semi-perishable food and non-perishable or stable food. The category of food should be known before applying preservation techniques. Also there are many intrinsic and extrinsic factors of food that should be considered before application of a preservation method.

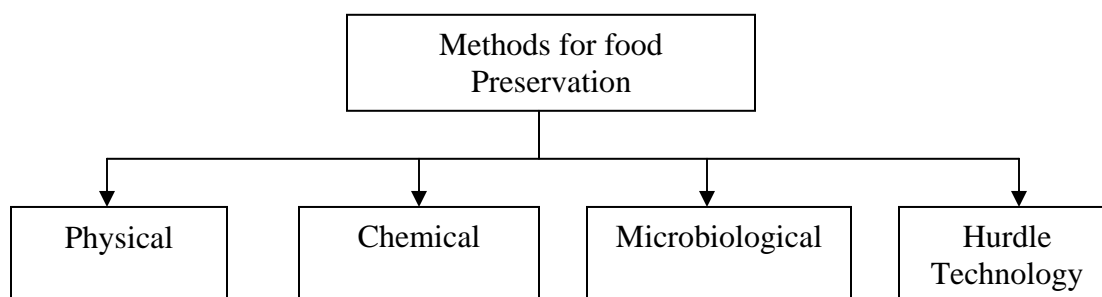


Fig.1: Methods for food preservation

Intrinsic parameters involve the natural state of food. Disturbing these parameters will affect the growth and death of microorganisms in food. Intrinsic factors related to pH includes acidity, alkalinity, total dissociated and non-dissociated inorganic and organic acids and buffer capacity of the food. The other intrinsic factors are moisture, nutrients, geo and redox potential. Most microorganisms have absolute demand for water and the water requirement is best expressed in terms of available water or water activity a_w . The water activity of various food is variable. It is more in vegetables, fruits, fresh meat and less in honey, dried eggs, dry fruits. The nutrient levels and content are also variable. More food are rich in carbohydrates like fruit juices and cereals. Some food are rich in proteins like meat, fish and pulses. Most food are rich in minerals and vitamins that promote microbial growth. Some food could have inhibitory substances like lysozyme of egg white, lactoferin in milk, etc.

The extrinsic factors that should be considered are production conditions, harvesting methods and period, processing conditions, etc. The production conditions could be temperature, humidity, aeration etc. The harvesting methods could be manual, mechanized and the period of harvest may be in dry season or in rainy season etc. The food material could be processed differently both for consumption and preservation under aseptic or normal conditions. The processing conditions include grinding, mixing, fermenting, cooking, baking and several other methods of food preparation. Preservation of processed/prepared food or food materials is necessary for food to be available to consumer as and when required. It could be short term or long term preservation of food or food materials depending on the type and necessity.

Preservation of food is necessary for their availability to the consumer as and when required. It could be short duration or long term preservation during which food or food materials should not lose their nature in terms of composition, nutritive value and microbiological quality. To protect these values, variety of methods are practiced depending on necessity and practicability.

Various principles of food preservation are involved in accomplishment of preservation by different methods. These methods are based on self decomposition of food or decomposition by microorganisms. The decomposition of food by the above reasons is prevented for preservation methods as given below.

1) *Prevention or delay of self decomposition of food*

Prevention or delay of self decomposition of food is achieved by various methods such as:

- a) Destruction or inactivation of food enzymes (Blanching)
- b) Prevention or delay of purely chemical reactions (prevention of oxidation by use of anti oxidants)
- c) Prevention of damage by insects, animals mechanical causes.

Food or food materials have their self life depending on their composition and water content. Several food or food materials have different types of enzymes in them which are responsible for changes brought about in food items. Some of these enzymes such as amylases, proteases present in food grains in inactive form under dry conditions are activated by absorption of water from the environment. These enzymes degrade the substrates like starch and proteins present in food grains making them easily decomposable by various factors including microbial attack and spoilage. Such enzymes are inactivated by drying (dehydration) or other physical or chemical methods.

Food are made up of various chemical compounds, organic or inorganic in nature, which ultimately serve as nutrients to the consumer. As all these are together, slow chemical reactions take place among the chemical groups which are mainly non enzymatic in nature. Oxidation of chemical compounds or groups present is the important phenomenon which is prevented by the addition of antioxidants.

Food grains or prepared food are damaged by insects, animals or mechanical causes resulting in destruction of their natural makeup in terms of structure and content. Several insects make their way in food and food grains and grow causing spoilage of food and food grains for consumption. Insect damage is prevented by use of insecticides mostly by external application. Food grains are damaged by animals like rodents and other related animal species causing damage in quantity and quality. These are prevented by mechanical devices and anti-rodent chemicals. Food grains and prepared food items are damaged while harvesting, processing and preparation, packing etc due to mechanical damage making them unattractive or unfit for consumption. This is prevented by good quality mechanical devices and good manufacturing practices.

2) *Prevention or delay of microbial decomposition of food*

Prevention or delay of microbial decomposition of food is achieved by various methods such as:

- a) Keeping out micro organisms (asepsis)
- b) Removal of micro organisms (filtration)
- c) Hampering microbial growth (low temperature treatment, drying, food additives, anaerobic conditions)
- d) Killing the micro organisms (heat treatment, radiation treatment)

Food is the good medium for growth of microorganisms. Microorganisms make their entry at different stages of food preparation and consumption. Growth of microorganisms in food changes their quality and content. Presence of pathogenic microorganisms in food is detrimental to health. Therefore microorganisms are kept away from food by practicing hygienic methods at various stages of making or processing food.

Microorganisms present in food are removed by different methods. Method of removing microorganisms from food depends on their type like solid, semisolid or liquid food. Microorganisms present in liquid food are removed by physical separation using filtration methods. Various types of filters and filtration methods are practiced depending on the size of microorganisms to be removed, thickness and consistency of liquid food. Once microorganisms are removed, food are protected from reentry so that the life of food can be enhanced for their quality.

Growth of microorganisms in food can be arrested by several means. Microbial growth is affected by various physical factors like temperature, water content, aeration etc. Growth of microorganisms in food is prevented by altering one or more of such physical factors. Low temperature hampers growth of microorganisms in food by reducing enzyme activities in them. Therefore, preservation of most of the perishable food is practiced by keeping them at refrigerators or cold storage facilities in large quantities. Preservation of food or food materials at low temperature also protects them for physical quality and nutritional value for longer durations. Water content of food or food materials influences growth of microorganisms in them. Removing water from grains for long term storage by drying is conventionally practiced method by farmers. Dry food have long life than food with more water content. Foods are also protected from microbial spoilage by addition of antimicrobial agents as food preservatives. This is the method of preventing microbial growth in processed food. Different chemicals like organic acids, inorganic salts are normally used as food preservatives which prevent growth of microorganisms directly, inhibiting them or creating a condition in food which becomes inhibitory to their growth. Use of salts as food preservatives is to reduce water activity (a_w) or water availability for microbial growth. Salts act as good solutes and take water in food making it not available for microbial growth. Mostly food are processed and preserved under aerobic conditions. Majority of known microorganisms in food grow under aerobic conditions. Preserving food away from atmospheric air (anaerobic conditions) inhibits growth of aerobic microorganisms like fungi and bacteria. However, growth of anaerobic bacteria occurs under anaerobic preservation if proper care is not taken.

Microorganisms present in food are killed by subjecting them to high temperature treatment or radiation. Cooking food at high temperatures removes microorganisms present in them. Pasteurization is the most commonly used method of high temperature treatment for liquid food and milk. Most of the processed food under packing are subjected to radiation treatment using gamma radiation to kill microorganisms present in them. Any method of high treatment or radiation of food should not affect food and its nutritional quality.

Methods of food preservation

Food are preserved by different methods depending on the type of food, its composition and period of preservation required. Some of the commonly practiced preservation methods based on above explained principles are given below. A method of preservation is practicing a principle of food preservation.

Asepsis

It is the process of keeping out microorganisms to avoid spoilage. Food, whether fruits or vegetables, if in healthy condition, are free from microbes and are contaminated if damaged during processing or if the protecting external covering is damaged. The load of microorganisms in food is described as “bioburden”. The type and numbers of organisms comprising the bioburden influence food spoilage and this also decides the quality of food. Asepsis in food industry is important to reduce bioburden of food to avoid spoilage. Some methods of ‘asepsis’ are packaging of food, hygienic handling and processing of food, adoption of sanitary methods in slaughtering, handling and processing of meat.

Removal of microorganisms

It is not an effective method of food preservation but helpful during processing of food for juice and soft drink production. The methods followed are filtration, centrifugation, sedimentation, washing and trimming. Filtration is used for fruit juices, beer, wine and water. Centrifugation is used for milk and sedimentation for water. Washing and trimming are followed for fruits and vegetables. Washing food may be dangerous if the water used is contaminated or has pathogenic enterobacteriaceae members like *E. coli*, *Salmonella*, *Shigella*, etc.

Maintenance of anaerobic conditions

Food is preserved by sealing, packing, canning to create anaerobic condition. The seals, packs or cans are completely filled, evacuated of air leading to creation of vacuum or they are replaced with inert gases like CO₂, N₂, and H₂. Anaerobic conditions created prevent growth of aerobes but obligate anaerobes could grow like *Cl. botulinum* which is the most dangerous organism as it produces a potent neurotoxin. Precautions should be taken to avoid contamination by anaerobes when anaerobic conditions are used for preservation.

Use of high temperature

Exposure to heat denatures proteins leading to inactivation of enzymes required for metabolism. The heat treatment necessary to kill microorganisms or their spores varies with the kind of organism, its physiological state and the environment during heating. The heat treatment selected depends on kind of organisms to be killed, other preservative methods to be employed and the effect of heat on food. Certain factors affect the heat resistance of cells or spores like

- a) Initial concentration of cells / spores in food to be heated.
- b) Previous history of cells/ spores, that is the conditions under which they have been grown like culture medium, temperature, growth phase, desiccation etc.

Culture medium components influence heat resistance of microorganisms. Glucose increases heat resistance, phosphate and magnesium ions reduce heat resistance in bacteria. Temperature of incubation influences heat resistance of microorganisms. Resistance increases if the cells are incubated at optimum temperature of growth. Growth phase of bacteria has influence on their

sensitivity to heat. Bacterial cells are resistant to heat during late log and stationary phases. They are most susceptible during log phase. Water content in cells influences their sensitivity to heat. Desiccation makes them to resist high temperatures. Therefore spores are more resistant to heat than their vegetative cells. Composition of substrate in which cells / spores are heated influences response of microorganisms to high temperature. Moisture of substrate increases effectiveness of heat as moist heat is more effective in killing microorganisms than dry heat. Substrate at neutral pH is less susceptible to heat than that at acidic or alkaline pH. Solutes containing salt and sugar reduce susceptibility of cells or spores to heat. For efficient application of high temperature used for food preservation, heat resistance of microorganisms in terms of thermal death time and thermal death point have to be taken into consideration.

Thermal death time

It is defined as the time taken at a given temperature to kill specified number of microorganisms under defined conditions.

Thermal death point

It is the temperature necessary to kill all microorganisms present in a given substance in defined time (minutes).

For efficient application of high temperature for preservation of food, the rate of heat penetration into food should be efficient and this is influenced by material of the container, consistency, size and shape of contents, initial temperature of food, prior treatments if any etc. Material of container like glass has slower rate of penetration than metal. Consistency of can contents and size and shape of food item, like larger one takes longer time, smaller one takes shorter time. Food items could retain their identity, lose their form and become viscous, or layer out. Initial temperature of food, like lower the initial temperature, longer time of exposure and higher the initial temperature, shorter the time of exposure required for treatment. Prior treatments like asepsis, removal of microorganisms etc. affect by reducing the bioburden of food items.

Heat treatments employed in processing food

The temperature and time used in heat-processing of food will depend on what effect heat has on the food and what other preservation methods are to be employed. Some food, such as milk and peas can be heated to only a limited extent without undesirable changes in appearance or loss in palatability. Whereas, others like corn or pumpkin can undergo a more rigorous heat treatment with out marked change. The greater the heat treatment, the more microorganisms will be killed, as a result heating will produce sterility of the product.

The various degrees of heating used on food can be classified as (a) pasteurization (b) heating at about 100°C and (c) heating above 100°C .

Pasteurization

Pasteurization is a heat treatment that kills part but not all the microorganisms present and usually involves the application of temperatures below 100°C . The heating may be by means of steam, hot water, dry heat, or electric currents and the products are cooled promptly after the heat treatment. Pasteurization is used under these conditions like (i) when more rigorous heat

treatments might harm the quality of product, as with market milk, (ii) when the aim is to kill pathogens, as with market milk, (iii) when the main spoilage organisms are not very heat resistant, such as the yeasts in fruit juices, (iv) when any surviving spoilage organisms will be taken care of by additional preservative methods to be employed, as in the chilling of market milk, and (v) when competing organisms are to be killed, allowing a desired fermentation, usually by added starter organisms, as in cheese making.

Preservative methods used to supplement pasteurization include (a) refrigeration, e.g., milk, (b) keeping out microorganisms, usually by packaging the product in a sealed container as in case of fruit juices, (c) maintenance of anaerobic conditions, as in evacuated, sealed containers, (d) addition of high concentrations of sugars in sweetened condensed milk, and (e) presence or addition of chemical preservatives, e.g., the organic acids on pickles.

Times and temperatures used in the pasteurizing process depend on the method employed and the product treated. The high-temperature-short-time (HTST) method employs a comparatively high temperature for a short time whereas the low-temperature- holding (LTH) for long-time method uses a lower temperature for a longer time. The minimal heat treatment of market milk is at 72⁰C for 16 sec like in the HTST method; and at 137.8⁰C for at least 2 sec in the ultra pasteurized method. Ice cream mix may be heated at 71.1⁰C for 30 min or at 82.2⁰ C for 16 to 20 sec. Grape wines may be pasteurized for 1 min at 82⁰ to 85⁰ C in bulk. Dried fruits usually are pasteurized in the package at 65.6⁰ to 85⁰ C in for 30 to 90 min. The average heat treatment for carbonated juices would be 65.6⁰ C for 30 min.

Heating at about 100⁰C

A temperature of approximately 100⁰C is obtained by boiling a liquid food, by immersion of the container of food in boiling water, or by exposure to flowing steam. Some very acid food, e.g., sauerkraut, may be preheated to a temperature some what below 100⁰C, packaged hot, and not further heat-processed. Blanching fresh vegetables before freezing or drying involves heating briefly at about 100⁰C. During baking, the internal temperature of bread, cake, or other bakery products approaches but never reaches 100⁰C as long as moisture is present, although the oven is much hotter. The temperature of unsealed canned goods heated in the oven cannot exceed the boiling temperature of the liquid present. Simmering is incipient or gentle boiling, with the temperature reaching about 100⁰C. In roasting meat the internal temperature reaches only about 60⁰ C in rare beef, upto 80⁰C in well-done beef, and 85⁰C in a pork roast. Frying gets the outside of the food very hot, but the center ordinarily does not reach 100⁰C .

Heating at above 100⁰C

Temperatures above 100⁰C usually are obtained by means of steam under pressure in steam-pressure sterilizers (autoclaves) or retorts. With no pressure, the boiling temperature of water at sea level is 100⁰C; with 5 lb of pressure it is 109⁰C, with 10 lb it is 115.5⁰C, and with 15 lb it is 121.5⁰C. Milk can be heated to temperatures up to 150⁰C by use of steam injection or steam infusion followed by “flash evaporation” of the condensed steam and rapid cooling.

Canning

Canning is defined as the preservation of food in sealed containers and usually implies heat treatment as the principal factor in the prevention of spoilage. Most canning is in “tin cans,” which are made of tin-coated steel, or in glass containers, but increasing use is being made of

containers that are partially or wholly of aluminum, of plastics as pouches or solid containers, or of a composite of materials. Therefore, the word “canning” is a general term and is often replaced by “hermetically sealed containers.” A Frenchman, Nicolas Appert, who has been called the “father of canning,” due to his experiments on the heating of food in sealed containers and for publishing directions for preservation by canning. Appert worked out methods that were good enough to be followed for years after by home and commercial canners, who named the process as “appertization” after its developer. Most modern cans are made of steel plate coated with tin. The trend is toward a thinner and more even coating of tin. Enamels are coated onto flat sheets of plate before the manufacture of cans to prevent or slow discoloration or corrosion. Sanitary, or standard, enamel is used for cans for highly colored fruits and berries or for beets to prevent the fading of color caused by tin plate. Special enamels are employed for certain products, e.g., milk, meats, wine and beer, soups and some fruit juices.

The heat processes necessary for the preservation of canned food depend on the factors that influence the heat resistance of the most resistant spoilage organism and those which affect heat penetration. The heating ordinarily is done in retorts, with or without steam pressure as the food demands. HTST heat processes, now used for some fluid food, require special equipment for sterilizing the food in bulk, sterilizing the containers and lids, and filling and sealing the sterile containers under aseptic conditions.

Other ways of heating cans are by means of a direct gas flame, by steam injection, by heating in a fluidized bed in case of granular solids, and by the hydrostatic sterilizer, which consists of a vertical tank with conveyors that carry cans down through a water leg, up into live steam, and then up and out through a second water leg. Heat is also being combined with other preservative agencies, e.g. antibiotics, irradiation, or chemicals, e.g. hydrogen peroxide for improving preservation methods.

Use of low temperatures

Low temperatures are used to retard chemical reactions and action of food enzymes and to slow down or stop the growth and activity of microorganisms in food. The lower the temperature, the slower will be chemical reactions, enzyme action, and microbial growth. A low enough temperature will prevent the growth of any mesophilic or thermophilic microorganism. As the temperature drops from its optimal temperature toward the minimal, the rate of growth of the microorganism decreases and is slowest at the minimal temperature. Cooler temperatures will prevent growth of an organism but slow metabolic activity may continue. Therefore, the cooling of food from ordinary temperatures has a different effect on various organisms present.

In general, freezing prevents the growth of most food-borne microorganisms and refrigeration temperatures slow growth rates. Commercial refrigeration temperatures, i.e., lower than 5 to 7.2⁰C, effectively retard the growth of many food-borne pathogens. One notable exception is *Clostridium botulinum* type E, which has a minimum temperature for growth of about 3.3⁰C, *Yersinia enterocolitica* can survive and grow at temperatures as low as 0 to 3⁰C. The molds, *Cladosporium sp* and *Sporotrichum sp* have been found to be growing on food at -6.7⁰C and *Penicillium* and *Monilia* at -4⁰C. Growth by one yeast has taken place at -34⁰C, and two others grew at -18⁰C. Different methods commonly employed in use of low temperature for preservation are given below:

Common, or cellar, storage

The temperature in common, or cellar, storage usually is not much below that of the outside air and seldom is lower than 15⁰C. Root crops, potatoes, cabbage, celery, apples, and similar food can be stored at these temperatures for limited periods.

Chilling or cold storage

Chilling storage is at temperatures not far above freezing and usually involves, cooling by ice or by mechanical refrigeration. Most perishable food, including eggs, dairy products, meats, seafood, vegetables, and fruits, may be held in chilling storage (commonly used deep freezers) for a limited time with little change from their original condition. Factors to be considered in connection with chilling storage include the temperature of chilling, the relative humidity, air velocity and composition of the atmosphere in the store room, and the possible use of ultraviolet rays or other radiations.

Temperature

The lower the temperature of storage, the greater the cost, Therefore, although most food will be kept best at a temperature just above their freezing point, they are not necessarily stored at this low temperature. The chilling temperature is selected based on the kind of food, the time and conditions of storage. Some varieties of apples undergo “ low-temperature breakdown” at temperatures near freezing, and sweet potatoes keep best at 10 to 12.8⁰C, and banana cannot be refrigerated but stored at 13.3 to 16.7⁰C.

Relative Humidity

Too low a relative humidity results in loss of moisture and hence of weight. It also leads to the wilting and softening of vegetables and the shrinkage of fruits. Too high a relative humidity favors the growth of spoilage microorganisms. The highest humidity required for surface growth of bacteria on food is above 92%, for yeasts it is 90-92% and for molds 85-90%.

Ventilation

Ventilation or control of air velocities of the storage room is important in maintaining a uniform relative humidity throughout the room, removing odors, and preventing the development of stale odors and flavors.

Composition of storage atmosphere

The amounts and proportions of gases in the storage atmosphere influence preservation by chilling. Nowadays increased attention has been given to “gas storage” of food, where the composition of the atmosphere has been controlled by the introduction of carbon dioxide, ozone (experimentally) or other gas or the removal of carbon dioxide. The presence of optimal concentrations of carbondioxide or ozone will influence food preservation variably like (i) a food will remain unspoiled for a longer period, (ii) a higher relative humidity can be maintained without harm to the keeping quality of certain food, or (iii) a higher storage temperature can be used without shortening the keeping time of the food that is possible with ordinary chilling storage.

Irradiation

Use of ultraviolet irradiation along with chilling storage helps preserve some food at higher temperature or by use of higher humidity. Ultraviolet lamps are normally installed in rooms for storage of meat and cheese.

Freezing or frozen storage

The storage of food in the frozen condition has been an important preservative method for centuries where outdoor freezing temperatures were available. With the development of mechanical refrigeration and the quick-freezing processes, the frozen food industry has expanded rapidly. Even in the home, the freezing of food has become extensive, now that home deep freezers are readily available. The lower the storage temperature, the slower will be any chemical or enzymatic reactions, but most of them will continue slowly at any temperature now used in storage. To avoid this as a common practice the enzymes in vegetables are inactivated by scalding or blanching. The efficiency of freezing as a method of preservation depends on various factors like selection and preparation of food, method of freezing, condition of food and its method of harvest etc.

Selection and preparation of food for freezing

The quality of the food to be frozen is of prime importance. Fruits and vegetables are selected on the basis of their suitability for freezing and their maturity and are washed, trimmed cut, or otherwise pretreated as desired. Most vegetables are scaled or blanched, and fruits may be packed in a syrup. Meats and seafood are selected for quality and are handled so as to minimize enzymatic and microbial changes. Most food are packaged before freezing. But strawberries, may be frozen before packaging.

Freezing of food

The rate of freezing of food depends on a number of factors, such as the method employed, the temperature, circulation of air or refrigerant, size and shape of package, and kind of food. Sharp freezing usually refers to freezing in air with only natural air circulation or at best with electric fans. The temperature is usually -23.3°C or lower but may vary from -15 to -29°C , and freezing may take from 3 to 72 hrs. also termed slow freezing. Quick freezing is variously defined, but in general implies a freezing time 30 min or less and usually involves the freezing of small packages or units of food. Quick freezing is accomplished by one of three general methods: (i) direct immersion of the food or the packaged food in a refrigerant, as in the freezing of fish in brine or of berries in special syrups, (ii) indirect contact with the refrigerant, where the food or package is in contact with the passage through which the refrigerant at -17.8 to -45.6°C flows (iii) air-blast freezing, where frigid air at -17.8 to -34.4°C is blown across the materials being frozen.

Certain fruits and vegetables, fish, shrimp, and mushrooms now are being frozen by means of liquid nitrogen at -196°C . For dehydro-freezing, fruits and vegetables have about half their moisture removed before freezing. The condition of the food at the time of freezing will determine the potential quality of the frozen food. This is in turn dependent on condition of food at harvesting or slaughter and methods of handling or processing etc.

Changes during freezing

The quick-freezing process rapidly slows chemical and enzymatic reactions in food and stops microbial growth. The physical effects of freezing are of great importance. There is an expansion

in volume of the frozen food, and ice crystals may form and grow in size. These crystals usually are larger with slow freezing, and more ice accumulates between tissue cells than with quick freezing and may crush cells. Water is drawn from the cells to form such ice, with a resultant increase in the concentration of solutes in the unfrozen liquor. The increased concentration of solutes in the cells hastens the salting out, dehydration, and denaturation of proteins and causes irreversible changes in colloidal systems.

Changes during storage

Meat, poultry, and fish proteins may become irreversibly dehydrated, the red myoglobin of meat may be oxidized, especially at surfaces, to brown metmyoglobin. Fats of meat and fish may become oxidized and hydrolyzed. The unfrozen, concentrated solution of sugars, salts etc., may ooze from packages of fruits or concentrates during storage as a viscous material called the metacryotic liquid. Fluctuation in the storage temperature results in growth in the size of ice crystals and in physical damage to the food. Desiccation of the food is likely to take place at its surface during storage. When ice crystals evaporate from an area at the surface, a defect called freezer burn is produced on fruits, vegetables, meat, poultry, and fish.

Changes during thawing

Thawing is reverse of freezing when temperature is increased. Most of the changes that seem to appear during thawing are the result of freezing and storage but do not become evident earlier. When the ice crystals melt, the liquid either is absorbed back into the tissue cells or leaks out from the food. Slow, well-controlled thawing usually results in better return to the cells than does rapid thawing and results in a food more like the original food that was frozen. The pink or reddish liquid that comes from meat on thawing is called drip, or bleeding, and the liquid oozing from fruits or vegetables on thawing is termed leakage. The wilting or flabbiness of vegetables and the mushiness of fruits on thawing are chiefly the result of physical damage during freezing.

Lethal effects of freezing

One of the most widely used techniques for the preservation of cultures is by freezing and frozen storage, usually in liquid nitrogen. Lethal effects are thought to be the result of denaturation or flocculation of essential cell proteins or enzymes possibly as a result of the increased concentration of solutes in the unfrozen water or perhaps in part because of physical damage by ice crystals. Repaid cooling of cells from an optimal temperature to 0⁰C can also result in death, referred to as cold shock and is thought to be related to alterations of lipids in the membrane with damage to the permeability of the cell or to the release of repair enzyme inhibitors, e.g., a ribonuclease inhibitor.

Response of microorganisms to freezing

Various factors influence microorganisms variably during low temperature preservation. Some microorganisms die, some are injured, and some are not damaged. The factors that influence are given below:

1. The kind of microorganism and its state - Resistance to freezing varies with the kind of microorganism, its phase of growth, and whether it is a vegetative cell or a spore.
2. The freezing rate - Faster freezing rates are less destructive than slower rates since critical range would be passed quickly.

3. The freezing temperature - High freezing temperatures are more lethal. More organisms are inactivated at -4 to -10°C than at -15 to -30°C .
4. The time of frozen storage - The initial killing rate in freezing is rapid, but later there is gradual reduction of microorganisms and this is called storage death. Storage of frozen food in the critical range of temperatures results in more rapid reduction than at higher or lower freezing temperatures.
5. The kind of food - Composition of the food influences rate of death of organisms during freezing and storage. Sugar, salt, proteins, colloids, fat, and other substances may be protective, whereas high moisture and low pH may hasten killing.
6. Alternate freezing and thawing - Alternate freezing and thawing is reported to hasten the killing of microorganisms but apparently does not always do so.

As the temperature is lowered, more and more water freezes, and the remaining or unfrozen free water in cells or food items at each temperature becomes more and more concentrated with solutes (salts, proteins, nucleic acids, etc.) This can change the pH of cellular matter, concentrate electrolytes, alter colloidal states, denature proteins, and increase viscosity. Ice crystals can form outside the cell ("extra cellular ice") and draw water out of the cell with a resulting dehydration or concentration effect. Intracellular crystals may form and grow or crystallize right through the cell, resulting in altered permeability or "holes" in the membrane and cell wall. Intracellular ice is thought to be more harmful to cells than are extra cellular ice crystals.

Preservation by drying

Preservation of food by drying has been practiced for centuries. Drying usually is accomplished by the removal of water, but any method that reduces the amount of available water i.e., lowers a_w in a food is also a form of drying. For example sugar may be added, as in sweetened condensed milk will reduce the amount of available moisture. Moisture may be removed from food by different methods, ranging from the ancient practice of drying by sun rays to the modern artificial ones like freeze-drying.

Drying can be accomplished by various methods. In sun-dried food, moisture is removed by exposure of food to the sun rays without any artificially produced heat and without controlled temperatures, relative humidities, or air velocities. A dehydrated or desiccated food has been dried by artificially produced heat under controlled conditions of temperature, relative humidity, and air flow. Condensed usually implies that moisture has been removed from a liquid food, and evaporated may have a similar meaning or may be used synonymously with the term dehydrated. Different methods of drying are solar drying, mechanical drying, freeze-drying and smoking.

Solar drying

Solar drying is limited to mostly the tropical climates with a hot sun and a dry atmosphere. It is applicable to certain fruits, such as raisins, prunes, figs, apricots, nectarines, pears and peaches.

The fruits are spread out on trays and may be turned during drying. Fish, rice and other grains may also be sun-dried. Many household preparations like papads are also sun-dried.

Drying by mechanical dryers

Most methods of artificial drying involve the passage of heated air with controlled relative humidity over the food to be dried or the passage of the food through such air. A number of devices are used for controlled air circulation. The simplest dryer is the evaporator or kiln. Forced-draft drying systems employ currents of heated air that move across the food, usually in tunnels.

Liquid food, such as milk, juices, and soups, may be evaporated by the use of comparatively low temperatures and a vacuum in a vacuum pan or similar device. These food could also be drum-dried by passage over a heated drum, with or without vacuum or spray-dried by spraying the liquid into a current of dry, heated air.

Freeze drying

Freeze drying, or the sublimation of water from a frozen food by means of application of both vacuum and heat is being used for a number of food, including meats, poultry, seafood, fruits, and vegetables. Frozen thin layers of food of low sugar content may be dried without vacuum by sublimation of moisture during passage of dry carrier gas.

Smoking

Food items like fish are subjected to smoking which removes moisture and also helps in preservation as smoke like woods smoke has antimicrobial compounds.

Other Methods

Electronic heating has been suggested for the removal of still more moisture from a food already fairly well dried. Foam-mat drying, in which liquid food is whipped to foam, dried with warm air, and crushed to a powder, is significantly being used for most food. Another method is pressure-gun puffing of partially dried food to give a porous structure that facilitates and hastens drying. Tower drying is a process in which dehumidified air at 30⁰C or lower is used and has been successful with tomato concentrate, milk, and potatoes.

Factors that influence or control drying

Various factors influence the method of drying as given below:

- a) The temperature employed varies with the food and the method of drying taking into consideration the sensitivity of food to temperature.
- b) The relative humidity of the air influences drying and is varied with the food, the method of drying and also with the stage of drying. It usually is higher at the start of drying than it is at later stage of drying.
- c) The velocity of the air influences by removal of moisture at faster rate if velocity is more and slower rate if velocity is less.

- d) The time of drying determines the extent of dehydration. More the time, more is the drying, but it again depends on other three factors.

Improper control of these factors may cause case-hardening, resulting from more rapid evaporation of moisture from the surface than diffusion from the interior, with a resulting hard, horny, impenetrable surface film that hinders further drying.

Pre-treatments of food before drying

Many of the food need pretreatments before drying for an effective effect of drying method on the microbial population. These pretreatments may include:

- (a) selection and sorting for size, maturity, and soundness
- (b) washing, especially of fruits and vegetables
- (c) peeling of fruits and vegetables by hand, machine, lye bath, or abrasion
- (d) subdivision into halves, slices, shreds, or cubes
- (e) alkali dipping, which is used primarily for fruits such as raisins, grapes, and prunes (for sun drying) and employs hot 0.1 to 1.5 percent lye or sodium carbonate
- (f) blanching or scalding of vegetables and fruits
- (g) sulfuring of light-colored fruits and certain vegetables. Fruits are sulfured by exposure to sulfur dioxide gas produced by the burning of sulfur so that a level of 1,000 to 3,000 ppm, depending on the fruit, will be absorbed. Vegetables may be sulfured after blanching in a similar manner or by dipping into or spraying with sulfite solution. Sulfuring helps maintain an attractive light color, conserve vitamin C and perhaps vitamin A, and repel insects; it also kills many of the microorganisms present.

Post-treatments of food after drying

The procedures after drying vary with the kind of dried food. They could be sweating, packing, pasteurization.

Sweating

“Sweating” is a process of storage, usually in bins or boxes, for equalization of moisture or re-addition of moisture to a desired level. It is used primarily with some dried fruits and some nuts (almonds, English walnuts).

Packaging

Most food are packaged soon after drying for protection against moisture loss, contamination with microorganisms, and infestation with insects, although some dried food (e.g. fruits and nuts) may be held as long as a year before packaging.

Pasteurization

Most dried fruits are subjected to pasteurization to kill pathogens and spoilage organisms. Fruits in package are treated generally at 65.6 to 85⁰C for 30 to 70 min at 70-100% relative humidity.

Changes during the drying process

Heat applied during the drying process causes a reduction in total numbers of microorganisms, but the effectiveness varies with the kinds and numbers of organisms originally present and the drying process employed. Usually all yeasts and most bacteria are destroyed, but spores of

bacteria and molds commonly survive, as do vegetative cells of a few species of heat – resistant bacteria. More microorganisms are killed by freezing than by dehydration during the freeze-drying process. The dried food item remains un-spoiled after drying if storage conditions are adequate. Microorganisms decrease in number but some resistant forms like spores of bacteria and molds could survive and also increase, during storage.

Chemical Preservatives and Food Additives

A food additive is a substance or mixture of substances, other than the basic food stuff, which is present in food as a result of any aspect of production processing, storage or packaging. Those food additives which are specifically added to prevent the deterioration or decomposition of food have been referred to as chemical preservatives. Preservatives may inhibit microorganisms by interfering with their cell membranes, their enzyme activity, or their genetic mechanisms. Other preservatives may be used as antioxidants to hinder the oxidation of unsaturated fats, as neutralizers of acidity, as stabilizers to prevent physical changes, as firming agents, and as coatings or wrappers to keep out microorganisms, prevent loss of water, or hinder undesirable microbial enzymatic and chemical reactions.

An ideal anti-microbial preservative should have wide range of microbial activity, should be non-toxic to consumers, should be economical, should not affect organoleptic properties of food, should not be inactivated by food, should not promote the growth of resistant strains, and should kill rather than inhibit microbes.

Factors that influence the effectiveness of chemical preservatives in killing microorganisms or inhibiting their growth and activity are: (i) concentration of the chemical, (ii) kind, number, age, and previous history of the organism, (iii) temperature, (iv) time, and (v) the chemical and physical characteristics of the substrate in which the organism is found (moisture content, pH, kinds and amounts of solutes, surface tension, and colloids and other protective substances). A chemical agent may be bactericidal at a certain concentration, only inhibitory at a lower level, and ineffective at still greater dilutions.

Antimicrobial preservatives added to food can be grouped as follows:-

1. *Those added preservatives not defined as such by law* - Natural organic acids (lactic, malic, citric, etc.) and their salts, vinegars (acetic is a natural acid), sodium chloride, sugars, spices and their oils, woods smoke, carbon dioxide, and nitrogen.
2. *Substances generally recognized as safe (GRAS) for addition to food* - Propionic acid and sodium and calcium propionates, caprylic acid, sorbic acid and potassium, sodium, and calcium sorbates, benzoic acid and benzoates and derivatives of benzoic acid such as methylparaben and propylparaben, sodium diacetate, sulfur dioxide and sulfites, potassium and sodium bisulfite and metabisulfite, and sodium nitrite. (Limitations on the use of some of these should be considered during usage).
3. *Chemicals considered to be food additives* - These include all not listed in the first two categories. They can be used only when proved safe for humans or animals, and they then fall into group 4.
4. *Chemicals proved safe and approved by the Food and Drug Administration.*

Preservatives added to inhibit or kill microorganisms may be classified on various other criteria, such as their chemical composition, mode of action, specificity, effectiveness, and legality.

Organic acids and their salts

Lactic, acetic, propionic, and citric acids or their salts may be added to or developed in food. Citric acid is used in syrups, drinks, jams, and jellies as a substitute for fruit flavors and for preservation.

Propionates

Sodium or calcium propionate is used most extensively in the prevention of mold growth and rope development in baked goods and for mold inhibition in many cheese food and spreads. Propionic acid is a short-chain fatty acid ($\text{CH}_3\text{CH}_2\text{COOH}$) and, like some other fatty acids, perhaps affects the cell-membrane permeability, although its precise mode of fungistatic action is not known.

Benzoates

The sodium salt of benzoic acid has been used extensively as an anti-microbial agent in food. It has been incorporated into jams, jellies, margarine, carbonated beverage, fruit salads, pickles, relishes, fruit juices, etc. The mechanism of action of the benzoates is not clear; it is known, however, that the effectiveness of the benzoic acid esters increases with an increase in the chain length of the ester group.

Sorbates

Sorbic acid, as the calcium, sodium, or potassium salt, is used as a direct anti-microbial additive in food and as a spray, dip, or coating on packaging materials. It is widely used in cheeses, cheese products, baked goods, beverages, syrups, fruit juices, jellies, jams, fruit cocktails, dried fruits, pickles, and margarine. Sorbic acid and its salts are known to inhibit yeast and molds but are less effective against bacteria.

Acetates

Derivatives of acetic acid, e.g., monochloroacetic acid, peracetic acid, dehydroacetic acid, and sodium diacetate, have been recommended as preservatives.

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

Nitrites and nitrates

Combinations of these various salts have been used in curing solutions and curing mixtures for meats. Nitrites decompose to nitric acid, which forms nitrosomyoglobin when it reacts with the pigments in meats and thereby forms a stable red color. Nitrate probably only acts as a reservoir for nitrite. Nitrites can react with secondary and tertiary amines to form nitrosamines, which are known to be carcinogenic. Recent work has emphasized the inhibitory property of nitrites towards *Clostridium botulinum* in meat products, particularly in bacon and canned or processed hams. Nitrates have a limited effect on limited number of organisms and would not be considered a good chemical preservative.

Sulfur dioxide and sulfites

The Egyptians and Romans burned sulfur to form sulfur dioxide as a means of sanitizing their wine-making equipment and storage vessels. Today sulfur dioxide and sulfites are used in the wine industry to sanitize equipment and to reduce the normal flora of the grape must. In aqueous solutions, sulfur dioxide and various sulfites, including sodium sulfite, potassium sulfite, sodium bisulfite, potassium bisulfite, sodium metabisulfite, and potassium metabisulfite, form sulfurous acid, the active antimicrobial compound. Many mechanisms for the action of sulfurous acid on microbial cells have been suggested, including the reduction of disulfide linkages, formation of carbonyl compounds, reaction with ketone groups, and inhibition of respiratory mechanisms.

Ethylene and propylene oxide

Ethylene oxide kills all microorganisms, propylene oxide, although it kills many microorganism, is not as effective as that of ethylene oxide. They are thought to act strong alkylating agents attacking labile hydrogen. They have also been used successfully in dried fruits, dried eggs, gelatin, cereals, dried yeast, and spices.

Sugar and salt

Sodium chloride is used in brines and curing solutions or is applied directly to the food. Salt has been reported to have its action like (a) Causes high osmotic pressure and hence plasmolysis of cells, the percentage of salt necessary to inhibit growth or harm the cells is varying with the microorganism. (b) It dehydrates food by drawing out and tying up moisture as it dehydrates microbial cells (c) It ionizes to yield the chlorine ion, which is harmful to organisms (d) It reduces the solubility of oxygen in the moisture (e) It sensitizes the cell against carbon dioxide and (f) it interferes with the action of proteolytic enzymes.

Sugars, such as glucose or sucrose, owe their effectiveness as preservatives to their ability to make water unavailable to organisms and to their osmotic effect. Examples of food preserved by high sugar concentrations are sweetened condensed milk, fruits in syrups, jellies, and candies etc.

Alcohol

Ethanol, a coagulant and denaturizer of cell proteins, is most germicidal in concentrations between 70 and 95 percent. Flavoring extracts, e.g., vanilla and lemon extracts, are preserved by their content of alcohol.

Formaldehyde

The addition of formaldehyde to food is not permitted, except as a minor constituent of woods smoke, but this compound is effective against molds, bacteria, and viruses and can be used where its poisonous nature and irritating properties are not objectionable.

Wood smoke

The smoking of food usually has main purposes like adding desired flavours and preserving. Woods smoke contains a large number of volatile compounds that may have bacteriostatic and bactericidal effect. Formaldehyde is considered the most effective of these compounds, with phenols and cresols next in importance. Other compounds in the smoke are aliphatic acids from formic through caproic; primary and secondary alcohols, ketones, and acetaldehyde and other aldehydes; waxes; resins; guaiacol and its methyl and propyl isomers; and catechol, methyl,

catechol and pyrogallol and its methyl ester. Wood smoke is more effective against vegetative cells than against bacterial spores, and the temperature varies with the kind of wood employed.

Spices and other condiment

Spices and other condiments do not have any marked bacteriostatic effect in the concentrations customarily used but may help other agents in preventing the growth of organisms in food. The inhibitory effect of spices differs with the kind of spice and the microorganism being tested. Mustard flour and the volatile oil of mustard, for example, are very effective against *Saccharomyces cerevisiae* but are not as potent as cinnamon and cloves against most bacteria. The essential oils of spices are more inhibitory than the corresponding ground spices. Cinnamon and clove, containing cinnamic aldehyde and eugenol, respectively, usually are more bacteriostatic than are other spices.

Other food additives

Halogens are added to water for washing food or equipment, for cooling, and for addition to some products, i.e., washing butter; water for drinking may be chlorinated by the direct addition of chlorine, or hypochlorites or chloramines may be used. Idoine-impregnated wrappers have been employed to lengthen the keeping time of fruits. Iodophors, which are combinations of iodine with non-ionic wetting agents and acid, are being used in the sanitization of dairy utensils. Halogens kill organisms by oxidation, injury to cell membranes, or direct combination with cell proteins.

Antibiotics

Most of the better-known antibiotics have been tested on raw food, chiefly proteinaceous ones like meats, fish, and poultry, in an endeavor to lengthen the storage time at chilling temperatures. Aureomycin (chlortetracycline) has been found superior to other antibiotics tested because of its broad spectrum of activity. Terramycin (oxytetracycline) is almost as good for lengthening the time of preservation of food.

Developed preservatives

Preservatives could be produced in food by microbes like lactic acid, alcohol, bacterixin, etc. Their preservative effect is mostly supplemented by one or more additional preservative methods like low temperature, high temperature, anaerobic conditions, sodium chloride, sugar etc. Developed acidity plays a part in preservation of sauerkraut, pickles, green olives, fermented milk and cheese.

Preservation by radiation

Radiations are of various types and are divided into two categories one on each side of visible light. Low-frequency, long-wavelength, low-quantum-energy radiation ranges from radio waves to infrared. The effect of these radiations on microorganisms is related to their thermal agitation of the food. Conversely, the high-frequency, shorter-wavelength radiation have high quantum energies and actually excite or destroy organic compounds and microorganisms without heating the product. Microbial destruction without the generation of high temperatures is referred by the term "cold sterilization". Different types of radiations used in food preservation are ultraviolet radiation, ionizing radiations like X-rays, gamma rays, beta (β) rays and microwaves.

Ultraviolet irradiation

Ultraviolet irradiation has been the most widely used in the food industry. It is a short wavelength, less penetrating radiation with wavelengths near 260 nm is absorbed strongly by purines and pyrimidines and is therefore the most germicidal. Ultraviolet radiation around 200nm is strongly absorbed by oxygen, this may result in the production of ozone, and so could be ineffective against microorganisms. The usual source of UV radiation in food industry is from quartz-mercury vapor lamps, that emit radiation at 254nm. The lamps are available in various sizes, shapes and power. UV radiation induces pyrimidine dimer formation leading to replication errors followed by mutations that could be lethal.

Various factors influence the effectiveness of UV radiation such as

Time

The longer the time of exposure to a given concentration, the more effective the treatment.

Intensity

The intensity of the rays reaching an object will depend on the power of the lamp, the distance from the lamp to the object, and the kind and amount of interfering material in the path of the rays.

Penetration

Nature of the object or material being irradiated has an important influence on the effectiveness of the process. Penetration is reduced even by clear water, which also exerts a protective effect on microorganisms. Dissolved mineral salts, especially of iron, and cloudiness greatly reduce the effectiveness of the rays.

Distance

The intensity of destruction of microorganisms falls proportional to the square of the distance from the lamp.

Action on Microorganisms

Each microorganism has a characteristic resistance to ultraviolet irradiation. This can vary with the phase of growth and the physiological state of the cell. This is given in the following table. UV radiation results in damage to the DNA of the cells and death.

Applications of UV radiation in the food industry

Examples of the successful use of these rays include treatment of water used for beverages, aging of meats, treatment of knives for slicing bread, treatment of bread and cakes, packaging of sliced bacon, sanitizing of eating utensils, prevention of growth of film yeast on pickle, vinegar, and sauerkraut vats, killing of spores on sugar crystals and in syrups, storage and packaging of cheese, prevention of mold growth on walls and shelves, and treatment of air used for, or in, storage and processing rooms.

Microorganism	Dose needed for 1 log cycle reduction or 1 D value $\mu\text{W sec} \times 10^3$
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Gram-negative bacteria	
Facultative anaerobes	0.8-6.4
Aerobes	3.0-5.5
Phototrophic	5.0-6.0
Gram-positive bacteria	
Bacillus	5.0-8.0
Bacillus spores	8.0-10.0
Micrococcus	6.0-20.0
Staphylococcus	2.2-5.0
Molds	10.0-200.0
Yeasts	3.0-10.0

Table 2: Ultraviolet radiation doses to destroy certain groups of microorganisms

Ionizing radiations

Radiation classified as ionizing includes x-rays, gamma rays, cathode or beta rays, protons, neutrons, and alpha particles. These are called ionizing radiations as they cause ionization in the material exposed.

Terminology in usage of ionizing radiation

Rad - It is a unit of radiation dosage, being equivalent to the absorption of 100 erg per gram of irradiated material. A *Megarad* (Mrad) is 1 million rad, and a *Kilorad* (Krad) is 1000 rads.

Gray(Gy) - It equals 100 rads and is being used as a term to replace rads.

Radappertization - It is a term used to define "radiation sterilization". The resulting product is shelf-stable.

Radurization - It refers to "radiation pasteurization", where low-dose treatments are used to extend the shelf life of products.

Radication - It is "radiation pasteurization", but used with intention of elimination of a particular pathogen.

X-Rays

X-rays, gamma rays, and cathode rays are equally effective in sterilization for equal quantities of energy absorbed. X-rays and gamma rays have good penetration, while cathode rays have comparatively poor penetration. Though effective, X-rays are not commonly used for treatment in food preservation because of the harmful effects while handling and cost.

Effects on microorganisms

The bactericidal efficacy of a given dose of irradiation depends on the following:

The kind and species of organism

The lethal doses of radiations are variable for various organisms like for example for humans it is 0.0056-0.0075 KiloGrays, for yeast 4-9, molds 1-4, bacterial spores 12-20.

The numbers of organisms (or spores) originally present

The more organisms there are, the less effective a given dose will be.

The composition of the food

Some constituents, e.g., proteins, catalase, and reducing substances (nitrites, sulfites, and sulfhydryl compounds), may be protective.

The presences or absence of oxygen

The effect of free oxygen varies with the organism, ranging from no effect to sensitization of the organism.

The physical state of the food during irradiation

Both moisture content and temperature affect different organisms in different ways.

The condition of the organisms

Age, temperature of growth and sporulation, and state-vegetative or spore – may affect the sensitivity of the organisms.

Effect of ionizing radiation

It is supposed that irradiated microorganisms are destroyed by passage of an ionizing particle or quantum of energy through them, or in close proximity to, a sensitive portion of the cell, causing a direct “hit” on this target, ionization in this sensitive region, and subsequent death of the organism (this is called the target theory). It is assumed also that much of the germicidal effect results from ionization of the surroundings, especially of water, to yield free radicals, some of which may be oxidizing or reducing and therefore helpful in destruction of the organisms. Irradiation also may cause mutations in the organisms present. Radiation doses, if heavy, have been found to be producing undesirable side reactions in many food like undesirable colours, odors, tastes etc. Some changes are such as:

- (i) Meat - Rise in pH, destruction of glutathione, increase in carbonyl compounds, H₂S, methyl mercaptan.
- (ii) Fats - Destruction of natural antioxidants, oxidation, polymerization, increase in carbonyl compounds.
- (iii) Vitamins of food - Reduction in levels of thiamine, pyridoxin, vitamin B₁₂, C,D,E & K, riboflavin and niacin are fairly stable.

Type of food	Radiation Dose in kilograys	Effect of treatment
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Meat, poultry, fish shellfish, Some vegetables, baked goods, Prepared food	20-70	Sterilization, Treated product can be stored at room temperature without spoilage, Treated product is safe for hospital patients who require Micro-biologically sterile diets
Spices and other seasonings	8.30	Reduces number of microorganisms And insects. Replaces chemicals Used for this purpose
Meat, poultry, fish	1-10	Delays spoilage by reducing the number of microorganisms in the fresh, refrigerated Product. Kills some types
Strawberries and some other fruits	1-4	Extends shelf life by delaying mold growth
Grain, fruit, vegetables, and other	0.1-1	Kills insects or prevents them from reproducing Could partially replace Fumigants used for this purpose
Bananas, avocados, mangos Papayas, guavas, and certain Other noncitrus fruits	0.25-0.35	Delays ripening
Potatoes, onions, garlic	0.05-0.158	Inhibits sprouting
Pork	0.08-0.15	Inactivates trichinae
Grain, dehydrated vegetables, Other food	various doses	Desirable physical and chemical Changes

Table 3: Applications of food irradiation

Microwaves

Microwave heating and processing of food is becoming increasingly popular, particularly at the consumer level. Microwaves are electromagnetic waves between infrared and radio waves. Specific frequencies are usually at either 915 MHz (megacycles) or 2,450 MHz (megacycles). The energy or heat produced by microwaves as they pass through a food is a result of the extremely rapid oscillation, or intermolecular friction, which generates heat. The preservative effect of microwaves or the bactericidal effect produced is really a function of the heat that is generated.

Mechanical destruction of microorganisms

The microbes in food items are destroyed by subjecting them to mechanical stress. This could destroy the cells or lead to their disintegration. The methods followed are grinding, high pressures etc. They are not generally used at industrial level but followed at domestic levels. Some examples of this method are grinding of batter for idli, wet milling of malt in beer preparation, mincing of meat, lysis of cells by application of pressure etc.

Combined methods or Hurdle Technology

Combination of two or more methods could be used to preserve food items as only rarely a single method is effective. When preservative methods are combined, the required intensity of each is reduced to less than that when a single method is used like for example, when benzoate or sorbate is added to fruit juices, less heat is required to sterilize them. If salt, sugar and vinegar are all added to pickles, sauces, each can be used at a lower concentration than only if one were added. Food previously irradiated with gamma rays or treated with antibiotic tylosin require less heat for sterilization than food not so treated.

Suggested Readings

1. Microbiology. Pelczar, M.J., Chan, E.C.S. and Krieg, N.R. Publ: McGraw-Hill Book Company, New Delhi
2. Food Microbiology. Frazier, W.C. and Westhoff, D.C. Publ: Tata McGrah-Hill Publishing Company, New Delhi
3. Modern Food Microbiology. James M. Jay. Publ: CBS Publishers & Distributors. New Delhi