

# 1 Methods of Food Preservation

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## 1. Introduction

Virtually all foods are derived from living cells from animals and plant origin and in some cases from some microorganisms by biotechnology methods. Thus, foods are for the most part composed of “edible biochemicals”.

One of the most important goals of the food scientist is to make foods as safe as possible whether they are used fresh or processed. The judicious application of food processing, storage and preservation methods helps prevent outbreaks of foodborne illness, that is the occurrence of disease or illness resulting from the consumption of contaminated food. The processed food industry has an outstanding record preventing such cases when it is considered that billions of cans, jars, packets and pouches of processed and fresh food products are consumed annually. Occasionally, however, this excellent record has been broken by limited outbreaks in which persons do succumb to the effects of toxic foods.

Food preservation is an action or method of designed to maintain foods at a desired level of quality. A number of new preservation techniques are being developed to satisfy current demands of economic preservation and consumer satisfaction in safety, nutritional and sensory aspects (Potter and Hotchkiss, 1995).

## 2. Why Do We Need to Preserve?

The preservation, processing and storage of the food are vital for the continuous supply of foods during seasons and off-seasons. One very important consideration that differentiates the agricultural from all other industrial processes is their

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seasonal nature. The main reasons for food processing and preservation are: to overcome seasonal production in agriculture; to produce value-added products; and to provide variety in diets. People like to eat wide varieties of foods, having different tastes, flavours, nutritional, dietetic and other characteristics. Unfortunately it has been estimated that as many as 2 billion people do not have enough to eat and that perhaps as many as 40 000 die every day from diseases related to inadequate diets, including the lack of sufficient food, protein or specific nutrients. Inadequate nutrition in extreme cases can produce in children an advanced state of protein deficiency known as kwashiorkor or the more widespread protein.

Major processes of food deterioration are caused by environmental factors such as temperature, humidity, oxygen and light which can be reason for several reaction mechanisms that may lead to food deterioration to such an extent that they are either rejected by or harmful to the consumer. Microbial effects are the leading cause of food deterioration and spoilage (Desai, 2000).

### *2.1. The necessity to preserve*

Foods are perishable or deteriorative by nature. Based on the mode of action, major food preservation techniques can be categorised as: slowing down or inhibiting chemical deterioration and microbial growth; directly inactivating bacteria, yeast, moulds and enzymes and avoiding recontamination before and after processing. A number of techniques or methods from these categories are presented in Figure 1.

## 3. Conventional Food Preservation Methods

### *3.1. Food preservation by heat treatment*

Heat is by far the most commonly used method of food preservation. There are various degrees of preservation by heating that ultimately dictate the type of final product manufactured, the terms used are pasteurisation and sterilisation. However, to be effective, these processes must be carried out under a combination of strict temperature and time control to ensure the killing of pathogenic and non-pathogenic microorganisms. These same factors also cause thermal inactivation of food enzymes and some destruction of food constituents (Heldman and Lund, 1992).

#### 3.1.1. Heat resistance of microorganisms

Heat resistance of microorganisms is a basic topic of thermobacteriology, which is a very important part of microbiology including food microbiology. The most heat resistant pathogen found in foods, especially those that are canned and held under anaerobic conditions is *Clostridium botulinum*. It is spore forming, proteolytic anaerobe, which is able to produce the most harmful known toxin since

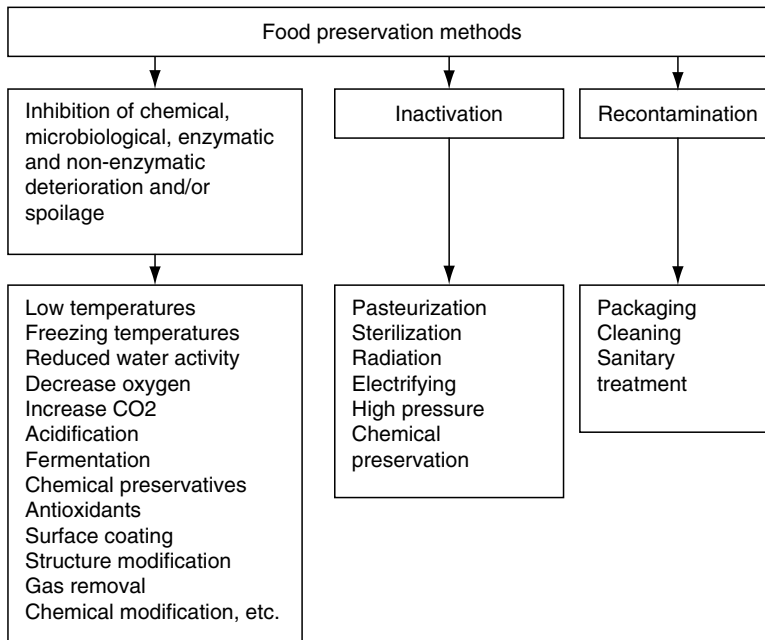


FIGURE 1. Major food preservation methods.

amount of about  $10^{-6}$  –  $10^{-8}$ g is able to kill one person. However, there are non-pathogenic, spore forming food spoilage bacteria, such as the putrefactive anaerobe *Clostridium sporogenes* 3679 (PA3679) and *Bacillus stearothermophilus* (FS1518) which are more heat resistant than spores of *Cl. botulinum*. This means that if a heat treatment inactivates spores of these spoilage microorganisms, the spores of *Cl. botulinum* and all others pathogens will be also killed (Bell and Kyrakides, 2000).

### 3.1.2. Kinetics of heat destruction of microorganisms

#### • Thermal death time

The thermal death time is the time of heating required to kill all vegetative cells of microorganisms. Theoretically this is not possible but this expression is used in thermobacteriology for practical purposes.

Microorganisms are killed by heat at a rate that is very nearly proportional to the number of cells of a specified organism (expressed on a logarithmic basis) present in the system (food, laboratory nutritive medium, water, etc.) being heated. This is referred to as a logarithmic order of death. A typical thermal death rate curve is shown in Figure 2. It provides data on the rate of destruction of specific microorganisms in specific media or food at specific constant temperature,

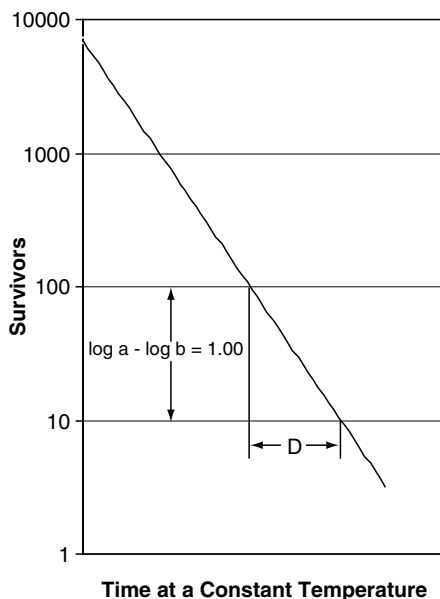


FIGURE 2. Bacterial destruction rate curve showing logarithmic order of death. D, decimal reduction time.

which is able to kill the corresponding microorganism – pathogenic, toxicogenic, or spoilage organism of the specific food.

Figure 2 shows the logarithmic dependence between the time ( $\tau$ ) and number of the killed cells ( $C$ ) at constant temperature, or  $\lg C = f(\tau)$  at  $t^\circ\text{C} = \text{constant}$ . The logarithmic order of thermal killing is valid for all spores and vegetative cells but latter are killed faster. It is valid also for yeast and moulds. The D-value is real kinetic constant determined at  $t^\circ\text{C} = \text{constant}$ . That is why it is normally written as  $D_t$ . For example, if  $t = 100^\circ\text{C}$  then it should be  $D_{100}$  which means that the value of D is determined at  $100^\circ\text{C}$  and it is valid only for this temperature and product in which the cells have been suspended (Ray, 2000).

This “D-value”, or decimal reduction time, is defined as the time, in minutes, at specified temperature required to destroy 90% of the cells at the respective microbial population. During each time interval (1 min or 3 min or 6 min) numbers of the cells is reduced 10 times, let say from 1000 to 100 or from 100 to 10, etc. This means that 90% of the cells are killed during each interval. In each case  $1000/100 = 100/10 = 10$  which is the reason it is called the decimal reduction time. In other words, the D-value represents the time for the number of cells to be reduced by one logarithmic cycle, for example from  $10^6$  to  $10^5$  cells per 1 g.

TABLE 1. Effective time-temperature relationships for destruction of *Clostridium botulinum* spores

Temperature (°C)	Time (min)
100	330
104	150
110	36
116	10
118	5.27
121	2.78
124	1.45
127	0.78

### • Dependence between the thermal death time and temperature

For example, if the time-temperature combinations required for destruction of *Clostridium botulinum* spores in low-acid media (i.e pH >4.5) are taken from this type of relationship. The time-temperature relationships that will be equally effective are shown in table 1.

From such data, the dependence of thermal death time on the temperature can be presented graphically in semi-logarithmic co-ordinates called an Arrhenius plot where the heating time is plotted in a logarithmic scale. This shows (Figure 3) that for any one initial concentration of the cells, time-temperature relationship is linear and can be described by the equation:

$$\lg \tau = f(t^{\circ}\text{C}), \text{ at } C = \text{constant}$$

This figure illustrates two terms or kinetic constants, the “Z-value” and the “F-value”. The “Z-value” is the number of degrees required to pass through one log cycle, which means that the thermal death time is changed by factor of 10, let say from 100 to 10 min or from 10 to 1 min. The “F-value” is defined as the number of minutes at a specific temperature, required to destroy the desired number of cells of any microorganism. The “F-value” is a measure of the capacity of any heat treatment applied to a specific food product in order to sterilise it. When it is written as  $F_0$  it means that the tested microorganism is spores of *Cl. botulinum* when they are treated at 121.1°C (Ray, 2000).

The dependence of thermal death times on the temperature has been determined for many important pathogens and food spoilage microorganisms. Such curves for putrefactive anaerobe *Clostridium sporogenes* (PA3679) and *Bacillus stearothermophilus* (FS1518) are shown in Figure 4. This figure shows the length of time it takes to kill these microorganisms at a chosen temperature. For example it would take about 60 min at 105°C to kill the specified number of spores of PA 3679. At 121.1°C, the same numbers of spores are killed in a little over 1 min (Shapton and Shapton, 1991).

It has been shown that the criterion for commercial sterilisation is that the population of spores of *Cl. botulinum*, should be reduced by 12 log cycles or 12D. This means that if one can contains  $10^6$  spores before heating, which is unusually high, then after a 12D heat treatment, out of 1 million cans, 999 999 cans will be

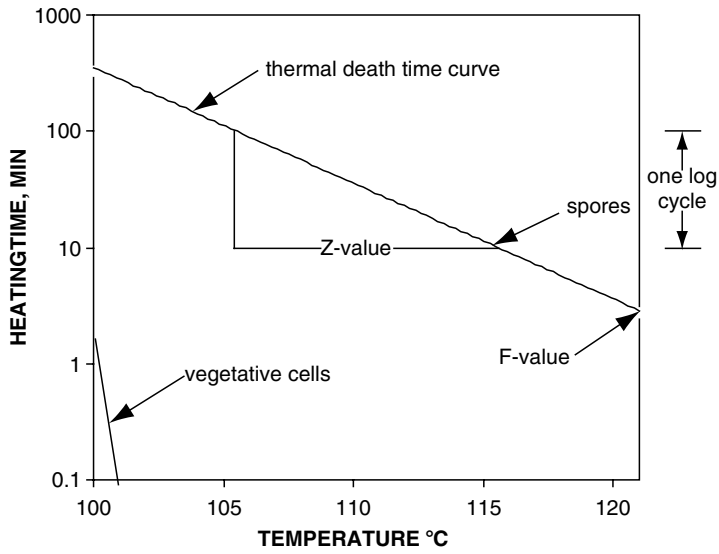


FIGURE 3. Typical thermal death time curves for bacterial spores and vegetative cells.

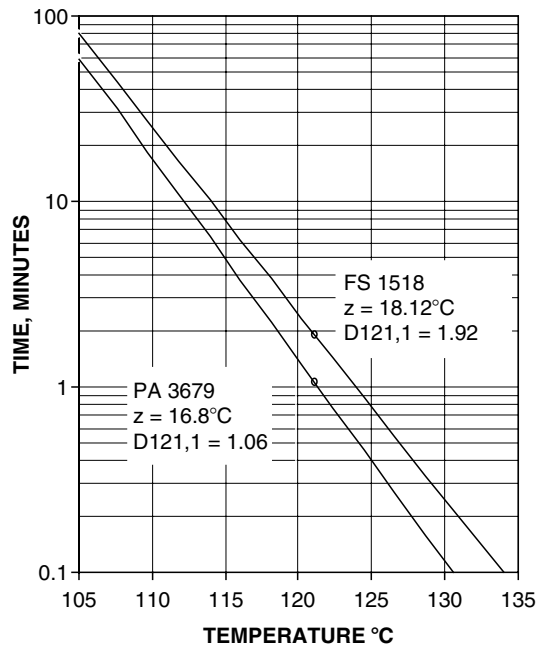


FIGURE 4. Thermal death time curves for test microorganisms PA 3679 and FS 1518.

sterile. For spores of *Cl. sporogenes* PA 3679 and *Bacillus stearothermophilus* FS 1518, in low acid foods, a 5D heat treatment is equivalent to 12D values against *Cl. botulinum*.

For food with  $\text{pH} < 4.6$  (higher acid foods) requirement 12D is not valid since *Cl. botulinum* does not grow in these foods (although spores may survive).

In container sterilisation time required to sterilise food is influenced by:

- heat resistance of microorganisms and/or the enzymes in the food, when  $\text{pH} < 4.6$ ; heating method – steam, water, flame, etc.;
- pH of the food;
- the size of the container;
- chemical composition of the food;
- physical state of the product;
- mechanism of heat exchange (convection or conduction);
- initial product temperature;
- temperature of sterilisation;
- state of the containers during sterilisation – static, shaking, rotating, etc.

The mechanisms of heat inactivation and injury of microorganisms is not very well identified since heat will bring about so many changes in biological material, such as microbial cells, that is why identification of the event that causes death or injury of the cells is difficult to predict (Larousse and Brown, 1997).

Thermal death time depends on the microbial cell concentration (Figure 5).

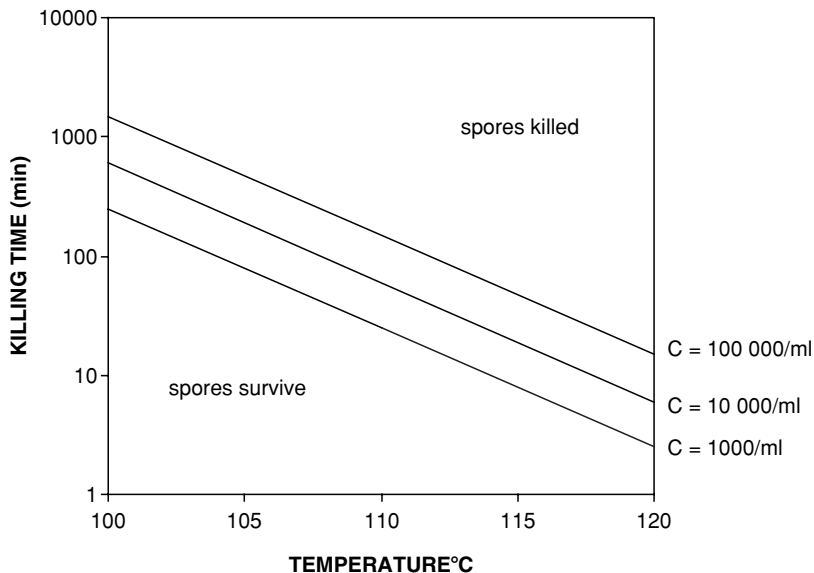


FIGURE 5. Thermal death curves for bacterial spore suspensions of different initial concentrations.

### 3.2. Preservation by low water activity ( $a_w$ )

Water activity can be reduced by partial removal of the water (drying, reverse osmosis, concentration) or by adding substances which increase the osmotic pressure of the food or media such as sugars, ethanol, glycerol, salts, etc. (Booth, 1998). The majority of microorganisms are sensitive to the water status in their immediate environment and they can remain metabolically active only in a narrow range of high water activities. There is a lot of information about low water activity limits for the growth of microorganisms. However it is typical that those organisms that are tolerant to low  $a_w$  be also tolerant of very high osmotic pressures. The lowest  $a_w$  limits for growth recorded up to now illustrate the enormous range of tolerances that exist. The most  $a_w$  tolerant species are able to grow when osmotic pressure is as high as about 800 MPa. They can grow slowly below  $a_w$  0.62. The nature of the solute exerts additional affect on potential for growth. Ionic solutes such as NaCl and KCl are more inhibitors than non-ionic solutes such as sugars. Solutes such as glycerol, unlike the salts and sugars, rapidly permeate most bacteria but not yeast, e.g. *Saccharomyces rouxi* and *Debaryomyces hansenii*. However, for the more low  $a_w$ -tolerant species this simple relationship is no longer valid. *Staphylococcus aureus*, for example, is extremely salt-tolerant and more sensitive at a higher  $a_w$  in glycerol than in sodium chloride (Shapton and Shapton, 1991).

Lowering the  $a_w$  by various means may also influence the rate of enzymatic and chemical changes in foods. Whilst all microbiological growth is completely stopped below about  $a_w = 0.6$ , some enzymatic reactions that cause food spoilage continue and some reactions, such as lipid oxidation, may even be accelerated at very low  $a_w$  values (Shapton and Shapton, 1991).

### 3.3. Preservation by low pH and organic acid

#### 3.3.1. Preservation by low pH

Foods are classified according to their acidity as follows: non-acid – 7.0-5.3; low or medium acid – 5.3-4.6; acid I – 4.6-3.7 and acid II – 3.7 and lower.

Microorganisms have a characteristic range of pH values within which they can grow. Most bacteria have an optimum pH near 6.8 and may grow at pH values ranging from 4.0 to 8.0. A small number bacterial species can multiply when  $\text{pH} < 4.0$  or  $\text{pH} > 8.0$ . Yeast and moulds can sometimes grow at pH less than 2.0. Usually the growth rate decreases as the pH drops below the optimum value. Approaching the lower limiting pH for growth, cells are first inhibited and eventually killed. The degree of inhibition increases as pH decreases and this relationship is linear. The differences in inhibition and/or lethal effects of organic acids used for pH reduction having different pK values are well known. The pK values of some acids are as follows: citric – 3.08; malic – 3.4; tartaric – 2.98; acetic – 4.75 (it is usually used as an effective preservative). The theory of food canning accepts a pH of 4.5 or 4.6 (for USA) as the bor-



derline between acid and low acid foods, which respectively do not need and do need the minimum botulinum cook known to be 12D. However, this assumption does not take into account the ability of *Cl. botulinum* to grow at pH levels near 4.0 and in very specialised environmental conditions as well as the ability of *Staphylococcus aureus* and several Salmonella strains which would also be of significance if container leakage occurred when these organisms are present in water used for cooling of the sterilised containers (Shapton and Shapton, 1991).

The pH limits of growth differ widely among microorganisms. In general heterotrophic bacteria tend to be least acid tolerant among common food microorganisms. Approximate pH range for bacteria is 4.0-9.0; for yeast is 1.5-8.0; for moulds is 1.5-11.0. Bacteria that grow outside of these ranges are well known but are rarely significant food spoilage organisms. The pH limits for growth in laboratory media are often much wider than those observed in the foods.

The exact details of how microorganisms interact with pH are not fully understood. As with other physiological parameters, pH is not also an absolute determining factor in potential for spoilage of food. The type of microorganisms and acids presented into the product also will affect the outcome, as will others environmental factors, which reduce microbiological activity.

Yeast and moulds are very acid-tolerant and frequently the pH range for growth extends well below pH values commonly encountered in foods. For example the pH range for *Saccharomyces cerevisiae* growth is 2.35-8.6, for *Acetobacterium* spp. is 2.8-4.3, for *E. coli* is 4.4-8.7, for *Bacillus acidocaldarius* is 2.0-5.0, etc. Lowering of cytoplasmic pH is probably the major cause of inhibition of growth by weak acid used as food preservatives. However, mechanistic basis of inhibition of pH homeostasis is still not clear.

### 3.3.2. Preservation by organic acids

Some organic acids and their esters are found naturally in many foods or as a product of microbial metabolism in fermented foods. Many foods are preserved by the addition of relatively low concentrations of such compounds, all of which show marked pH-dependant activity as preservatives. These compounds are primarily active against yeast and moulds at low concentration but bacteria are affected also. Lowering the pH increases the proportion of undissociated acid molecules, which increases the antimicrobial effectiveness of all such organic acids. It has therefore been generally assumed that the antimicrobial activity of these acids is directly related to the concentration of their undissociated molecules. The sensitivity of microorganisms to weak organic acids is a significant species-dependant parameter. Organic acids and esters cover a large group of substances but only a limited number are used as food.

Acetic acid has only a limited action as a preservative. Its main action is linked to its pH-reducing capacity. Inhibitory action is effective when concentration is from 0.04% and/or pH = 4.9 for *Salmonella anthracis* to 2.4% and pH = 4.5 for *Saccharomyces ellipsoideus*.

Propionic acid: only the sodium and calcium salts are used as food preservatives. They are mainly used against moulds in cheese and bakery products. Effective concentrations are from 440 to 850 mM.

Lactic acid is generally viewed as being less effective than other organic acids. It is excellent inhibitor of spore-forming bacteria at pH = 5,0 although totally ineffective against yeast and moulds. It is found that aflatoxin and sterigmatocytin formation by fungi are prevented by lactic acid.

Sorbic acid is used either as such or as the sodium, potassium and calcium salts but most commonly as the potassium salt. It is more effective against moulds and yeast than bacteria. Growth inhibition of bacteria occurs at concentration of between 50 and 10 000 ppm, for yeast between 25 and 500 ppm and for moulds between 100 and 1000 ppm. Generally has been assumed to possess antimicrobial activity in the undissociated state only.

Benzoic acid is used as such or as its sodium salt, commonly against yeast (20 to 7000 ppm), moulds (20 to 10 000 ppm) and bacteria (50 to 1800 ppm) Bacteria are more variable in their sensitivity.

Parabens are esters of *p*-hydroxybenzoic acid. The most common are methyl, ethyl, propyl and butyl parabens. For bacteria the minimum inhibitory concentration (in ppm) decreases as follows: methyl > ethyl > propyl > butyl. This means that effectiveness increases in the opposite direction. For *B. cereus* effective concentrations of these preservatives are respectively 2000, 1000, 125 and 63 ppm. The same rule is valid for yeast and moulds. Compared with the weak acids, parabens as preservatives are effective at significantly lower concentrations. Their activity is practically pH-independent. Some gram-negative bacteria are resistant to parabens with longer side chains (Russel and Gould, 1991).

The production of organic acids by food fermentation plays a significant part in preservation of foods. Many dairy products rely upon the metabolic activities of lactobacilli to prevent the growth of spoilage microorganisms. This is believed to be due to the production of lactic and acetic acids but the production of hydrogen peroxide may also be an important factor. Concerning meat, it is believed that the reduction of pH, and not the production of lactic acid, is primarily responsible for the preservative action. In dairy fermentation, flavour production is very important. It has been noted that different rates of acid production may be modulated by temperature, salt concentration and starting pH. The presence of glucose in meat has been suggested to be a major factor in the rate of spoilage.

### *3.4. Preservation by carbon dioxide, sulphite, nitrite and nitrate*

#### *3.4.1. Carbon dioxide (CO<sub>2</sub>)*

It is recognised that CO<sub>2</sub> has a major role in modifying microbial growth. Modified atmospheres enriched with CO<sub>2</sub> are a widespread natural means of extending the shelf life of a variety of non-sterile refrigerated foods.

Concentration of CO<sub>2</sub> in normal air is 0.03% but when is more than 5%, it is particularly effective against the psychrotrophic microorganisms which cause spoilage of chilled foods (Gould, 1995). Significant preservative effects have been demonstrated with fresh fermented meats and fish and also fruits and milk. Mechanisms of inhibition of microorganisms by CO<sub>2</sub> are not fully understood. The most likely mode of action is the inhibition of the decarboxylation reaction in living cells.

#### 3.4.2. Sulphur dioxide (SO<sub>2</sub>)

Sulphur dioxide, sulphite ([SO<sub>3</sub>]<sup>2-</sup>), bisulfite ([HSO<sub>3</sub>]<sup>-</sup>) and metabisulphite ([S<sub>2</sub>O<sub>4</sub>]<sup>2-</sup>) are used as preservatives in wine, fruit juices, sausages and other foods (Tapia de Daza et al., 1996). As antioxidants they are used to inhibit various enzyme-catalysed reactions notably enzymatic and non-enzymatic browning. The precise mechanisms of action are not known. It has been suggested that the undissociated sulphurous acid is the active molecular species since the inhibitory effect is enhanced at low pH. Bisulfite has been shown to accumulate in yeast at concentrations 50 fold greater at pH = 3.6 than at higher pH. The bisulfite ion has greater inhibitory activity towards bacteria and fungi than the sulphite ion.

#### 3.4.3. Nitrite and nitrate

Nitrite and nitrate, as their sodium and potassium salts, are widely used in fermentation of meat products and the curing of pork during ham producing and bacon. Originally added together with sodium chloride these compounds are important because they stabilise the red meat colour and inhibit the growth of pathogenic and spoilage microorganisms. Many bacteria reduce nitrate to nitrite and it is the latter that helps to prevent microbial spoilage. The antibacterial effectiveness of nitrite increases as pH is lowered. Nitrite inhibits the growth of *Cl. botulinum*, which would otherwise present an unacceptable risk in such products. Nitrite also helps to prevent rancidity in cured meats (Rozum, 1995).

### 3.5. *Preservation by modified and controlled atmospheres*

The effect of the food's the gaseous environment on microorganisms is less well understood by microbiologists and food technologists than other factors that influenced microbial growth like pH, a<sub>w</sub>, etc. The maintenance of a constant gas phase is difficult to achieve but modification of the atmosphere is used mainly for larger storage of fresh and partly processed food including meat, fish, fruits, vegetables, etc. though individual packs are often gas-flushed.

Deliberate attempts to modify the atmosphere in order to aid food preservation occur at three levels of sophistication:

- 1) Controlled atmospheres. They are mainly used for bulk storage or transportation. The gas composition, humidity and temperature can be controlled to provide optimal conditions for long-term storage of fruit, meat and other foods.

2) Gas packaging. This method is used for bulk storage and retail packs. Gas mixtures are used. During storage, gas content of CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> may subsequently change as a consequence of pack permeability, biological activities of packed product, chemical reaction, for example, of oxygen with some components of the foods like vitamin C.

3) Vacuum packaging. This method is predominantly used for retail packs. The original air atmosphere is evacuated and the atmosphere, which develops during storage, is mainly the result of biological activities of the products itself.

Anaerobic growth rates of different bacteria are reduced from 8% (*Lactobacillus* 173) to 67% (*Bacillus cereus*) when 100% CO<sub>2</sub> is used in comparison with atmosphere containing 5% CO<sub>2</sub> and 95% N<sub>2</sub>. Carbon dioxide alone or in mixture with N<sub>2</sub> and/or oxygen is most important for food preservation.

A reduction in respiratory activity in presence of CO<sub>2</sub> is observed for five species of meat spoilage bacteria but *Enterobacter* and *B. thermosphacta* are not affected under aerobic conditions.

In current commercial practice, N<sub>2</sub>, O<sub>2</sub> and CO<sub>2</sub> in various combinations are the only gases widely used for food preservation (Gould, 1995).

By combination of ultra low level oxygen (0,5-1%), 2-3% CO<sub>2</sub> and 1-2°C, Elstar apples can be stored almost a whole year without unacceptable quality loss. In the case of dynamic controlled atmosphere packaging, gas levels are not controlled at pre-set levels but are continuously adapted to the physiological response of the stored product. In this way an optimal match is made between the physiological demand and tolerance of the product from one side and storage condition to the other side (Rooney, 1995).

### 3.6. Irradiation preservation of the foods

The effects of ionising radiation on biological materials are direct and indirect. In direct action, the chemical events occur as a result of energy deposition by the radiation in the target molecule. The indirect effects occur as a consequence of reactive diffusible free radical forms from the radiolysis of water, such as, the hydroxyl radical (OH<sup>-</sup>), a hydrated electron, hydrogen atom, hydrogen peroxide and hydrogen. Hydrogen peroxide is a strong oxidising agent and a poison to biological systems, while the hydroxyl radical is a strong reducing agent. These two radicals can cause several changes in the molecule structure of organic matter, including foods.

Irradiation is used mainly for:

- Disinfection using low radiation dose of 0.15-0.50 kGy, for damage insects at various stages of development that might be present in some food likes grain;
- Self-life extension by inhibiting sprouting of potatoes, onions and garlic at 0.2-0.15 kGy;
- Delaying ripening and senescence of some tropical fruits such as bananas, avocado, papayas and mango at 0.12-0.75 kGy;
- Extending storage of beef, poultry and seafood by destroying spoiling microorganisms;

- Delaying microbiological spoilage of fruits and vegetables;
- Pasteurisation of seafood, poultry and beef using low dose (1.0-2.0 kGy);
- Sterilisation of poultry, spices and seasoning using higher dose 93.0-20 kGy);
- Product quality improvement for example decreasing gas producing factors in soya beans by using dose of 7.5 kGy;
- Reduction in the need for nitrate during production of some meat products.

Ionisation irradiation affects bacteria, yeast and moulds by causing lesions in the genetic material of the cell. Factors that affect the susceptibility of microorganisms to irradiation are dose level, temperature, atmosphere composition, medium including foods and type of organism. In general the higher the dose applied the lower number of survivors. At lower temperatures the rate of chemical reactions, such as the formation of radicals from water molecules is lower. If the product is frozen, radical formation is practically inhibited. The D-value increases from 0.16 kGy at 5°C to 0.32 kGy at 30°C when *Campylobacter jejuni* is inoculated into ground beef. This D-value means the dose by which concentration of microorganisms is reduced 10 times, say from 1000 to 100 or from 500 to 50, etc. Bacteria become more resistant to ionisation radiation in frozen state as well in the dry state. The composition of irradiating product will affect the survival of microorganisms. As a rule, the simpler the life form, the more resistant it is to the effect of radiation. For example viruses are more resistant than bacteria, which are more resistant than moulds, which are more resistant than human beings. Also some genera of bacteria are more resistant and bacterial spores are more resistant than their corresponding vegetative cells by a factor of about 5-15.

The effectiveness of irradiation to control foodborne parasite depends on the type of organism. Minimum effective doses (kGy) for representative protozoa: *Toxoplasma gondii* 0.09-0.7 and *Entamoeba histolytica* 0.251. Killing cyst stages for Trematodes: *Fasciola hepatica* 0.03; *Clonorchis senensis* 0.15-0.20; *Opisthorchis viverrini* 0.10; *Paragonium westermani* 0.10. For Cestodes: *Taenia* >3.0, for complete inactivation of larvae; 0.40 to prevent development in humans; 0.3 to eliminate infectivity of *Taenia solium*; 0.2-0.7 to eliminate infectivity of *Echinococcus granulosus*. For Nematodes, the doses are: *Trichinella spiralis*, 0.10-0.66 for elimination of infectivity; 0.11 for sterilisation of female *Angiostrongylus cantonensis*; 2.0-4.0 for decreasing infectivity of *Gnathostoma spinigirum*; 7.0 for reducing larval penetration (Potter and Hotchkiss, 1995).

D-values (kGy) for various food borne pathogens are 0.4-0.6 for *Listeria*; 0.4-0.5 for *Salmonella*; 0.25-0.35 for *E. coli* 0157:H75; 0.14-0.32 for *Campylobacter*; 0.14-0.21 for *Yersinia*; 0.14-0.19 for *Aeromonas*.

### 3.7. Preservation by low temperatures

Food preservation by cooling and freezing are the oldest methods using natural low temperatures. In 1875 the ammonia refrigeration system, that was capable of supporting commercial for foods refrigeration and freezing, was invented. Starting from 1920, the modern frozen food industry grew rapidly. Refrigeration

today markedly influences the practices of marketing and food industry and sets the economic climate in agro-food industry (Gould, 1995).

Chilling is used to reduce the rate of biochemical and microbiological changes and hence to extend the shelf life of fresh and processed foods. Chilled foods are grouped into three categories according to their storage temperature range as follow:

- 1) From  $-1^{\circ}\text{C}$  to  $+1^{\circ}\text{C}$ , fresh fish, meats, sausages, ground meats, etc.
- 2) From  $0^{\circ}\text{C}$  to  $+5^{\circ}\text{C}$ , pasteurised milk, cream, yoghurt, prepared salads, sandwiches, baked goods, fresh pasta, fresh soup and sausages, pizzas, etc.
- 3) From  $0^{\circ}\text{C}$  to  $+8^{\circ}\text{C}$ , fully cooked meats, fish, pies, cooked and uncooked cured meats, butter, margarine, cheeses, fruits and vegetables, etc.

The rate of biochemical changes of foods, caused by microorganisms or naturally occurring enzymes increase logarithmically with temperature increasing. Chilling therefore reduces the rate of enzymatic and microbiological changes and retards respiration of fresh foods. The factors that control the self-life of fresh crops during chilling storage include:

- Type of food and variety;
- The part of crop; the fastest growing parts have the highest metabolic rates and the shortest storage life. For example, the relative respiration rate of asparagus is 40, of mushrooms is 21, of spinach is 11, of carrots is 5, of potatoes and garlic is 2, of onions is 1, etc. When relative rate of respiration is higher than 17, at  $2^{\circ}\text{C}$  storage time is a maximum of 4 days, when the rate is 2 to 1, storage time is 25-50 weeks, etc.;
- Conditions of food at harvest, for example degree of microbial contamination, degree of maturity, etc.;
- Temperature of harvest, storage distribution, retail display, etc.;
- The relative humidity of the storage atmosphere which influence dehydration losses.

The rate of respiration of fresh fruits is not constant at constant storage temperature. For example fruits which undergo "climacteric" ripening show a short but abrupt increase in the rate of respiration that occurs near to the point of optimum ripeness. Examples of climacteric fruits are apple, apricot, avocado, etc. and non-climacterics fruits are cherry, cucumber, etc. Temperature has a strong influence on the rate of respiratory activity, for example for apples at  $0^{\circ}\text{C}$  it is about 4-6 times less than at  $10^{\circ}\text{C}$ .

Undesirable changes to some fruits and vegetable occur when the temperature is reduced below a specific critical level. These changes are called chilling injury, for example internal or external browning, failure to ripen, etc. The reasons for these changes are not fully understood. For example, for apples such a temperature is less than  $2-3^{\circ}\text{C}$ , for avocado it is less than  $13^{\circ}\text{C}$ .

In animal tissues aerobic respiration rapidly declines when the supply of oxygenated blood is stopped after the animal is slaughtered. Anaerobic respiration of glycogen to lactic acid then causes the pH of the meat to fall and the onset of rigor

mortis in which the muscle tissue becomes firm and inextensible. Cooling during anaerobic respiration is necessary to produce the required texture and colour of meat and to reduce bacterial contamination. However, cooling must not be too rapid otherwise cold shortening can occur, which results in tough meat (Potter and Hotchkiss, 1995).

A reduction in temperature below the minimum necessary for microbial growth extends the generation time of microorganisms and prevents or retards reproduction. There are four broad categories of microorganisms based on the temperature range for growth, as follows:

- Thermophilic (minimum 30-40°C, optimum 55-65°C);
- Mesophilic (minimum 5-10°C, optimum 30-40°C);
- Psychrotrophic (minimum <0-5°C, optimum 20-30°C);
- Psychrophilic (minimum <0-5°C, optimum 12-18°C).

Chilling prevents the growth thermophilic and many mesophilic microorganisms. The main microbiological concerns with chilled foods are a number of pathogens that can grow during extended refrigerated storage below 5°C or as a result of any increase in temperature (temperature abuse) and this may cause food poisoning. But it is now known that some pathogenic species can either grow to large numbers at these temperatures or are sufficiently virulent to cause poisoning after ingestion of only a few cells. For example *Aeromonas hydrophilica*, *Listeria* spp., *Yersinia enterocolitica*, enteropathogenic *E. coli*, etc. *E. coli* O157:H7) may cause haemorrhagic colitis after ingestion of as few as 10 cells.

Freezing is method of food preservation in which the temperature of food is reduced below its freezing point and a proportion of the water undergoes a change in state to form ice crystals, resulting in concentration of dissolved solutes in unfrozen water respectively reducing the water activity ( $a_w$ ) and pH values. Preservation is achieved by a combination of low temperatures, reduced water activity in some foods, pre-treatment by blanching and also retarding growth of microorganisms (Ray, 2000).

The proportion of water that remains unfrozen at the temperature used in commercial freezing depends on the type and composition of the food and temperature of storage. For example, at storage temperature -20°C the percentage of water frozen is 88% in lamb, 91% in fish and 93% in egg albumin.

#### 4. Potential Food Preservation Methods

Consumer trends and food markets are changing and will change more in future towards foods with high quality and more fresh like attributes, consequently fewer external treatments and/or fewer additives will be used. This means less heat and chill damage, more freshness, less acid, salt, sugar and fat, etc. To satisfy these demands some changes in the traditionally used preservation techniques must be achieved. From microbiological point of view, these changes have

important and significant implications since food safety is an aspect of increasing importance. The safety and quality of food should be well balanced in every one of the new methods of food preservation. From scientific and technological point of view, the following methods have quite interesting prospects:

#### 4.1. Pulsed electric fields

The inactivation of microorganisms is attributed to a shock wave generated by an electric arc that prompts the formation of highly reactive free radicals from various chemical species in the food. In general, an increase in the electric field intensity and number of pulses has been found to lead to an increase in the inactivation of microorganisms. Formation of pores in cell membranes may be a reason for killing of microorganisms. Mechanisms of pore formation are not entirely understood. Applying the dielectric rupture theory, it is concluded that membrane rupture is caused by an induced transmembrane potential approximately 1V larger than the natural potential of the cell membrane. The reversible or irreversible rupture, termed electroporation, of the cell membrane depends on factors such as intensity of electric field, number of pulses and duration of the pulses (electroporation is used to open cell membranes temporarily in order to allow the entry of various molecular species including genetic material). For example, when the pulsed electric field is 9.2 kV/cm the number of survivors of *Staphylococcus* cells is 100%, at 14.25 it is 35%, at 19.50 it is 0.9%, at 27.50 it is 0.6%. Other factors that influence microbial inactivation by pulsed electric fields are the treatment temperature, pH, ionic strength and conductivity of the medium containing the microorganisms.

Raw and reconstituted apple and peach juices, skim milk, beaten eggs and pea soup have been exposed to pulsed electric fields at 25-45 kV/cm. *E. coli* inoculated in skim milk and exposed to 60 pulses of 2  $\mu$ s at 45 kV/cm and 35°C was reduced by 2D. A reduction of 6D was observed in liquid egg inoculated with *E. coli* and treated with pulsed electric fields of 25.8 kV/cm and 100 pulses of 4  $\mu$ s at 35°C. *E. coli* and *B. subtilis* inoculated in pea soup and exposes to electric field of 25-33 kV/cm (10-30 pulses of 2  $\mu$ s) provided a limited inactivation (< 1.5D) when the process temperature of pea soup was below 53°C while microbial inactivation was 4.4D with process temperature was between 53°C and 55°C (Barbosa-Canovas et al., 1998).

#### 4.2. High pressure

One “new” or emerging technology receiving a great deal of attention is high hydrostatic pressure. The basis of this process is Le Chatelier’s principle, according to which, any reaction, like conformational change or phase transition that is accompanied by decrease in volume will be favoured at high pressure, while reactions involving an increase in volume will be inhibited (Knor, 1996).

Hydrostatic pressure technology is novel non-thermal food processing technology, whereby foods are subjected to high hydrostatic pressure, generally in



the range of 100-600 MPa at or around room temperature. In general, the vegetative microbial forms are inactivated by pressure between 400 and 600 MPa, while spores of some species may resist pressures higher than 1000 MPa at ambient temperatures. Gram-positive bacteria are more pressure resistant than gram-negative ones. Among the gram-positive bacteria, *Staphylococcus* is one of the most resistant and can survive treatment at 500 MPa for more than 60 min. High pressure can inactivate also enzymes. The extent of microbial inactivation achieved at a particular pressure depends on a number of interacting factors including type and number of microorganisms, magnitude and duration of high pressure treatment, temperature and composition of the suspension media, etc.

The first historical data on effects of high pressure on microorganisms were most likely compiled by Certes (1883) who found viable bacteria in water samples obtained from 5100 m depth. (Approx. 50 MPa). Hite (1899) applied hydrostatic pressure-treatment of food for the first time and he reported the reduction of spoilage microorganisms in milk and meat products and later on (1914) in fruit and vegetables.

The higher resistance of spores to high pressure than vegetative cells was shown in 1965 and that pressure can induce germination of bacterial spores was demonstrated in 1970. High hydrostatic pressure induces a number of changes to the morphology, biochemical reactions, genetic mechanisms, cell membrane and wall of microorganisms. At 0.6 MPa intracellular gas vacuoles can collapse (Cheftel and Culioli, 1997).

Most bacteria are capable to grow at pressure around 20-30 MPa. *B. aerophiles* can grow at pressure higher than 40-50 MPa and baroduric or barotolerant can survive for prolonged periods at pressures > 200 MPa. In pork slurry, vegetative forms of *E. coli* are not affected at pressure lower than 203 MPa but more than 6 log cycles are reduced at pressures higher than 405 MPa for 10 min. For *S. cerevisiae* less than 2 log cycles reduction is observed at pressures lower than 304 MPa and more than 6 log cycles at pressures higher than 405 MPa. *B. cereus* spores are not reduced considerably (less than 1 log cycle) even with treatment at 608 MPa for 10 min.

Generally an increase in pressure increases microbial inactivation. However, increasing the duration of the treatment does not necessarily increase the lethal effect. Above 200-300 MPa, inactivation of vegetative cells increases with pressure or process time. The temperature during pressurisation can have significant effect on the inactivation of microbial cells (Barbosa-Canovas et al., 1998).

Pressure inactivation of yeast and moulds has been reported in citrus juices. Juices pressure treated at 400 MPa for 10 min at 40°C did not spoil during 2-3 months of storage. A population of 10<sup>6</sup> CFU/ml of *Listeria monocytogenes* was inactivated by exposure to 340 MPa at 23°C in UHT milk and the same population of *Vibrio parahaemolyticus* was killed by exposure to 170 MPa within 10 min at 23°C in clam juice. *Salmonella senftenberg* 775 W, a very heat resistant vegetative microorganism, could be reduced in chicken puree by high-pressure treatment but was shown to recover in chicken based medium.

It is reported that a linear relationship was found between the water activity depression factor ( $1-a_w$ ) and the log of *Z. bailii* survival fraction ( $N/N_0$ ) after a high-pressure treatment at 345 MPa for 5 min. It was found that  $\log(N/N_0) = -4.599 + 45.538(1-a_w)$ . This relationship indicates that as the  $a_w$  of the model system decreases, the number of surviving *Z. bailii* increase. For *Saccharomyces cerevisiae* in mandarin juice at 250 MPa for 5 min, the decimal reduction (D) is about 2D, after 10 min it is about 4D and after 30 min it is about 6D. After 5 min at 300 MPa it is 5D and after 10 min is 6D.

The patterns of high hydrostatic pressure inactivation kinetics observed with different microorganisms are quite variable. Some investigators indicate first order kinetics in the case of several bacteria and yeast. Other authors observed a change in the slope and a two phases inactivation phenomenon, since the first fraction of the population being quickly inactivated, whereas the second fraction to be much more resistant. Pressure, temperature and composition of the medium influence the patterns of inactivation kinetics. Some authors have stated that there is clear evidence that pressure mediating death is not first order and inactivation curves often present pronounced survivor tails. Thus the D and Z concepts commonly used in thermal processing cannot be usefully applied to describe pressure processes.

Current industrial applications of high pressure treated food include:

- Jams, fruit dressing, fruit sauce (topping), yoghurt, fruit jelly by the following processing conditions: 400 MPa, 2-20 min, 20°C;
- Grapefruit juice, 120-400 MPa, 2-20 min, 20°C;
- Mandarin juice, 300-400 MPa, 2-3 min, 20°C;
- Beef (tenderization only), 100-150 MPa, 30-40 min, 20°C;
- Rice cake, 400 MPa, 45-70°C, 10 min.

### 4.3. Ultraviolet radiation

Ultraviolet fraction of sunlight has long been known to be the major factor in the bactericidal action of light. It is mainly used in sterilising air and thin liquid films due to low penetration dept of the waves. When it is used in high dosage there is a marked tendency toward flavour and odour deterioration before satisfactory sterilisation of the foods is achieved. But low-level radiation at carefully applied doses can often usually extend the shelf life of foods, without damaging quality. Using UV radiation to kill off bacteria in water is well known. This radiation is safe, environmentally friendly and more cost effective to install and operate than conventional chlorinating. It does not affect the taste of the water, as does chlorine. High intensity UV-C lamps have become available which can increase the potential of destroying surface bacteria on food.

Ultraviolet irradiation is being applied commercially in the use of bactericidal ultraviolet lamps in various food applications: tenderizing of meat, curing and wrapping of cheeses, prevention of surface mould growth on bakery products, air purification in bottling and food processing establishment and over pickle vats.

The lethal effects of ultraviolet light on microorganisms have been well documented. The practical application of this has been controversial because of the type and intensity of radiation, methods of estimating lethality of microorganisms and other factors. It is found that 3-83% of the yeast and 33-72% of the moulds are killed in apple cider through layers varying from 2 to 25 mm in thickness. Colouring agents of the foods absorbed a greater part of the light. Incident energy levels of light at  $\lambda = 253.7$  nm, inhibit 90% of *Bacillus megatherium* at 1100 mWs/cm<sup>2</sup> and 90% of *Sarcina lutea* at 19800 mWs/cm<sup>2</sup>. There was a 90% or 1D reduction in microbial count of apple juice. Coupled with refrigeration, this could be of commercial significance.

It is generally agreed that the wavelength for maximum germicidal effect is 260 nm. The lethal action varies with the time of exposure and intensity of light and other factors like temperature, pH and the number of cell unit area exposed. The relative humidity affects the death rate of bacteria suspended in air but this is more valid for humidity greater than 50% (Barbosa-Canovas et al., 1998).

Spores of bacteria are generally more resistant to UV light than vegetative bacteria. Spores of *B. subtilis* are reported to be 5-10 times more resistant than *E. coli*. Moulds are more resistant than vegetative bacteria, while yeast differ less from bacteria in this respect. Dark pigmented spores are more resistant to UV irradiation than non-pigmented.

This type of radiation affects bacteria and fungi such as *Penicillium* and *Aspergillus* in grapes and oranges. Onion that had been UV irradiated showed the greatest percentage of marketable product and reduction in post-harvest rot compared with gamma rays and electron beams. Sprouting is also controlled. In addition UV radiation dose of  $1.50 \times 10^{-3}$  J/mm<sup>2</sup> controls soft and dry rot diseases of potato tubers in short term storage of 3 months (Gould, 1995).

UV radiation at a wavelength of 253.7 nm was effective in destroying surface bacteria on fresh meat by 2 log cycles (99% reduction) on a smooth surface beef after a radiation dose of 150 mWs/cm<sup>2</sup> but dose level to 500 mWs/cm<sup>2</sup> reduced bacteria 3 log cycles. A significant increase in shelf life may be obtained by the exposure of beef muscle and fat surface to UV light for 2 min at  $\lambda = 366$  nm

UV radiation (0.2-0.24  $\mu$ W/cm<sup>2</sup>) at 0°C and relative humidity 99.3%, resulted in extension of log phase of *Pseudomonas* and of the moulds *Thamnidium* and *Penicillium* but not of the yeast *Candida scotii*.

The use of UV radiation at  $\lambda = 254$  nm and dose 300 mWs/cm<sup>2</sup> reduced surface microbial count of mackerel by 2 or 3 log cycles.

UV radiation does not penetrate most opaque materials and it is less effective on rough surfaces.

#### 4.4. Sound in food preservation

Ultrasound is sound energy with frequency range from 20 kHz (upper limit of human hearing) to hundreds of MHz.

Bacteria and especially spores are very resistant to ultrasound requiring hours of ultrasonification to have any effect. At low frequency an increase in total amount of cells was reported during treatment of milk.

It has been found that the reduction of *Salmonellae* during treatment follows a logarithmic relationship. Ultrasound is used to free bacteria adhering to surfaces to facilitate the removal of flora contaminating foods and for machinery washing. It has been shown that bacteria are firmly attached to poultry skin and, although bactericides in processing water are lethal to *Salmonella*, they do not seem to access bacteria that are firmly attached to or entrapped in poultry skin. Most bacteria from milk films on metal surfaces can be affected by ultrasonic waves of 80 kHz. *Salmonella* cells attached to broiler skin are reduced 1-1.5 log cycles by sonication in peptone at 20 kHz for 30 min and by less than 1 log cycle by chlorine alone but by 2.5-4 log cycles by sonicating skin in chlorine solution (Gould, 1995).

The mechanical destruction of cells by very intense currents and cavitation generated by ultrasound is the main lethal effect on microorganisms rather than any chemical effect (Barbosa-Canovas et al., 1998).

Strains of *Staphylococcus aureus* are much more susceptible to damage by the combined affect of ultrasonic (20 kHz) and heat treatments than by either treatment separately. There is a marked decrease in heat resistance (without killing) of *Bacillus stearothermophilus* spores by ultrasound at 20 kHz regardless of the heating temperature and the storage time between both treatments. After sonication its heat resistance is reduced from one half to one third of its original value. The heat resistance at 105°C of *Bacillus cereus* and *Bacillus licheniformis* decrease after a previous treatment with ultrasound at 20 kHz.

#### 4.5. Application of bacteriocins in food preservation

A bacteriocin is defined as a protein that has a bactericidal action against a limited range of microorganisms, which are mostly closely related. They were discovered many decades ago. Bacteriocins produced by pathogenic bacteria (for example certain *E. coli* produce colicin, some *Staphylococcus aureus* strains produce epidermin) are not suitable for food application. More interesting sources of bacteriocins are the lactic acid bacteria. For centuries these microorganisms have been used in food fermentation to produce stable food products ranging from dairy (cheese), meat (sausages) and vegetables (sauerkraut) mainly through the preservative action of the lactic acid they produce (Davidson and Branen, 1998).

Ever since the identification of the inhibitory activity of a strain of *Lactococcus lactis* subsp. *lactis* in 1928, lactic acid bacteria have been increasingly scrutinised for bacteriocin production. The inhibitory agent was later termed nisin, the first known and most extensively studied bacteriocin of these bacteria. Nisin has been used for:

- Prevention of blowing faults of Swiss type cheese, caused by *Clostridia*;
- Extension of the shelf life of milk;

- Lower heat processing of tomato juice;
- Control of flat sour spoilage of some canned foods caused by thermophilic spoilage bacteria;
- Optimising starter function during sauerkraut production by improving competitiveness;
- Inhibition of beer and wine spoilage by lactic acid bacteria.

Pediocin is the name given to bacteriocins produced by bacterial genus *Pediococcus*. The first report on pediocin production dates back to 1975, when it was found that *Pediococcus pentosaceus* inhibited the growth and acid production of *Lactobacillus plantarum*, which is undesirable competitor in mixed brine cucumber fermentation. The active agent was designated as pediocin A and it inhibits a broad range of lactic acid bacteria, as well as several *Clostridia*, *Staphylococcus aureus* and *Bacillus cereus*. Pediocin PA-1 produced by a strain of *Pediococcus acidilactici* inhibits growth of *Listeria monocytogenes*, when inoculated into cottage cheese, half and half cream and cheese sauce for 1 week at 4°C but rapid growth to high cell densities was observed in the control samples.

Sakacins, a group of bacteriocins produced by *Lactobacillus sake*, owe their discovery probably to the intensive search for natural antimicrobial compounds capable of increasing shelf life of raw meat by inhibiting growth of meat spoilage microorganisms and controlling *Listeria monocytogenes* (Gould, 1995).

Today about 30 different bacteriocins, produced by some 17 species of lactic acid bacteria have been identified and their bactericidal activity has been studied. For food preservation, the advantageous features of several bacteriocins are their relatively high heat resistance and inhibition of gram-positive foodborne pathogens and spoilage microorganisms. Much attention has been given to the inhibition of *Listeria monocytogenes* which may give rise to a high mortality rate and may occur in many different foods causing problems, specifically in dairy (soft cheese) and meat products (paté, sausages). The bacterial impact of several bacteriocins on spore forming bacteria has been subject of research for many decades and indicates the great potential these bacteriocins could have in food preservation.

Bacteriocins can be used in three basic ways:

- A pure culture of the viable bacteriocin producing lactic acid bacteria;
- A (semi-) purified preparation of the bacteriocin;
- A crude bacteriocin preparation.

The production of lactic acid and bacteriocins by lactic acid bacteria may well provide for a safe and natural means of (bio) preservation.

## 5. Combined Methods for Food Preservation

The microbial stability and safety of most traditional and novel food is based on the combination of several preservative factors (called hurdles) which microorganisms present in the food are unable to overcome. This is illustrated

by so-called hurdle effect, introduced by Leistner (1978). The hurdle effect is of fundamental importance for the preservation of foods, since hurdles in a stable product control microbial spoilage, food poisoning, as well as desired fermentation processes. In fact, the hurdle concept illustrates only the well-known fact that complex interaction of temperature, water activity, pH, redox potential, etc. are significant for the microbial stability of foods. The relation between technology and homeostasis of microorganisms is well established (Leistner and Gorris, 1994). Foods preserved by this method are safe, stable, nutritious, tasty and economical. From an understanding of the hurdle effect, hurdle technology was derived, which allows improvements in the safety and quality of foods using deliberate and intelligent combination of hurdles. In industrialised countries, hurdle technology is currently of practical interest for minimally processed foods, whereas in developing countries foods storable without refrigeration, due to stabilisation by hurdle technology, are at present of paramount importance. The application of deliberate and intelligent hurdle technology is increasing rapidly world-wide. This concept is also referred to as food preservation by combined methods, combined processes, combination of preservations or combination of techniques. At present, the term hurdle technology is most often used.

Many preservation methods are used for making foods stable and safety, e. g. heating, chilling, freezing, drying, curing, salting, sugar addition, acidification, fermentation, smoking, oxygen removal, etc. However these processes are based on relatively few parameters or hurdles, i. e. high temperature (F value), low temperature ( $t^{\circ}\text{C}$  value), water activity ( $a_w$ ), acidification (pH), redox potential (Eh), preservatives, competitive flora, etc. In some of the preservation methods mentioned, these parameters are of major importance; in other they are only secondly hurdles (O'Connor-Shaw, 1995; Tapia de Daza et al., 1996).

The critical values of these parameters for the death, survival or growth of microorganisms in foods have been determined in recent decades and are now the basis of food preservation. However it must be kept in mind that critical value of a particular parameter changes if other preservative factors are presented in food. For example, the heat resistance of bacteria increases at low  $a_w$  and decreases in the presence of some preservatives, whereas a low Eh increases the inhibition of microorganisms caused by a reduced  $a_w$ . Furthermore, as mentioned earlier, the microbial stability and safety of many foods is based on the combined effects of hurdles. For instance heated canned foods called "Half-preserved" or "three-quarter preserved", need refrigeration during storage; fermented sausages are only stable and safe if both the  $a_w$  and pH are in an appropriate range (Ray, 2000).

Therefore, in food preservation the combined effect of preservative factors must be taken into account, which is illustrated by the hurdle effect. More than 40 hurdles of potential use for foods of animal or plant origin which improve the stability and/or qualities of these products have hitherto been identified (Hudson, 1990).

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