

FOOD AND INDUSTRIAL MICROBIOLOGY

Food Preservation

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Keywords

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Introduction

Most foods deteriorate in quality following harvest, slaughter or manufacture, in a manner that is dependent on food type, its composition and storage conditions. The principal quality deterioration reactions of foods may be

Microbiological

The microorganisms present in a food may be contributed by its own natural microflora or from the processing conditions like in the course of harvesting/manufacturing, storage, and transport. In some cases the microflora has no discernible effect on the food quality and food safety while in others, this may affect the quality in several ways like causing food spoilage, food borne illness or food fermentations. While food fermentations are desirable transformations of food but food spoilage, food borne infections and intoxications may result into huge economic losses as in cases where a particular batch of food has been found to be involved in an outbreak of a disease or has low shelf life as desired and hence the complete batch has to be recalled back from the market and destroyed. In developing countries like India, losses due to microbial spoilage have been estimated between 10-25% in various types of foods, which adds to the problems of acute shortage of food supply in these countries.

Enzymatic

Enzymes native to plant and animal tissues or from microorganisms are responsible for changes in the texture, color, smell and appearance of foods e.g. microbial enzymes cause hydrolytic reactions, rancidity and browning in foods, and plant enzymes may cause over ripening of fruits and vegetables rendering them unsuitable for consumption.

Chemical

Chemical reactions like oxidative rancidity, oxidative and reductive discoloration, non enzymatic browning and destruction of nutrients contribute to the deterioration of foods if not stored in a proper environment.

Physical

Physical changes are responsible for loss of texture, flavors and structural damage. The most serious forms of quality deterioration include those due to microorganisms, following the survival and/or growth of spoilage, infectious pathogenic bacteria or the growth of toxinogenic ones. In this chapter we are going to study how these losses due to microbial spoilage of foods can be minimized and how foods are made safe for our consumption. The techniques employed to achieve these targets are called food preservation.

Principles of food preservation

The food preservation methods by which the microbial decomposition of foods can be delayed or prevented include

1. Restrict access of microorganisms to foods (packaging and aseptic packaging),
2. Removal of microorganisms (by filtration or centrifugation),

3. Slow or prevent the growth and activity of microorganisms (reduction in temperature, water activity and pH, removal of oxygen, modified atmosphere packaging and addition of preservatives) and
4. Inactivation of microorganisms (by heat, radiations, high hydrostatic pressures, ultra sound and pulsed electric fields).

These methods usually are also effective against enzymatic activity or chemical reactions in the food, responsible for its self-decomposition. Changes in the requirement of consumers in recent years have included a desire for foods which are more convenient, higher quality, fresher in flavor, texture and appearance, more natural with fewer additives and nutritionally healthier than hitherto. Food industry reactions to these changes have been to develop less severe or minimal preservation and processing technologies with less intensive heating or use of less chemical preservatives. However, minimal technologies tend to result in a reduction in the intrinsic preservation of foods, and may, therefore, also lead to a potential reduction in their microbiological safety. A major trend is to apply these techniques in new combinations, in ways that minimize the extreme use of any one of them, and so improve food product quality. This has formed the basis of hurdle technologies or combination preservation systems proposed by Leistner (2000) that have fostered the development of new routes to food preservation around the world. Thus an ideal method of food preservation has the following characteristics:

1. it improves shelf-life and safety by inactivating spoilage and pathogenic microorganisms,
2. it does not change organoleptic (smell, taste, color, texture, etc.) and nutritional attributes,
3. it does not leave residues,
4. it is cheap and convenient to apply and
5. it encounters no objection from consumers and legislators.

These methods of food preservation are being discussed under two headings:

1. physical methods of preservation and
2. chemical methods of preservation.

Physical methods of food preservation

The foods to be preserved are physically processed or treated in such a way that the metabolic activity of microorganisms and their spores either slowed down or completely arrested. These various physical methods used for the preservation of foods are as follows.

Asepsis

Keeping quality of foods can be increased by introducing as few spoilage organisms as possible i.e., by reducing the amount of contamination. In nature, there are numerous examples of asepsis or removal of microorganisms as a protective factor. The presence of a protective covering surrounding some foods e.g. shells of nuts, shells of eggs, skin of fruits and vegetables and fat on meats and fish, prevents microbial entry and decomposition until it is damaged.

In food industries, contamination is prevented by packaging foods in a wide variety of artificial coverings ranging from a loose carton or wrapping to the hermetically sealed containers of canned foods. Moreover, practicing sanitary methods during the processing and handling of foods reduces total microbial load and thus improves the keeping quality of food. Both flexible

and rigid packaging materials, alone or in combination with other preservation methods, have been developed to offer the necessary barrier, inactivation, and containment properties required for successful food packaging. Rigid packaging materials such as glass and metal packages are considered absolute barriers, preventing contamination. However, the economic and functional disadvantages of metal and glass have led to the development of flexible packaging materials made from composites of polyester, nylon, polypropylene, polyethylene and polyvinyl. The microbiology of the flexible packaged foods is influenced by the permeability of the packaging material to oxygen, carbon dioxide and water vapor. Packaging with certain additional conditions like controlled atmosphere, modified atmosphere, and vacuum packaging can produce microbiostatic effect, which is more effective with further decrease in storage temperature.

Controlled atmosphere packaging conditions are defined as the alteration of a gaseous atmosphere over a food product regardless of environmental or temperature fluctuations encountered by the product throughout its distribution. This extends the microbial lag phase, depresses microbial and product respiration, and minimizes adverse changes in sensory and textural qualities of stored fruits and vegetables, while inhibiting the growth of certain spoilage organisms.

Vacuum packaging is accomplished by evacuating all the air before sealing, either by inserting a vacuum probe into the neck of the package, or by placing the package into a chamber and evacuating. The absence of oxygen from vacuum packed foods will not only prevent oxidative transformations in both plant or animal tissues and aerobic microbes, but also control oxidative rancidity of fats. Vacuum packaging retards the growth of common aerobic spoilage bacteria such as *Pseudomonas* species, on refrigerated fresh meat, poultry and fish, reducing putrefaction and slime formation. Therefore, It has become the method for packing table-ready meat items. However, it may permit conditions suitable for the growth and toxin production by anaerobic and facultative pathogenic organisms.

Modified atmospheres are generated during packaging by the initial alteration of the gaseous environment in the immediate vicinity of the product. This is achieved by filling the headspace of the food packages by 20-60% carbon dioxide, which will further vary depending on the type of fruits and vegetables and the targeted microorganisms. Modified atmospheres slow down the respiration rate of food as well as microbial growth and reduce the enzymatic degradation. Under these conditions, a variety of spoilage organisms, including *Pseudomonas* spp., *Acinetobacter* spp., and *Moraxella* spp. are inhibited, yet lactic acid bacteria grow slowly.

Removal of microorganisms

The removal of microorganisms is not a very suitable and effective way of food preservation, though it may be helpful under special conditions. Removal may be accomplished by filtration, centrifugation, washing, or trimming. Filtration through a previously sterilized filter made of asbestos pads, sintered glass, diatomaceous earth or similar materials has been used successfully for fruit juices, beer, soft drinks, wine, and water. Centrifugation or sedimentation, generally is not very effective in removing all microorganisms, though is applied for the treatment of water and clarification of milk. The bacteria- removing - centrifuge called bactofuge is used to remove heat-resistant and other bacteria from the milk prior to pasteurization (Fig. 1). This includes the spores of heat resistant bacteria such as *Clostridia* sp. and *Bacillus* sp., which can remain active

in the milk after pasteurization. By using bactofuge, milk has a longer shelf life, better taste, lower bacterial cell counts and reduced impurities.



Fig. 1: Bactofuge at Douglas Dale Dairy, Johannesburg, South Africa

Washing raw foods can be helpful in their preservation if the water used is not contaminated with spoilage and pathogenic organisms. Washing fresh fruits, vegetables and food handling equipments is an essential and effective procedure for removing microorganisms.

High temperatures

Every microorganism has an optimal temperature requirement for growth. When the temperature is increased above the maximum for growth, cells are injured and killed due to thermal inactivation of their key cellular components like cytoplasmic membranes, enzymes, RNA, ribosome and DNA. The heat treatment necessary to kill organisms or their spores varies with the kind of organism, its number, physiological state, and the environment during heating. The heat treatment selected will also depend upon the other preservative methods to be employed and the effect of heat on food texture, aroma and taste. Foods are subject to thermal processes in a number of different contexts. e.g. cooking (baking, boiling, frying, grilling, roasting), pasteurization, appertization etc.

Cooking

Cooking does not have a defined heat treatment process and is vaguely used term for all heat processes done to foods to make them palatable and consumer friendly. The heat treatments like baking, frying, grilling, boiling, roasting, etc. have been included in the process of cooking. Often the objective of cooking is not the destruction of microorganisms in the product, although this is an inevitable and frequently useful side effect. During baking the internal temperature of the bread and cake never reaches 100°C, which is sufficient to kill all the yeast, their ascospores and other vegetative cells but not the spores of bacteria. Similarly, during boiling of canned foods, temperature cannot exceed the boiling point of the liquid present i.e. water. In frying, grilling or roasting, though the external temperatures of the food product are too high, their internal temperatures do not reach 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 to:

1. eliminate a specific pathogen or pathogens associated with a product as with milk, bulk liquid egg, ice cream mix, cream etc.
2. eliminate a large proportion of potential spoilage organisms as in beers, fruit juices, pickles and sauces
3. kill competing organisms allowing a desired fermentation by starter cultures as in cheese making
4. extend further shelf-life by using other preservative methods like aseptic packaging and cooling and
5. avoid the rigorous heat treatments that might harm the physico-chemical organoleptic and nutritional quality of the product.

Pasteurization may be achieved by either employing the high-temperature-short-time (HTST) method or low-temperature-long-time (LTLT) method. The time and temperature combinations employed in these two methods of pasteurization for different foods are as follows. The minimal heat treatment applied to market milk is 62.8°C for 30 minutes in LTLT method and 71.7°C for 15 seconds in HTST method (Fig. 2). These treatments are sufficient to destroy the most heat-resistant non-sporeforming pathogenic organisms- *Mycobacterium tuberculosis* and *Coxiella burnetti*, which are generally associated with milk.

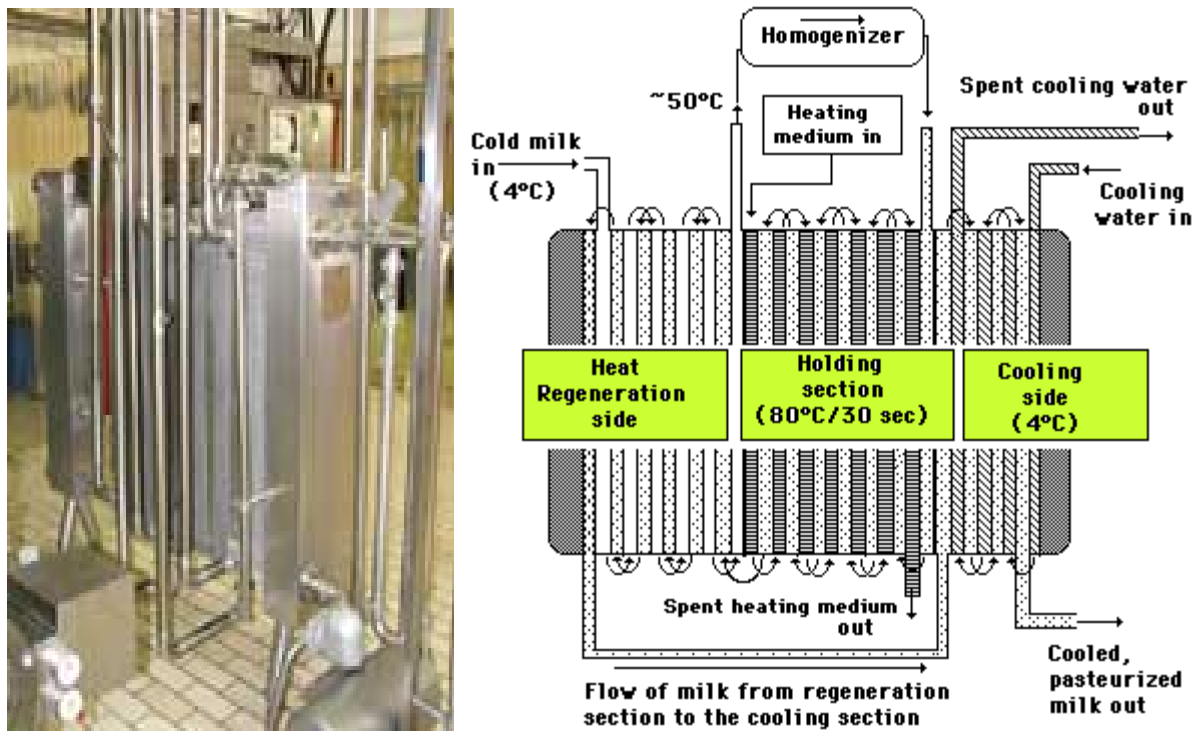


Fig. 2: HTST plate pasteurizer for milk

Ice cream mix is heated at 71.1°C for 30 minutes and at 82.2°C for 20 seconds in LTLT and HTST methods, respectively. Grape wines are pasteurized for 1 minute at 82-85°C; beer may be heated at 60°C or above, the time varying with temperature. The aim of pasteurization of wine and beer is to inactivate the yeast and its spores. Dried fruits are usually pasteurized in the packages at 65.6 to 85°C for 30-90 minutes to get rid of non-spore forming spoilage bacteria, yeast and molds.

Appertization

Appertization refers to the processes where the only organisms that survive processing are non-pathogenic and incapable of developing within the product under normal conditions of storage. As a result, appertized products have a shelf life even when stored at ambient temperature. Appertization involves heating food above 100°C for example canning of low acid foods (pH above 4.5) and UHT treatment of milk. Low acid foods are generally canned at 121°C for 2.52 minutes so that a spore of *Clostridium botulinum* will survive only in one can out of 10¹² cans. This heat treatment will make the can of food “commercially sterile” where the food is not necessarily sterile – completely free from viable organisms, but free from organisms capable of growing in the product under normal storage conditions. The UHT processing of milk involves heat processing at 138-142°C, for 2-3 seconds followed by “aseptic packaging” in a sterile environment. The shelf life of such UHT milk is about 6 months at room temperature compared to the shelf life of 2 days under refrigeration for pasteurized milk. Aseptic packaging is used to preserve and package everything from milk, juice, and drinks of all kinds to scrambled egg mix, tomato sauce, soups, and other liquid foods (Fig. 3).



Fig. 3: UHT treated and aseptically packaged milk and juice

Aseptic packaging has many benefits:

- Convenience -- because aseptic packages are portable, lightweight, and shatterproof, you can take them anywhere.
- Food Safety -- the aseptic process and carton together ensure that the liquid food or beverage inside is free from harmful bacteria and contaminants.
- No refrigeration -- you can store beverages and liquid foods in cabinets instead of a refrigerator, so you'll save energy and always have what you need on hand.

- More nutrition -- because the aseptic process places less heat stress on foods and beverages than traditional canning processes, products can retain more nutrients as well as natural taste, color and texture.

Aseptic processing and packaging also create minimal effects on the environment. Aseptic processing, for instance, is a very energy-efficient method of heat treating food products. Aseptic packaging, on the other hand, is a good example of source reduction—not only using minimal energy, but minimal materials as well. Aseptic packages are made from paper (75%) which provide stiffness, strength and efficient brick shape, polyethylene (20%) that forms the seal on the innermost layer that makes the package liquid-tight, and a protective coating on the exterior keeps the package dry, and aluminum foil (5%) that forms a barrier against light and oxygen, eliminating the need for refrigeration and preventing spoilage without using chemical preservatives. Paper, the largest component, is a renewable resource. Most paper used to make today's aseptic packages comes from forests that are professionally managed and replanted.

Relative Heat Resistance of Microorganisms

In general, the heat resistance of organisms is related to their optimum growth temperatures. Psychrophiles are the most heat sensitive of the three temperature groups, followed by mesophiles and thermophiles. Spore forming bacteria are more heat resistant than non-sporeformers, while thermophilic sporeformers are in general more heat resistant than mesophilic sporeformers. With respect to Gram reaction, gram-positive bacteria tend to be more resistant than gram-negative, with cocci being more resistant than non-sporeforming rods. Bacteria that clump considerably or form capsules and have high lipid content are harder to kill than those, which do not. Yeast and molds tend to be fairly sensitive to heat, with yeast ascospores being only slightly more resistant than vegetative yeasts. The ascospores of yeast need only 5-10° C more heat for their destruction than the vegetative cells from which they are formed. Similarly the asexual spores of molds are slightly more heat resistant than mold mycelia. Sclerotia are the most heat resistant of these types and cause trouble in canned fruits if survive. The heat resistance of endospores of *Bacillus* and *Clostridium* spp. is of special interests in the thermal processing of foods. This heat resistance of endospores is due to their spore coat, dehydrated nature of the cortex and spore core, and high contents of calcium and dipicolinic acid (DPA).

Determination of Thermal Death Time (Heat Resistant)

In order to decide a time and temperature combination to destroy an organism present in food, it is necessary to know its heat resistance in that food which can be determined by thermal death time (TDT) and its associated concepts. Thermal death time is the time necessary to kill a given number of organisms at a specified temperature. By this method, the temperature is kept constant and the time required to kill all the cells is determined. The procedure for determining TDT is to place a known number of cells or spores in a sealed container such as a tube, can, capillary tube or a flask and then expose them to a specific temperature, in an oil bath for a required time period. At the end of the heating period, containers are removed and cooled quickly in cold water. The cells or spores are removed and plated on the respective growth media to find out how many of them have survived this heat process. The experiment is continued at the same temperature by drawing samples at different time intervals and finding the survivors in each sample by plate count. If you now plot the data as log number of survivors against time you get a

straight line as shown in Fig. 4. The graph produced is called a survivor curve, which is useful to calculate the D values for a spore or a vegetative cell.

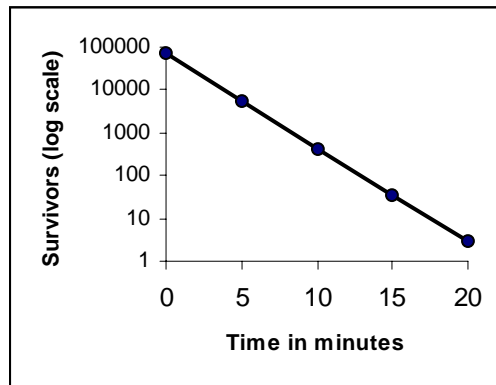


Fig. 4: Survivor curve for Salmonella spp. at 60° C. 10⁵ number of Salmonella cells were heated at 60°C for 5, 10, 15 and 20 minutes. The number of survivors was plotted against time on log scale

D value is the decimal reduction time or the time required to kill 90% of the bacterial population or the time in minutes required for the survivor curve to traverse one log cycle as shown in Fig 5. D values are frequently written with a subscript that defines the temperature, for example, D₁₂₁ is the time required to kill 90% of a population of microorganisms at 121°C. D values are invaluable for comparing the relative heat resistance of organisms and calculating process times at a specific temperature. For example the most heat resistant spores of thermophilic bacteria, *Bacillus stearothermophilus*, have a D value at 121°C between 4.0-5.0 minutes compared to the D value of 0.01-0.07 for spores of mesophilic bacteria, *B. coagulans* at the same temperature. Heat resistance of microorganisms and their D values vary considerably which are given in Table1.

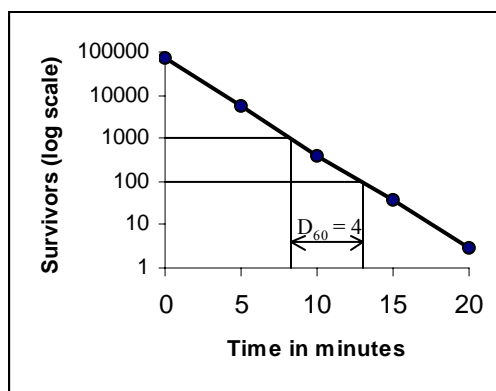


Fig. 5: Calculation of D values from Survivor curve at 60°C. Survivor curve was plotted as shown in Figure 4. D values were calculated in minutes that are required by the log survivor curve to traverse one log cycle i.e. it takes 4 minutes at 60°C to reduce the number of Salmonella cells from 10³ to 10², one log cycle. This time will remain same whether one log cycle decrease is from 10⁵ to 10⁴ or 10⁴ to 10³ or 10² to 10¹ at a certain fixed temperature

Table 1: Heat Resistance of food borne pathogenic and food spoilage organisms

Food Borne pathogens	D (mins)	Temperature (°C)	z value (°C)
<i>Listeria monocytogenes</i>	5.0-8.3	60	7.0
<i>Campylobacter jejuni</i>	1.0	55	12.3
<i>Salmonella spp.</i>	0.58-0.98	60	9.1
<i>Staphylococcus aureus</i>	2.0-15.0	60	8.8
<i>Escherichia coli</i>	0.1	65	10.6
<i>Yersinia enterocolitica</i>	1.0-3.0	60	6.2
<i>Clostridium botulinum</i> spores	0.21	121	10.2
<i>Bacillus cereus</i>	2.0-8.0	100	12.8
<i>Vibrio parahaemolyticus</i>	5.0	60	8.5
Food spoilage organisms			
<i>Bacillus stearothermophilus</i>	4.0-5.0	121	8.0
<i>Bacillus coagulans</i>	0.1-0.2	121	8.9
<i>Clostridium</i> <i>thermosaccharolyticum</i>	3.0-4.0	121	10.2
<i>Clostridium sporogenes</i>	0.1-3.0	80	8.0
Yeasts and Molds	0.5-3.0	65	3.5

Another interesting feature during heat processing is the effect of increasing temperature on the D values. D value or the heat resistance of an organism decreases as the temperature increases. Therefore, you would expect to have a different D value at each temperature. Now if we determine the D values at different temperatures and plot them against their temperature on a semi-log paper, again we get a straight line that passes through log cycles as shown in Fig. 6. The graph produced is called thermal death time curve where each log cycle represents a z value. The z value refers to the temperature change (°C) which results in a tenfold (1 log) decrease or increase in the D value. The z value provides information on the relative resistance of an organism to different destructive temperatures and can be used to determine the equivalent D values at different temperatures. Supposing an organism has a z of 15°C and a D value of 5 minutes at 121°C. We can use the z value to find the D value at any other temperature above its maximum growth temperature. If we raise the temperature from 121 to 136°C (121°C + z value 15°C), then the D value for the organism is 0.5 minutes that is the one log cycle decrease in D value. Similarly if we lower the temperature to 106°C (121°C - z value 15°C), then D equals to

50 minutes that is the one log cycle increase in D value. The z value can be calculated using the following equation

$$z = (T_2 - T_1) / (\log D_1 - \log D_2)$$

Where D_1 and D_2 are the D values at temperatures T_1 and T_2 respectively.

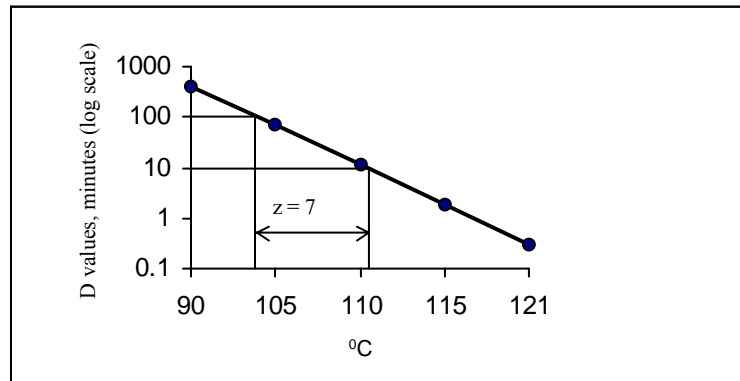


Fig. 6: Calculation of z value. D values are plotted on the log scale and degrees C, at which the D values are calculated, are plotted on the linear axis. z- value for this organism is 7 in degree C required to either decrease or increase the D values by one log cycle.

The z values normally ranges from 5-8°C for vegetative cells and 6-16°C for spores of bacteria. Heating process is neither uniform nor instantaneous. In order to compare the lethal effects of different processes, it is necessary for us to have some common currency to describe them. One parameter is F value which is the time in minutes required to destroy the organism in a specified medium at 121°C. The integrated lethal value of heat received by all points in a container during processing is designated as F_s or F_o . This represents a measure of the capacity of a heat process to reduce the number of spores or vegetative cells of a given organism per container. F_o may be derived as follows:

$$F_o = D_{121} (\log x - \log y)$$

Where x and y are the number of cells in the initial and the final population, respectively. For example, the widely accepted minimum lethality for a heat process applied to low acid canned foods is that it should produce 12 decimal reductions in the number of surviving *Cl. botulinum* spores at 121°C. This is known as the 12D concept or “botulinum cook” or “commercially sterile”. If D_{121} of *Cl. botulinum* is 0.21 minutes then a botulinum cook will have a $F_o = 0.21(\log 1 - \log 10^{-12}) = 0.21 \times 12 = 2.52$ minutes. The effect of applying a process with F_o to a product in which every can contains one spore of *Cl. botulinum* ($x = 1$) will be that a spore will survive in one can out of every 10^{12} thereby, making the product safe and stable at room temperature.

Canning

Canning is defined as the preservation of foods in sealed containers and usually implies heat treatment as the principal factor in the prevention of spoilage. The concept of canning was first

time introduced by a Frenchman Nicholas Appert (1795-1810) who has been called the “father of canning”. Appert gave exact directions for the preservation of a wide variety of foods in cork-stoppered, widemouthed glass bottles, heated for hours in boiling water, which he published in a book called “The Book for All Households” or “Art of Conserving all kinds of Animal and vegetable substances for many years”. Most modern canning is done in tin cans, which are made of tin-coated steel, or in glass containers, with increasing demand for containers made of aluminum, of plastics as pouches or solid containers or of a composite of materials.

Before canning, raw foods are freshly harvested, inspected, graded, thoroughly washed and blanched if vegetables (refer to freezing). Vegetables are canned in brine solution, consisting of either salt or salt and sugar both, while fruits are canned in sugar syrups. The heat process, a canned product receives is determined largely by its pH. The more acidic a product is, milder the heat process applied. For example, botulinum cook or 12D concept is applied to low acid foods with a pH above 4.5 (peas, corn, spinach, meats, fish, poultry, and milk) which get easily spoiled by heat resistant spoilage organisms (*B. sporogens*, a mesophile and *B. stearothermophilus*, a thermophile) and also pose a serious health risk to the consumers if the spores of *Cl. botulinum* survive the heat process. This is not a concern in acid foods with a pH between 4.5 and 3.7 such as canned tomatoes, pineapple and pears where F_0 applied is generally between 0.5-2.0. In higher acidity products with pH <3.7 such as canned citrus fruits, canned berries and sauerkraut (cabbage pickle), the heat process required is equivalent to a pasteurization.

In commercial canning, the heating ordinarily is done in retorts, with or without steam under pressure as the food demands. The foods may be heat treated first and then filled into sterile cans aseptically after cooling or may be filled into the cans and then heat treated. Canning at home can be accomplished by four methods (1) a bath of boiling water in which the containers are immersed, (2) a steamer in which the containers are exposed to flowing steam, (3) keeping cans in ovens and (4) using pressure cookers. Except the use of pressure cookers for the heat treatment of acid foods, other processes are not sufficient to prevent microbial spoilage.

The food industry has used heat processing for many decades as a method for producing safe stable foods, however, the simple application of a known temperature for a given time does not in itself assure a safe, stable product. According to Gaze (2005) the food microbiologist must understand many points, before deciding any time-temperature combination, including:

1. Knowledge of raw material microbiology.
2. An understanding of the product chemistry and its effects on microbial heat resistance.
3. An understanding of the product recipe, e.g. preserve and size of particles.
4. Packaging issues- type of pack, single or multi-component, potential for leakage, etc.
5. Correct microbial type and system to use for process validation.

Only by a full understanding of these factors can a correct process be given and the safety and stability of the food assured.

Drying

The preservation of foods by drying is based upon the fact that microorganisms and their enzymes require optimal water content for their activity. In this method, the moisture content of foods or a_w is lowered to a point where the activities of food spoilage and pathogenic

microorganisms are arrested. Desiccated, dried, or low moisture foods contain < 25% moisture with a_w between 0.00 and 0.60. Another category of shelf stable foods is the intermediate moisture foods (IMF) which contain moisture between 15 and 50% and a_w between 0.60 and 0.85. Moisture may be removed from foods by any of a number of methods, from the ancient practice of drying by sun's rays to the modern artificial ones that are discussed below.

Sun Drying

In sun drying or solar drying, moisture is removed by exposure of foods to the sun's rays without any artificially produced heat and without controlled temperatures, relative humidity and air velocities. Though, this method is easy and cheap, but limited to climates with hot sun and a dry atmosphere. Drying indoors over a fire is one way to avoid this problem. The foods are spread out on trays and may be turned during drying. Sun drying is widely practiced for products such as fruits (raisins, prunes, figs, apricots, nectarines, pears, peaches etc.), fish, coffee and grain (Fig. 7). In tropical countries like India with high humidity regions drying is usually slower so that products such as fish are often pre-salted to inhibit microbial growth during drying.

Drying by Mechanical Dryers

Mechanical drying is quicker, more reliable, albeit more expensive than sun drying. In this method, heated air with controlled relative humidity is allowed to pass over the food to be dried either in tunnels or the food is moved on conveyor belts through the heated air. Liquid foods, such as milk, juices, and soups, may be evaporated at low temperatures under vacuum or drum or spray dried (Fig. 7). In spray drying, the milk is pre-concentrated in vacuum evaporators to about 40-50% total solids before being sprayed into the stream of air heated to temperatures up to 260°C at the top of the tower. Milk in the form of fine droplets dry very rapidly and fall to the base of the tower where they are collected. Although the air temperature employed in the drier is very high the temperature experienced by the organisms in the wet droplets is reduced due to evaporative cooling. In drum drying the milk is spread on the surface of slowly rotating double jacketed metal drums which are heated inside by steam to a temperature of about 150°C. The film dries as the drum rotates and is scrapped off as a continuous sheet by a fixed blade close to the surface of the drum. Although drum drying gives greater lethality to organisms than spray drying, spray drying is widely used for milk drying because it provides a whiter product which is easier to reconstitute and has less of a cooked flavor.



Fig. 7: Sun dried peaches, apricots and resins. Spray dried milk powder

Freeze Drying

Freeze drying is the sublimation of water from a frozen food by means of a vacuum plus heat applied at the drying shelf. This technique is used for a number of foods including meats, poultry, seafood, fruits and vegetables, coffee, essences malted and other confectioneries.

Intermediate moisture foods (IMFs)

Intermediate moisture foods or reduced water activity foods are the commercially prepared foods that contain 15-50% moisture with a_w in the range of 0.6-0.85 and have non-refrigerated shelf stability. These foods are low in moisture, can be eaten without preparation or rehydration, and yet do not taste like dehydrated products. Few examples of some traditional IMFs are various soft candies, jellies, honey, jams, many dried fruits, sweetened condensed milk, cake and pastry (Fig. 8). The lower a_w in these foods is achieved by removal of water either by adsorption or desorption and /or by addition of humectants such as glycerol, sorbitol, propylene glycol in addition to salt and sugar. By adsorption, food is first dried and then subjected to controlled rehumidification until a desired product is achieved. By desorption, the food is placed in a solution of higher osmotic pressure so that at equilibrium, the desired a_w is reached. The human foods containing humectants were not received well in the market because of acceptability problems, although a number of moist pet food products (dog food) were successfully developed.

The general a_w range of IMF products prohibits the growth of gram-negatives, most gram-positive bacteria with the exception of cocci, some sporeformers and lactobacilli, yeasts and molds, giving the products an extended shelf life at ambient temperature. When spoilage does occur, it is often a result of incorrect storage in a high relative humidity environment. In addition to the inhibitory effects of lowered a_w , other factors such as pH, oxidation-reduction potential, chemical preservatives, competitive microflora also contribute to the extended shelf-life of IMFs.



Fig. 8: Intermediate moisture foods: IMFs (Jams, Fruit spread, Chocolates, etc.) with room temperature stability

Low Temperature

Freezing foods has a historical origin in China where ice cellars were used to preserve foods as early as 1000 BC. Later, the Greeks and Romans stored food in cellars in which snow had been compressed. In mid 1800s, Fish were purposely frozen in the United States using salt and ice, which became an important industry. In the late 1800s, frozen meat, poultry, shellfish and eggs were being shipped extremely long distances (Archer, 2004).

The lower the temperature, the slower the chemical reaction, enzyme action, and microbial growth, and a low enough temperature will prevent the growth of any microorganism allowing only slow metabolic activity. Since food spoilage is usually a result of chemical reactions mediated by microbial and endogenous enzymes, the life of many foods can be increased by storage at low temperatures. Storage temperatures are selected depending on the kind of food and the time and conditions of storage. For example banana keeps best at about 13.3-16.7°C and should not be kept in the refrigerator. In general there are three distinct low temperature ranges in which foods may be stored for preservation.

Common or Cellar Storage

The temperature in this type of storage is not much below that of outside air and is seldom lower than 15°C. Foods such as root crops, potatoes, cabbage, apples etc. are stored at these temperatures for limited periods. Although chemical reactions in the food carried out by microorganisms and endogenous enzymes are not prevented, they are slower than at outside temperatures.

Chilling or Cold Storage

Chilling storage of foods employs temperatures near, but above their freezing point typically 0-5°C. These chill temperatures are achieved either by cooling by ice or mechanical refrigeration i.e. refrigerators. Most of the perishable foods such as eggs, dairy products, meats, seafood, vegetables, and fruits are held at these temperatures for a limited time with little change from their original condition. Chill storage can change both the nature of spoilage and the rate at which it occurs by reducing the enzymatic and microbial changes in the foods considerably. Though psychrotrophs grow only relatively slowly, mesophiles cannot grow in chilled foods, thereby delaying the onset of spoilage. The ability of organisms to grow at low temperatures depends on the composition and architecture of their plasma membrane. As the temperature is lowered, the plasma membrane undergoes a phase transition from a liquid crystalline state to a rigid gel in which solute transport is severely affected. This phase transition is lower in psychrotrophs and psychrophiles due to the presence of unsaturated and short chain fatty acids in their membrane lipids making them resistant to low temperatures. In addition, rapid cooling of foods to chilling temperature will exert a cold shock on mesophiles causing cell death and cell injury due to damage to their membranes and DNA. Mesophiles that survive cooling can persist in the food in injured state for extended periods and may recover and resume growth under favorable conditions. Thus chilling will prevent an increase in the risk from mesophilic pathogens, but will not assure its elimination. Therefore, the use of good microbiological quality raw materials and hygienic handling are the key requirements for the production of safe chilled foods.

Freezing or frozen Storage

The storage of foods in the frozen condition is one of the most successful methods for long term preservation of food since the product largely resembles the fresh on thawing and retains its nutritive value. Frozen vegetables, fruits, meat and meat products, fish, cheeses, etc. have captured the frozen food market (Fig. 9). The temperature used in frozen storage is usually less than -18°C . Under the usual conditions of storage of frozen foods microbial growth is prevented entirely and the action of microbial and endogenous enzymes is greatly retarded. Therefore, it is a common practice to inactivate enzymes of vegetables by scalding or blanching before freezing. Blanching is achieved either by brief immersion of foods into hot water or the use of steam. This brief heat treatment is supposed to accomplish the following:

1. inactivation of most of the plant enzymes that might cause undesirable changes during frozen storage such as toughness, softness, change in color, loss of nutritive value and flavor
2. reduction in the numbers of microorganisms including pathogens in the food e.g. blanching of vegetables at 72°C for 1 second is enough to inactivate *Listeria monocytogenes*, a pathogen that survive low temperatures of freezing
3. enhancement of green color of vegetables such as peas, spinach etc.
4. facilitating the packing of leafy vegetables by inducing wilting
5. displacement of entrapped air in the plant tissues.



Fig. 9: Frozen vegetables

The two basic ways to achieve the freezing of foods are slow and quick freezing. Slow freezing is the process where the desired temperature in the range of -23°C or lower is achieved in 3-72 h using air. Quick freezing refers to the process where the temperature is lowered down within 30 minutes or less either by direct immersion of food in a refrigerant as in freezing of fish in brine or by indirect contact with the refrigerant or by air blast freezing. Quick freezing is preferred over slow freezing to achieve a better product quality on thawing which looks more like that of original food in texture and flavor. The advantages of quick freezing over slow freezing are that:

1. formation of small and intracellular ice crystals during quick freezing causes less mechanical destruction of intact cells of the food,
2. there is more prompt prevention of microbial growth and enzymatic activity,
3. cold shock effect on microbial cells is more prominent and does not allow adaptation to low temperatures,
4. upon thawing, there are less chances of drip in meats (pink or reddish liquid oozing from meats) and leakage in vegetables (liquid oozing from vegetables and fruits).

Although, freezing has been shown to kill certain microorganisms of importance in foods, this should not be regarded as a means of destroying food borne spoilage and pathogenic microorganisms. The type of organisms that lose their viability under these conditions differ from strain to strain and depend upon the types of freezing methods (quick or slow), the nature

and composition of the food (egg whites, glycerol, sucrose, high fat and protein contents in food increase freezing viability, while high moisture and low pH hastens killing), the length of time of freezer storage (the number of viable cells decreases with lengthened time of storage) and the temperature of freezing. That is why the survival rates after freezing have been variously recorded between 5 and 70%. In general there is a sudden mortality immediately on freezing after which survivors die gradually during storage. This decline is more rapid at temperatures just below the freezing point around -2°C and is usually slow below -20°C . All the microorganisms have been classified on the basis of their sensitivity to freezing as

1. susceptible that includes vegetative cells of yeasts, molds and protozoa and gram-negative bacteria,
2. moderately resistant like vegetative gram-positive bacteria and
3. insensitive organisms which are predominantly sporeformers.

During freezing free water is converted to ice and the solute concentration increases in the unfrozen water. This will change the pH of the cellular matter, concentrate electrolytes, alter colloidal states, denature cellular proteins, increase viscosity and lower down a_w of frozen foods. Both low temperature and reduced a_w are the inhibitory factors operating in frozen foods and are responsible for their increased shelf life.

Frozen foods have an excellent overall record of safety and illnesses associated with frozen foods are rare. However, in addition to preserving the quality of foods freezing also preserves the viability of some pathogenic microorganisms. Ice cream, particularly homemade ice cream, has been the historical vehicle for diseases associated with frozen foods. Commercial ice cream is pasteurized before freezing, and therefore, has an excellent record of safety, but illnesses have occurred nonetheless. It is clear from various outbreak investigations that certain human pathogens do not succumb to freezing, or that enough remain viable to pose a health threat.

Irradiation

The type of radiation of primary interest in food preservation is electromagnetic which includes microwaves, ultraviolet rays, and ionizing radiations.

Microwave radiations

The microwave region of the electromagnetic spectrum occupies frequencies between the infrared (10^9 Hz) and radio frequency (10^{12} Hz) and has relatively low quantum energy. Most food research has been carried out at two frequencies; 915MHz and 2450 MHz. Microwaves are generated using a magnetron, a device first developed in the UK during research into radar during the Second World War. Although microwaves are used both commercially and domestically in domestic microwave ovens and in catering, these have been slow to find industrial applications in food processing. Microwaves have been used to defrost frozen meats before cutting, in blanching of vegetables and fruits, destruction of molds in bread, pasteurization of beer and sterilization of wine.

Microwaves act indirectly on microorganisms through the generation of heat. When food-containing water is placed in a microwave field of 950 MHz, water molecules oscillate back and forth 915 million times/sec creating an intermolecular fraction. This kinetic energy is transmitted

to neighboring molecules leading to a rapid rise in temperature throughout the product. This heating effect is responsible for killing microorganisms in food exposed to microwave radiations.

UV Radiation

Ultraviolet light is a powerful bactericidal agent with the most effective wavelength being about 260 nm. It is absorbed by purine and pyrimidine bases causing the production of covalent bonds between adjacent thymine molecules giving thymine dimers. This may prevent the DNA replication in the normal way and disrupt gene functioning by creating new mutants. Although microorganisms have the capacity to repair this DNA damage, extensive damage may cross the limits of DNA repair mechanisms leading to cell death. The resistance of microorganisms to UV is largely determined by their ability to repair such damage. In addition to the repair mechanisms, some organisms such as micrococci also synthesize protective pigments. Generally, the resistance to UV irradiation follows the pattern: Gram-negative < Gram-positive < yeast < bacterial spores < mold spores < viruses. The UV D values for these groups are 3-4, 6-8, 6-10, 8-10, 20-100 and >200 ergs x 10² respectively.

High intensity ultraviolet radiation generated by low-pressure mercury vapor lamps is extremely effective in killing microorganisms. The poor penetrating capacity of UV light restricts its use in food applications. UV radiations are able to penetrate only to 300-500 cms in air, 30 cms in water, 0.1 cm in glass and 0.01 cm in milk. Therefore, the practical applications of UV light are limited to surface disinfections and air sterilization such as in hospital theaters, aseptic filling rooms in pharmaceutical industry, in food and dairy industry (in sterile packaging of UHT milk and in bakery to control mold spores). UV radiation is commonly used as an alternative to chlorination in the disinfections of water in water filters installed at homes and offices such as Aqua Guard, Aqua Care etc.

Ionizing Radiations

Ionizing radiations such as X-rays and gamma γ -rays generated by X-ray apparatus and radioisotopes such as cobalt 60 (⁶⁰Co and ¹³⁷Cs) respectively are highly effective in killing microorganisms. Since they destroy microorganisms without appreciably raising temperature, the process is termed "cold sterilization." Ionizing radiations can affect the cells directly by interacting with key molecules within the microbial cell. The main site of damage in cells is the chromosome where hydroxyl radicals cause single and double strand breaks in the DNA molecule as a result of hydrogen removal from deoxyribose sugar. Further cleavage of the molecule occurs by β elimination of phosphate. Ionizing radiations also have indirect inhibitory effect on cell constituents by generating free radicals produced by the radiolysis of water. Free radicals formed from water can combine with each other or oxygen molecules to give powerful oxidizing agents that can damage cell components. Thus in the absence of water and oxygen, radiation doses 2-3 times higher are required to obtain the same lethality.

Death of microorganisms caused by ionizing radiation is logarithmic, producing survivor curves that are similar to those produced by heat. In this case, the number of survivors is plotted against the radiation dose and D values are calculated as the dose required to kill 90% of the population. The radiation dose is currently measured in Gray (Gy), which is equivalent to 1 joule of energy absorbed/kg of material. Microbial resistance to radiation usually decreases in the order viruses > bacterial spores > pigmented mold spores > yeast and molds > Gram-positive bacteria > Gram-

negative bacteria. The most resistance organism is *Micrococcus radiodurans* which has a D value of >30 kGy.

The electromagnetic radiations (gamma rays) emitted from the excited nucleus of ^{60}Co or ^{137}Cs are the cheapest form of radiation for food preservation. Unlike UV light, gamma rays have excellent penetration power so that foods can be packaged and then irradiated to destroy contaminating microorganisms, making it potentially an ideal method of food preservation. Foods are irradiated by using gamma rays in the three following ways:

i) Radappertization

Radappertization is equivalent to radiation sterilization or “commercial sterility” of low acid foods which requires a dose of radiation capable of giving a 12D reduction in the number of spores of *Clostridium botulinum*. As the D value for *C. botulinum* is 3.5 kGy, the dose required will be 42 kGy to achieve 12D kill. The application of radappertization is restricted to only few food products such as bacon as the high doses of radiation may cause color changes and or production of off odors.

ii) Radicidation

Radicidation refers to reduction of the number of viable specific non-sporeforming bacterial pathogens such as *Salmonella* and is equivalent to pasteurization of milk. Irradiation levels of 2-5 kGy are effective in destroying non-sporeforming and non-viral pathogens. The foods such as fresh poultry, cod and red fish, and spices and condiments are preserved by irradiating at these levels.

iii) Radurization

Radurization refers to the enhancement of the keeping quality of a food by causing substantial reduction in the numbers of viable spoilage microorganisms especially gram-negative, non-sporeforming rods by low levels of radiation. Common dose levels are 0.075-2.5 kGy for fresh meats, poultry, seafood, fruits, vegetables, and cereal grains. The shelf life of seafood, fish and shellfish may be extended from two to six folds by radurization.

Not all foods are suitable for irradiation treatment. Softening and discoloration may occur in the case of some fruits. Milk may acquire an unpleasant taste. Certain protein foods are flavor sensitive to irradiation and may develop off-flavors. Another major limitation of irradiation processing of food is its slow acceptance by the consumers, due to a perceived association with radioactivity.

High Hydrostatic Pressures

During high hydrostatic pressure (HHP) processing, foods are subjected to pressures in the range 100 to 1000 MPa (megapascals). High pressures are known to have an antimicrobial effect which appears to be associated with the denaturation of cell proteins and damage to cell membranes. Membrane lipid bilayers have been shown to compress under pressure that alters their permeability. The application of high pressures for food processing is referred to as pascalization. Overall, HHP is very effective in inactivating vegetative cells of microorganisms, but pressure treatment alone does not achieve a substantial inactivation of spores and reduction in activity of certain enzymes. Although, vegetative bacteria, yeast and molds can be reduced by at least one log cycle by 400 MPa applied for 5 min, bacterial endospores can tolerate pressures

as high as 1200 MPa. Therefore, the commercial application of pascalization has been limited to only acid and high acid foods like fruit juices and sauces in which bacterial spores that survive processing are unable to grow. Foods preserved by this technology resemble very much to the fresh product and appears natural to the consumer with none of the negative associations of processes such as heat, irradiation and chemical preservation. Interest in using high-pressure technology to extend the shelf life of low acid foods is increasing by combining this treatment with other food preservation methods.

Relative resistance of microorganisms to HHP is as follows: Bacterial spores > Gram-positives (vegetative cells) > Gram-negatives \cong yeast and molds.

Ultrasound

Ultrasound is defined as sound waves with frequencies above that of human hearing (>16 kHz), which can be propagated in liquid media as alternating compression. If ultrasound has sufficient energy, a phenomenon known as cavitation occurs involving the formation, growth, and rapid collapse of microscopic bubbles. This cavitation is believed to be responsible for causing mechanical damage to the microbial cells. Ever since the lethal effects of ultrasound on microorganisms were first observed in 1929, its use has been continually suggested for disinfections and food preservation. However this technology has not been adopted commercially due to the long treatment needed for the substantial microbial inactivation in foods. Recently, its industrial application is gaining increased attention as it has been demonstrated that microbial inactivation increases when ultrasound treatment is applied under pressure up to 600kPa (known as manosonication) or with heat and pressure (manothermosonication). Resistance to ultrasound under pressure generally follows the following sequence: Bacterial spores > Gram-positives (vegetative cells) > Gram-negatives.

Pulsed Electric Fields

This technology involves applications of short duration (microseconds), high intensity electric field pulses to inactivate microbial cells in food. Pulsed electric field (PEF) technology has received increased attention in the last decade as a food preservation technique because of its potential to inactivate microorganisms at temperatures below those adversely affecting food quality. PEF is highly effective in killing vegetative cells of bacteria, yeasts and molds. However, PEF inactivation of bacterial spores and enzymes in food is unclear. Therefore, PEF treated food products must be stored under refrigeration to prevent enzymatic reactions and spore germination. PEF technology though not being used to preserve foods commercially at present, has great potential to extend the shelf-life of different foods such as apple juice, skim milk, beaten eggs, green peas soup, or orange juice. Relative resistance of microorganisms to PEF is very similar to monosonication: Bacterial spores > Gram-positives (vegetative cells) > Gram-negative > Yeast and molds.

Chemical Methods of Food Preservation

Chemical preservatives are the food additives that are specially added to prevent the deterioration or decomposition of a food by inhibiting, retarding or arresting the growth of

microorganisms. They do not include substances, which enhance the shelf life of foods by inhibiting a chemical reaction such as rancidity or discoloration. The chemical preservatives may be either intentionally added to the food or may be developed during the growth of microorganisms as in case of some fermentation (lactic acid, acetic acid, bacteriocins etc.). The use of chemical preservatives in foods may allow products to be subjected to less severe heat treatments, resulting in an improvement in product quality and consumer acceptability. While a large number of chemicals have been described that show potential as food preservatives, only a relatively small number are allowed in food products. This is due in large part to the strict rules of safety adhered to by Food and Drug Administration (FDA).

A chemical preservative should have a wide range of antimicrobial activity, should be non-toxic to humans or animals, should be economical, should not have an effect on flavor, taste, aroma of the original food, should not be inactivated by food and should not encourage the development of resistant strains. There are added chemical preservatives which are not defined as such by law such as natural organic acids (lactic, malic, citric etc.), vinegars, sodium chloride, sugars, spices and their oils, wood smoke etc. On the other hand, there are some chemical substances, which are generally recognized as safe (GRAS) for addition to foods such as organic acids and their salts (Propionic, sorbic and benzoic) sodium nitrite, sulfur dioxide and metabisulfites and nisin, a biopreservative. Most of the common antimicrobial additives used in foods and their current allowable levels are presented in Table 2.

Organic Acids and their Salts

Malic, citric, tartaric acids are found naturally in fruits and will inhibit most bacteria. Lactic and acetic acids are produced naturally by microorganisms in amounts sufficient to exert an effect on flavor and the pH of the product, thus potentiating their own action by increasing the proportion of undissociated acid present. Propionic, sorbic, benzoic acids and parabens (*para*-hydroxybenzoic acid esters) are not generally found naturally in foods or produced by microorganisms. There are exceptions, e.g. propionic acid is produced in Swiss cheese by *Propionibacterium spp* and benzoic acid is found in cranberries. These acids are sometime considered to be 'true' chemical preservatives.

Benzoic Acids and Parabens

Benzoic acids and its sodium salts are widely used as antimicrobial compounds in a large number of foods. The antimicrobial activity of benzoate is related to pH, the greatest activity being at low pH values and essentially ineffective at neutral values. This indicates that the antimicrobial activity resides in the undissociated molecule at pH between 2.5 and 4.0. This results in the restriction of benzoic acid and its sodium salts to high acid products such as apple cider, soft drinks, jams, jellies, fruit salads, pickles, tomato catsup. As used in acidic foods, benzoates and their sodium salts act mainly as a mold and yeast inhibitor.

Among parabens, ethyl and methyl parabens are extensively used in foods. Though, these compounds are similar to benzoic acid in their effectiveness, they have an added advantage of being effective at even higher pH values. Because of the esterification of the carboxyl group, the undissociated molecule is retained over a wider pH range exerting inhibitory effect even at neutral pH. This means that they can be used effectively in low and non-acid foods.

Table 2: Maximum levels of some GRAS chemical food preservatives permitted in foods

Preservatives	Maximum concentrations allowed	Organisms affected	Foods
Benzoic acids	0.1%	Yeasts and molds	Jams, jellies, salad dressings, apple cider, soft drinks, pickles, tomato catsup
Parabens (methyl-, propyl-, and heptyl esters of p-hydroxybenzoic acid)	0.1%	Yeasts and molds	Fruit drinks and beverages, bakery products, salad dressings, apple cider, soft drinks, pickles, tomato catsup
Propionic acid	0.32%	Molds	Bread, cakes, Swiss cheese
Sorbic acid	0.2%	Molds and yeast	Hard cheeses, baked goods, fruit cocktails, syrups, fruit juices, jams and jellies, dried fruits, margarine
SO ₂ and sulfites	200-300 ppm	Insects and microorganisms	Wines, molasses, fruit juices, lemon juice, dried fruits (not to be used in meats or other foods containing thiamine)
Nitrites and nitrates	100-120 ppm	Clostridia and molds	Meat and meat products as a meat curing agent
Ethylene and propylene oxide	700 ppm	Yeasts, molds and Clostridia	Fumigants for dried fruits, dried eggs, gelatin, cereals, dried yeast and spices
Ozone	>100 ppm 0.2-0.4 ppm 5-15 ppm	Viruses <i>Salmonella</i> , <i>Pseudomonas</i> <i>Botrytis</i>	Animal sanitation Fish and Poultry Vegetables
Nisin (biopreservative)	100 ppm	Gram+ve spore formers	Processed cheeses, canned fruits and vegetables, condensed milk

In the undissociated form these compounds are soluble in the cell membrane and act apparently as proton ionophores. As such they facilitate proton leakage into the cells thereby increasing the energy output of cells to maintain their usual internal pH. With this disruption in membrane activity, amino acid transport is adversely affected. These compounds have also been found to block the oxidation of glucose and pyruvate at the acetate level. Benzoates have also been found to inhibit the outgrowth of vegetative cells during endospore germination. Maximum concentration of benzoates permitted in foods is 0.1%.

Sorbic Acid

Sorbic acids and their calcium, potassium or sodium salts are permissible in foods at levels not to exceed 0.2%. Like benzoates, they are also most effective at low pH values when present in the undissociated form. These compounds are more effective than sodium benzoate at pH values

between 4.0 and 6.0. Sorbic acid and its salts are used either as a direct antimicrobial additive in foods or as a spray, dip, or coating on packaging materials. These are widely used in cheeses, cheese products, bakery products, beverages, syrups, fruit juices, jellies, jams, pickles and salad dressings. They are active against yeasts, molds and catalase-positive bacteria. Inhibition of mold growth by sorbates is due to the inhibition of the dehydrogenase enzyme system, several other Krebs cycle enzymes and the membrane function impairment affecting the cellular uptake of substrate molecules such as amino acids, phosphate and organic acids. Sorbic acid is also known to inhibit the germination and outgrowth of *C. botulinum* spores.

Propionic acid

Propionic acid and its calcium or sodium salts are permitted in breads, cakes, and certain cheeses as a mold inhibitor to maximum levels of not more than 0.32%. In bread and bread dough it prevents ropiness by inhibiting the rope forming bacilli e.g. *Bacillus subtilis* or *B. licheniformis*. The mode of action of these compounds on microorganisms is similar to that of benzoates and sorbates. Dissociation tendency of these compounds at high pH values makes them useful preservatives for low acid foods.

Nitrite

Sodium nitrate (NaNO_3) and sodium nitrite (NaNO_2) are used in curing formulae for meats since they stabilize red meat color, inhibit some spoilage and food poisoning organisms, and contribute to flavor development. In an acid environment nitrite ionizes to nitrous acid that further decomposes to nitric oxide. The nitric oxide co-ordinates to the haem ferrous ion in the muscle pigment myoglobin under reducing conditions converting it to the desirable red pigment nitrosomyoglobin. The antibacterial effect of nitrite increases with decreasing pH suggesting that nitrous acid is the active agent. This nitrous acid, being a powerful reducing agent, causes disruption of the cell metabolism and also inhibits the germination and outgrowth of endospores. Nitrite acts as a preservative by inhibiting a wide range of bacteria; including *Clostridium* spp (*C. botulinum* is of particular interest), *Bacillus* spp and *Staphylococcus aureus*. However nitrite is not very effective against lactobacilli or members of the enterobacteriaceae including salmonellae.

Interestingly, it has been shown that the ability of nitrite to inhibit these spore formers in cured, canned, vacuum packed meats and culture media will increase about ten fold if it is added before heating the product. This increased inhibitory activity of nitrite upon heating in a medium is due to the production of a substance referred to as 'Perigo factor'.

It is this Perigo factor that results from the heat processing or smoking of certain meats and fish products containing nitrite that warrants the continued use of nitrite in such products. Nitrite levels of 100 ppm or more in the presence of 3-5% sodium chloride are sufficient to impart an adequate flavor and antibotulinal and antilisterial (against *Listeria monocytogenes*, a bacterial food pathogen) effect in meat products. The only problem with the use of nitrite is their reaction with secondary amines forming nitrosamines that are known to be carcinogenic.

Sulfur Dioxide

Sulfur dioxide (SO₂) and the sodium and potassium salts of sulfite, bisulfite, and metabisulfite have been used as disinfecting agents in wine industry particularly to sanitize wine making equipment and storage vessels and to reduce the normal flora of the grape must. It is also used as an antioxidant to inhibit enzymatic and non-enzymatic browning reactions in some food products. Sulfur dioxide has also been used, in syrups, fruits juices and to treat most light colored dehydrated fruits. The unionized forms of SO₂, which can readily penetrate the cell, have the greatest antimicrobial activity. As a reducing agent it can break disulfide linkages in proteins, and interfere with redox processes. It can also form addition compounds with pyrimidine bases in nucleic acids, sugars and several key metabolic intermediates. However, it has been found to react and destroy the vitamin thiamine present in meat and meat products prohibiting its use in these products.

Sulfur dioxide is active against bacteria, yeasts and molds. Sulfur dioxide, sulfites and metabisulfites are used at 200-300 ppm levels in most of the foods to have their bactericidal effect on all types of microorganisms.

NaCl and Sugars

Both of these preservatives are similar in their mode of action in preserving foods. These compounds tend to tie up moisture and thus exert a drying effect on both food and microorganisms. Salts are added in brine and curing solutions or applied directly to foods to slow down and prevent the activity of food spoilage and pathogenic organisms. The addition of salts has the following effects on food and microorganisms:

1. It causes high osmotic pressure and hence, plasmolysis of cells,
2. It dehydrates foods and microbial cells by drawing out and tying up moisture,
3. It ionizes to yield the chlorine ion, which is harmful to organisms,
4. It reduces the solubility of oxygen in water,
5. It sensitizes the cell against carbon dioxide and
6. It interferes with the action of proteolytic enzymes. The concentration of salt in food varies with the taste of the consumer and type of food. In the absence of refrigeration, salting may effectively preserve fish and other meats.

Sugars such as sucrose exert the same preserving effect, as salt but requires in about six times higher concentrations than salt to affect the same degree of inhibition. The most common uses of sugars as preserving agents are in the making of fruit preserves, candies, chocolates, condensed milk, cakes and pies. The shelf stability of these products is due in large part to the preserving effect of high concentrations of sugar.

Gases

Gases can be used to sterilize materials, which can not withstand the high temperatures of heat sterilization like many organic compounds, volatile food flavors and some plastic material. Gaseous sterilization offers a means for packaging heat sensitive products that only affect airborne surface bacteria but also it can attack the microbial cells after penetrating the porous materials. Some of these gases used to inactivate microorganisms are ethylene oxide, propylene oxide, methyl bromide and formaldehyde.

Ethylene oxide

Ethylene oxide, cyclic ether, is the most commonly used gas for effective sterilization of packaged items, dry products etc., at room temperature because of its good penetration with little damage to materials. The microbicidal action of ethylene oxide gas is directly related to the alkylating activity of cellular enzymes and other proteins. It has been used to sterilize spices, cereals, fruits and dry fruits and dried yeast. However, it is flammable, expensive, and toxic and requires three hours or more for effective sterilization and may alter nutrients and other quality factors of foods.

Ozone

Ozone has recently gained the attention of food and agricultural industries, though it has been used effectively as a primary disinfectant for the treatment of municipal and bottled drinking water for 100 years. In 2001, the Food and Drug Administration (FDA) allowed for the use of ozone as a direct contact-sanitizing agent.

Because of its very high oxidation reduction potential, ozone acts as an oxidant of the constituent elements of cell walls before penetrating inside microorganisms and oxidizing certain essential components e.g., unsaturated lipids, enzymes, proteins, nucleic acids, etc. When a large part of the membrane barrier is destroyed causing a leakage of cell contents, the bacterial or protozoan cells lyse resulting in the destruction of the cell. Most of the pathogenic and food borne microbes are susceptible to this oxidizing effect.

In aqueous solutions, ozone can be used to disinfect equipment, process water, and some food products. It has been used to decontaminate poultry meat, salmon, apples, strawberries and cauliflower. In gaseous form it has been used to preserve eggs during cold storage, fresh fruits and vegetables, and fresh fish. Ozone can also be used during the washing of produce before it is packaged and shipped to supermarkets, grocery stores, and restaurants. In food industry, much attention is given to the cleaning and sanitizing operations of food-processing equipment. Water containing low concentrations of ozone can be sprayed onto processing equipment, walls or floors to both remove and kill bacteria or other organic matter that may be present.

The concentrations of ozone, which are large enough for effective decontamination, may change the sensory qualities and colour of some food products, such as meat, milk powder and fish cake due to lipid oxidation. Additionally, microorganisms embedded in product surfaces are more resistant to ozone than those readily exposed to the sanitizer. Ozone is a toxic gas and can cause severe illness, and even death, if inhaled in high quantity. Exposure restrictions to plant operators must be addressed with leak proof system design and process operation.

Biopreservatives

Artificial chemical preservatives are employed to limit the number of microorganisms capable of growing within foods, but increasing consumer awareness of potential health risks associated with some of these substances, has led researchers to examine the possibility of using natural additives. For instance, egg white lysozyme is employed at levels in excess of 100 tones per annum in some cheeses to prevent blowing (gas production) by lysing the vegetative cells of *Clostridium tyrobutyricum*. Activation of the lactoperoxidase system has been shown to be

useful to extend the keeping quality of milk in countries like India where pasteurization is not possible immediately after milking and refrigerated transport systems are poorly developed. Plant derived antimicrobials such as the extracts of herbs and spices are being commonly used in preservation of foods for controlling microorganisms. Microbial products like antibiotics and bacteriocins in particular whether produced by fermenting microorganisms or added from outside are being increasingly used in cheese and canned foods. The broad -spectrum antibiotics such as chlorotetracycline (CTC) or oxytetracycline (OTC) were permitted at 5-7 μ g/g in fish, poultry, shrimps, etc. till 1959. However due to the hazards of the development of resistant strains of pathogens, the potential of hypersensitivity of humans to the antibiotics, the presence of residual antibiotics after cooking, costs and difficulties in monitoring these aspects, the use of these antibiotics in foods was never appreciated.

Bacteriocins produced by lactic acid bacteria (LAB) are a heterogeneous group of antibacterial proteins that vary in spectrum of activity, mode of action, molecular weight, genetic origin and biochemical properties. The food products that have been targeted for use of bacteriocins or bacteriocin like inhibitory substances include meat and meat products, fish products, dairy products, cereals, fruits and vegetables, and beverages. The bacteriocins can effectively be used to inhibit some gram-positive bacteria, spore-forming bacteria, and food-borne pathogens. The major classes of bacteriocins produced by LAB include:

1. lantibiotics,
2. small heat stable peptides,
3. large heat labile proteins, and
4. complex proteins whose activity requires the association of carbohydrates or lipid moieties.

Out of these, first two groups have received increased attention as food biopreservatives.

The most studied member among lantibiotics is Nisin A, a 34-residue antibacterial peptide that is produced by several strains of *Lactococcus lactis* and strongly inhibits the growth of a wide range of Gram-positive bacteria. This mature peptide displays several unusual features, such as the dehydrated residues dehydroalanine, dehydrobutyrine, lanthionine and β -methyl-lanthionine residues. In Gram-positive bacteria nisin has been shown to act on energized membrane vesicles to disrupt the proton motive force, inhibit uptake of amino acids, and cause release of accumulated amino acids. Nisin A is being used at the concentrations of 100-200 ppm in the preservation of, dairy products such as cheeses and milk, meat products, and fish.

Microgard products are bacteriocins-like inhibitory substances produced by fermenting grade A skim milk with lactic acid bacteria. It has been approved by FDA and widely used as a biopreservative for more than a decade by the Cottage cheese industry. It is antagonistic toward most gram-negative bacteria and some yeasts and molds, but not against gram-positive bacteria.

Lacticin 481 produced by *L. lactis*, lactocin S produced by *Lactobacillus sake* and carnocin U149 produced by *Carnobacterium piscicola* are the other lantibiotics, which are being tried as food biopreservatives.

Class II LAB bacteriocins are small heat stable, non-lanthionine containing membrane-active peptides. Few examples of class II bacteriocins, which have been studied for their antibacterial

effect, are pediocin produced by pediococci (widely applied in the fermentation of meat and vegetables) and leucocin A produced by *Leuconostoc spp*, another LAB found in meat and vegetable fermentations. These peptides are active against broad range of gram-positive bacteria including *Listeria monocytogenes*.

Reuterin is a water-soluble non-proteinaceous product produced by *Lactobacillus reuteri*. It has been described to have antimicrobial effect against certain gram-negative and gram-positive bacteria, yeasts, fungi, and protozoa. It inhibits *Salmonella*, *Shigella*, *Clostridium*, *Staphylococcus*, *Listeria*, and *Trypanosoma*.

Bacteriocins exhibit a very narrow inhibiting spectrum, typically active against only one target microorganism. The bacteriocin activity is not stable and loss occurs when it interacts with food components by binding with food lipids and proteins or being degraded by proteolytic enzymes.

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