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# UNIT 5 CONCEPT, DETERMINATION OF PROCESS LETHALITY REQUIREMENTS AND IMPORTANCE

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## 5.0 OBJECTIVES

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After studying this unit, you should be able to understand:

- kind of foods based on their acidic reaction;
- relationship between pH of foods and heat resistance of microorganisms;
- difference in heat resistance of vegetative cells and spores of microorganisms;
- what is thermal death time and how it is determined?;
- how microorganisms behave under freezing and refrigeration conditions?; and
- the basic principle involved in various methods for controlling microorganisms.

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## 5.1 INTRODUCTION

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The foods, which we eat or drink are also excellent substrates (food) for microorganisms, which are present in air, water, soil, utensils and even in raw foods. Under suitable conditions of growth, particularly temperature and moisture, the microorganisms multiply using these food items and produce luxuriant growth. Many foods serve as carrier of various pathogenic and non-pathogenic microorganisms, which may spoil the food by their growth, change of chemical nature of food, release of unpleasant odour, production of various harmful enzymes and toxins. Such foods are unfit for human consumption. For these reasons, it is essential to prevent the entry and growth of microorganisms in our food if present, by suitable processing. Before using a suitable process, we should understand various factors which may influence the effectiveness of a process.

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## 5.2 CLASSIFICATION OF FOODS ACCORDING TO pH

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Most foods are derived either from plants or from animals. In this course, we are concerned with foods of plant origin and are known as vegetables or fruits based on their use. These foods have different pH and are classified as low acid foods, medium acid foods, acid foods and high acid foods.

### a) Low acid foods

The foods having pH above 5.3 are called low acid foods. For example: peas, corn, lima beans etc.

### b) Medium acid foods

The foods which have pH between 4.3 and 5.3 are called medium acid foods. For example: asparagus, beets, pumpkin, spinach etc.

### c) Acid foods

Foods which have pH between 3.7 and 4.5 are called acid foods. For example: pears, pineapple, tomatoes etc.

### d) High acid foods

Foods having pH 3.7 or lower are included in this category. For example: Berries and sauerkraut.

You must have noted that in general vegetables are low or medium acid foods while fruits are acid or high acid foods.

Most foods are subjected to heat treatment or cooked before use. The heat process is essential in the canning of foods to eradicate the microorganisms, which may be present in the raw food or may enter from the environment during processing; and may spoil the food if not eradicated.

The effect of pH of the food is complicated as the heating at high temperature causes decrease in the pH of low or medium acid foods. Higher the original pH, the greater the drop of pH by heating. Foods artificially adjusted to more alkaline pH give increasing protection to spores against heat as pH increases towards 9.

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### 5.3 RELATIONSHIP BETWEEN pH OF FOOD AND HEAT RESISTANCE OF MICROORGANISMS

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The pH of the foods influence the heat resistance of microorganisms. In general cells or spores are most heat resistance in a substrate that is at near neutrality. An increase in acidity or alkalinity hastens killing by heat. However, a change towards acidic pH is more effective than a corresponding change in alkalinity. This will be more clear from the Table 5.1, which shows the effect of pH on heat resistance of spores of *Bacillus subtilis*. Therefore low acid foods are heated under pressure (i.e. temperature above 100°C) while the high acid foods are heated up to 100°C for making free from microorganisms.

**Table 5.1: Effect of pH on heat resistance of spores of *Bacillus subtilis* in 1:15 M phosphate buffer (100°C)**

pH	Time of survival (min)
4.4	2
5.6	7
6.8	11
7.6	11
8.4	7

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### 5.4 HEAT RESISTANCE OF MICROORGANISMS AND SPORES

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The heat resistance of microorganisms varies widely within the species and their forms:

- Thermophiles are more resistance than mesophiles and psychrophiles are least resistance.
- Spores formers are more resistant than non-spore formers. Cocci are usually more resistant than rods.
- The bacteria that clump considerably or form capsules are more resistant to heat than those which do not.
- Cells high in lipid content are difficult to kill than cells having low lipid.

However, there are many notable exceptions to the above mentioned general statements.

Higher the optimal temperatures for growth, the greater the resistance to heat. Thermal death time of bacterial cells of a few microorganisms are exemplified in Table 5.2.

**Table 5.2: Thermal death time of bacterial cells**

<b>Bacteria</b>	<b>Time (min)</b>	<b>Temperature (°C)</b>
<i>Gonococcus</i>	2 – 3	50
<i>Salmonella typhosa</i>	4.3	60
<i>Staphylococcus aureus</i>	18.8	60
<i>Escherichia coli</i>	20-30	57.3
<i>Streptococcus thermophilus</i>	15	70-75
<i>Lactobacillus bulgaricus</i>	30	71

The heat resistance of microbial spores is much higher than the vegetative cells, and vary with the species of microorganism and conditions during sporulation. Resistance may vary from <1 min to 20 h at 100°C. Similar to non-spore forming species, the spore forming species which have higher optimal temperature for growth are more resistant to heat than those spore forming species having lower optimal growth temperatures.

Simultaneous growth of two spores formers enhances the resistance of spores having lower heat resistance, e.g. *Clostridium perferingens* growing with *C. sporogenes*. Thermal death times of spores of a few microbial species are given in Table 5.3.

**Table 5.3: Thermal death times of bacterial species**

<b>Species</b>	<b>Thermal death at 100°C (min)</b>
<i>Bacillus anthracis</i>	1.7
<i>Bacillus subtilis</i>	15-20
<i>Clostridium botulinum</i>	100-300
<i>Clostridium calidotolerans</i>	520
<i>Bacillus coagulans</i> (flat sour bacteria)	> 1030

Above examples of thermal death times of vegetative cells as well as of spores are at various concentrations of cells or spores in different substrates. These values may change to lower or higher under different conditions.

### **What happens to enzymes in food by heat treatments?**

Most foods and microbial enzymes are destroyed at 79.4°C, however some may withstand higher temperatures, especially if high temperature for short duration is employed. This is called **pasteurization**, which you will learn later in this course.

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## **5.5 THERMAL DEATH POINT (TDP)**

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Thermal death point is the lowest temperature at which all microorganism in a liquid suspension are killed in 10 minutes.

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## 5.6 THERMAL DEATH TIME (TDT OR $t_D$ )

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The thermal death time is defined as the time required, at a given temperature, for heat killing of a population of a single species of microorganism in aqueous suspension.  $t_D$  depends on the size of the population and on the pH of the suspension. It is an important factor for controlling the microorganisms by heat treatment or to determine the heat resistance of a microorganism.

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## 5.7 DETERMINATION OF THERMAL DEATH TIME ( $t_D$ )

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The description of all the procedure and equipments/apparatus, used in the determination of thermal death time is beyond the scope of this course. However, a simple glass-tube method, used in canning industry is discussed here.

### 5.7.1 Glass Tube Methods

A known population of cells of an axenic culture in a small volume (1 ml) of buffer solution is sealed in small glass tube. The tubes are heated in a thermostatically controlled bath to a selected temperature. The tubes are selected periodically, cooled immediately to 0°C and the population of viable cells is determined. In case of spores, the suspension is first pasteurized to kill the vegetative cells, if present, before subjecting the spore suspension to  $d_T$  test. This is necessary as the lysed vegetative cells may have protective effect on spore-population.

Care is also taken to break up the clumps and remove the growth medium by centrifuging and washing. The volume of microbial suspension added to the buffer is kept 1-2 percent to avoid the change in the composition of heating substrate and the vials containing the suspension are brought to constant temperature, usually 0°C before subjecting to heat treatment. If temperature above 100°C is selected, oil bath instead of water bath is used. The test is always made in multiple tubes. Viability of the surviving organism after heat treatment should be checked on appropriate medium containing all the nutrients, which support maximum growth of that organism.

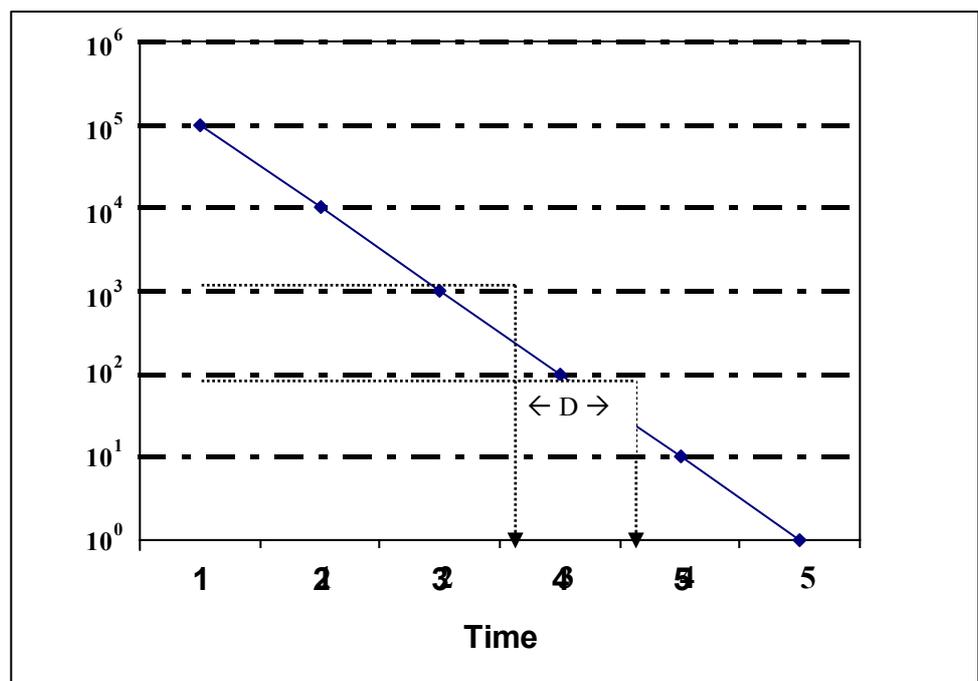
### 5.7.2 Decimal Reduction Time

When a microbial population is heated, the cells die at a constant rate. For example, suppose a population of 1 million ( $10^6$ ) cells has been heated to a high temperature for 1 minute and 90% has died. We are now left with 100,000 ( $10^5$ ) cells. If the leftover population is heated for another 1 minute, 90% of the population leaving 10000 ( $10^4$ ) survivors. Thus the each one minute of heat treatment will reduce 90% of the remaining population. This is shown in Table 5.4 and is known as decimal reduction time (DRT) and represented by  $D$ . It can be defined as the time of heating at a temperature to cause 90% reduction in the population of viable cells or spores.

**Table 5.4: Microbial death rate at constant temperature**

Time (min)	Death $\text{min}^{-1}$	Number of survivors
0	0	1,000,000
1	900,000	100,000
2	90,000	10,000
3	9,000	1,000
4	900	100
5	90	10
6	9	1

The D value (Decimal reduction time) may also be defined as the ‘time at given temperature for the surviving population’ to be reduced by 1 log cycl. Please refer Figure 5.1, if we extrapolate the times from  $10^3$  and  $10^2$ , the time difference is  $(3.5 - 2.5 = 1\text{min})$  is D). It means within 1 min initial population will decrease by 90 per cent (from 1000 to 100, Difference  $1000 - 100 = 900$ ).



**Figure 5.1:** A microbial death curve showing constant death rate of cells i.e. 90% per minute D value may be calculated from the curve by extrapolating lines from the Y axis and calculating the time difference.

### 5.7.3 Thermal Death Time Curve (TDT Curve)/Kinetics

The methods to construct TDT curve are: (i) The growth – no growth method (ii) classical end point method and (iii) based on  $D$  values. We shall here discuss the method based on  $D$ -values.  $D$  values can be calculated at different temperatures (refer 5.7.2). As the temperature is increased, the  $D$  value decreases. It means if we heat the sample at high temperature, it will take less time to kill the microorganism in a given food sample. If we plot  $\log D$  values against temperature, we will get a straight line. From this we can derive

another important parameter in heat processing  $Z$ , the temperature change which results in a ten fold (1 log) change in  $D$ .

$$Z = (T_2 - T_1)$$

$D$  value for a known population of cells or spores of a microbial species at several temperatures can be estimated. By plotting  $D$  values on the logarithmic scale against temperature TDT curves can be constructed (Figure 5.2).

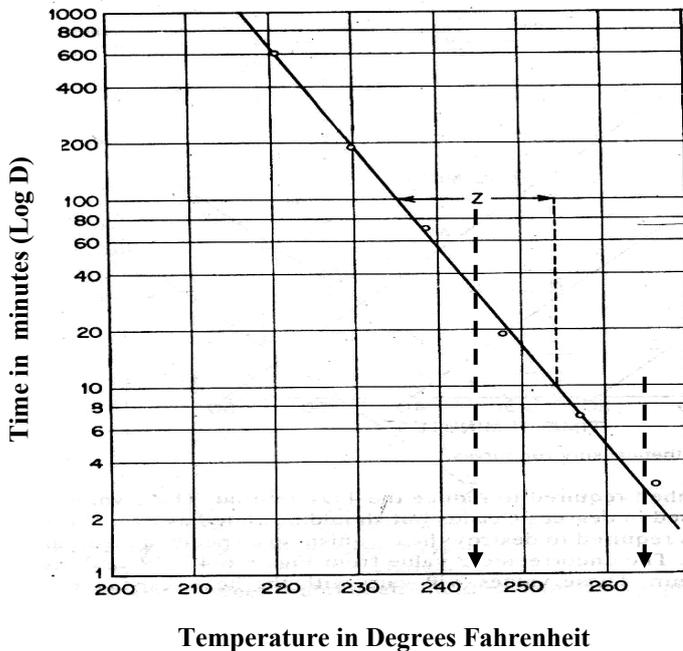


Figure 5.2: TDT curve for spores of flat sour bacteria; 115,000 spores per ml in corn at pH 6.1 ( $z = 19$ ).

If we are interested to process the food item so that it may be free from any spore or microorganisms, first we have to calculate  $D$ ,  $Z$  and  $F$  values.  $F$  is the time in minutes required to destroy the organism in a specified medium at  $121^\circ\text{C}$ . These values vary with the heat resistance and concentration of the test organisms and with the medium in which it is heated. From the  $Z$  and  $F$  values process times can be calculated.

#### 5.7.4 12D Concept

Canned foods are susceptible to the spores of the organism *Clostridium botulinum*, this organism causes botulism. As a safety measure, the canning industry use the 12D heat treatment for low acid foods. In this process enough heat is provided to reduce  $10^{12}$  spores of *C. botulinum* to 1 spores per ml. It can be explained as follows.

Assuming that  $D$  has a value of 0.21 minutes for spores of *C. botulinum* at  $121^\circ\text{C}$  and that out of 12 cans of food contains 1 spore. A heat process at  $121^\circ\text{C}$  for 2.52 min would reduce the spores to 1 spore in  $10^{12}$  cans. The value of 2.52 min has been arrived by the following formula:

$$\begin{aligned} F_0 &= D_{121} (\log a - \log b) \\ &= 0.21 (\log 1 - \log 10^{-12}) \\ &= 0.21 \times 12 \\ &= 2.52 \end{aligned}$$

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## 5.8 DETERMINATION OF PROCESS LETHALITY REQUIREMENTS AT LOW AND HIGH TEMPERATURE

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### 5.8.1 Heat Penetration

In heat/thermal processing of foods, the ratio of penetration of heat into a food is very important, because every part of food in a container must get adequate heat to prevent spoilage. The part which heats most slowly is the critical one; and this is near the centre of container. Solids are heated by conduction while liquids by convection. Foods which are semisolid are heated by combination of both conduction as well as convection. Conduction is slow in foods and rapid in metals.

The factor that determine the time required to bring the centre of the container of food upto the sterilizing temperature are as follows:

**i) Material of which the container is made**

Glass has slower rate of penetration than metal (tin can).

**ii) Size and shape of container**

Large container takes long time than small container. Less the radius of container faster the heating. For example: long slim cylinder will heat faster than compact wide cylinder of the same volume.

**iii) Initial temperature of food**

For the foods with the higher initial temperature the average temperature during heating is higher than in foods having lower initial temperature. A high initial temperature is important in processing canned foods that heat slowly. For example: pumpkin, cream style corn etc.

**iv) Initial temperature of retort (steam sterilizer)**

Fastest heating takes place in initially hot retort than retort having initially low temperature. Therefore, preheated retort should be used.

**v) Consistency of food, size and shape**

Consistency of food, size of food pieces and even their shapes affect the penetration of heat. The changes that takes place during heating (cooking effect) also affect the heat penetration. Penetration of heat in large pieces takes more time than in small pieces. This is applicable in foods, which retain their shape and even size during heating. For example: peas, plum, beats, whole grain corn etc.

Some foods become mushy or viscous during heating. In such foods penetration is slow. For example: Sweet potatoes, pumpkin, etc.

Pieces that layer like asparagus layers or spinach layers interfere with convection current

Sauces added on baked beans slow down heat penetration more than plain sauce. Starch interferes increasingly as the concentration is raised. Sodium chloride is never added in high concentration as it slows down rate of heating. Rate of heat penetration also decreases with increasing

concentration of sugars; however this effect is counteracted some what by the marked decrease in the viscosity of sugar solutions. (*Addition of sugar and salt slow down the rate of heating*)

#### vi) Rotation and agitation

The rotation and agitation of the container of food during heat processing hasten heat penetration, if food is in form of fluid. However, in some food such operation may cause undesirable physical changes.

### 5.8.2 Cooling of Food after Heat Processing

The cooling operation involves the same principles of heat transfer as the heating process. Rapid artificial cooling is recommended as slow cooling may cause overcooking of the food and may allow the growth of thermophiles.

### 5.8.3 Determination of Thermal Processes

To determine the thermal processes data on the following two aspects are required:

- i) TDT curve for most heat-resistant organism likely to be present in food. For example in low acid foods spores of *Bacillus coagulans* (flat sour organism), which is a thermophile, may be present.
- ii) Heat penetration and cooling curves for the food when packed in specific type of container of fixed size.

There are three methods to determine thermal processes:

- Graphical methods
- Formula method
- Nomogram method

The principle is similar for all the three methods; however the graphical method is most simple and therefore explained here:

#### Graphical method to determine thermal processes

1. The TDT curve for the most heat resistance organism likely to be encountered is determined in food being canned.

TDTs from this curve are converted to **lethal rates** for the various heating temperatures. The lethal rate for a temperature is the reciprocal of the TDT. If TDT is 400 min at 126.7°C to kill the spores in a food, the lethal rate would be 1/400 i.e. 0.0025.

2. Heat penetration and cooling curve for the food and can size involved are determined.
3. The lethal rates for different temperatures at the centre of can during the length of heating and cooling process are plotted on the heat penetration or cooling curves (see Fig. 5.3). In this figure the lethal rates are 0.01 units and times are 10 minutes for a square. An area equivalent to 10 squares under the lethality curve is unity. This means that the destruction of all the spores or cells has been accomplished. If this area is less than unity (i.e. less than 10 squares), the process is inadequate, and if more than 10 squares, it is greater than needed. The area beneath the curve is

measured by planimeter. In Figure 5.3 the heat treatment of 56 min at 126.7°C and 78 min at 121°C are adequate.

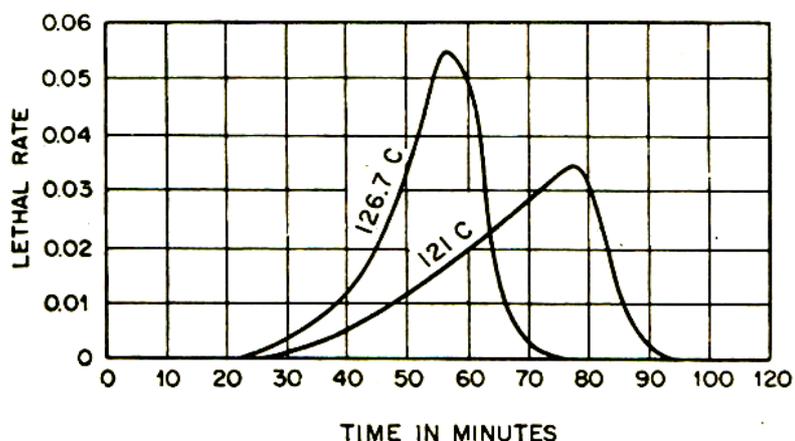


Figure 5.3: Equivalent lethality curves with retort at 126.7°C. (50,000 spores per ml)

## 5.9 BEHAVIOUR OF MICROORGANISMS UNDER FREEZING AND REFRIGERATION ENVIRONMENTS

Low temperatures are used to retard chemical reactions, and actions of food enzymes and to reduce slow down or stop growth and activity of microorganisms in food. The lower the temperature, the slower will be the chemical reactions, enzyme and microbial growth; and temperature below certain level will prevent the growth of a microorganism. Each microorganism has a specific **Cardinal temperature** i.e. the minimal temperature and maximal temperature at which it can grow and optimal temperature at which the growth is fastest in the shortest time. As the temperature drops below the optimal temperature towards the minimal, the rate of growth of organism decreases and is slowest at the minimal temperature. Below minimal temperature, the growth will stop but slow metabolic activity may continue. Therefore cooling down of a food from normal temperature has different effects on various microorganisms and slow down the growth of others; however the extent would vary with different species and even strains of microorganisms. A further decrease of 10°C would stop growth of more organisms, and make still slower the growth of the others. Therefore storage at low temperature influence the type of spoilage microorganisms which may predominate as illustrated in Table 5.5.

Table 5.5: Growth rate of *Pseudomonas fragii* at various temperatures

Temperature (°C)	Exponential growth rate (generation h <sup>-1</sup> )
0.0	0.09
2.5	0.13
5.0	0.20
7.5	0.29
10.0	0.38
20.0	0.92

### 5.9.1 Growth of Microorganisms at Low Temperature

In general, freezing prevent the growth of most food born microorganisms and refrigeration slow down growth rates except for *Clostridium botulinum* type E. Temperature below 5-6°C or less, effectively retard the growth of most food spoilage microorganisms. However, some of the microorganisms may survive at subfreezing temperature in frozen food. (Table 5.6 and 5.7)

**Table 5.6: Microorganisms able to grow at subfreezing temperatures**

Organisms	Temperature (°C)
Molds	
<i>Cladosporium</i>	-6.7
<i>Sporotrichum</i>	-6.7
<i>Penicellium</i>	-4.0
<i>Monilia</i>	-4.0
Yeast	
<i>Yeast (one strain)</i>	-34.0
<i>Yeast (two strains)</i>	-18.0
Bacteria	-5.0 to -17.8

**Table 5.7: Different microorganisms able to grow in different frozen foods**

Organisms	Food	Temperature (°C)
Bacteria	Meat	- 5.0
	Cured meats	-10.0
	Fish	-11.0
	Vegetables	-12.2
	Ice cream	-10.0
Yeast	Meat	- 5.0
	Oysters	-17.8
Molds	Meat	- 7.8
	Vegetables	- 7.8
	Barries	- 6.7

### 5.9.2 Effect of Freezing and Subfreezing Temperature on Microorganisms

Freezing usually results in a considerable reduction in the number of viable organisms in a food. The reduction in recoverable numbers can be the result of lethal or sublethal effects.

#### a) Lethal effects

Though several cells of microorganisms are killed by freezing, a few may remain viable with little or suspended metabolic activity. The lethal effects

are due to denaturation or flocculation of essential cell proteins or enzymes possibly as a result of the increased concentration of solutes in the unfrozen water or perhaps in part because of physical damage by ice crystal. Rapid cooling of cells from an optimal temperature to 0°C is most injurious and may lead to cell death due to cold shock. It is probably due to crystallization of the liquids in the membrane which damage to the permeability of the cell or due to the release of repair enzyme inhibitors, e.g. **ribonuclease inhibitors**.

### b) Sublethal effects

Freezing of food may cause cryo injury to the microorganisms present on food. Such injured cells are referred as freeze injured, frost injured, cryo-injured or metabolically injured. Such cells are not really dead and may recover to start refunctioning, if repair time is permitted or additional nutritional factors are added to the enumeration media. This fact of cryoinjury is of great significance in the microbiological examination of foods.

### 5.9.3 Factor Affecting Microorganisms during Freezing

1. The resistance or sensitivity of microorganisms vary with the kinds of microorganisms, their form and growth phase. For example:
  - a) Thermophiles are most sensitive and psychrophiles most resistance.
  - b) Spore formers are more resistance than non-spore formers.
  - c) Bacteria in logarithmic phase are more sensitive than in stationary phase.
  - d) Rods are more sensitive than cocci.
2. Microorganisms are classified on the basis of sensitivity to freezing:
  - i) **Susceptible:** Gram negative bacteria and vegetative cells of yeast and molds.
  - ii) **Moderately resistant:** Gram positive streptococci and enterococci.
  - iii) **Insensitive:** Spore formers.
3. Freezing parameters:
  - i) **Freezing rate**  
Rapid cooling upto 0°C is injurious to microbial cells.
  - ii) **Freezing temperature**  
Freezing temperature between -4 to -10 is more injurious than -15 to -30°C.
  - iii) **Times of frozen storage**  
Maximum death of microorganisms occur during the freezing process. Once the temperature is stabilized, the death is less and very low.
  - iv) **Kinds of food**  
The composition of food influence the rate of killing of microorganisms during freezing and storage. Sugar, salt, protein,

colloids, fats and other substance in foods provide protection to the microorganisms while low pH and high moisture hasten the death.

v) *Slow defrosting of food cause death* of microorganisms.

vi) *Freezing and thawing*

Maximum casualty of microorganisms occur if foods are repeatedly frozen and thawed.

#### 5.9.4 Effect of Freezing on Constituents of Microbial Cells

With the lowering of temperature, water in cell gets frozen. As a result the unfrozen fluids in cell gets concentrated with solutes (salts, proteins, nucleic acids, etc). This may change the pH of cellular matter, concentrate electrolytes, alter colloidal states, denature proteins and increase viscosity. Ice crystals also form outside the cell due to the freezing of water molecules in food. These extracellular ice crystals draw water from the cell causing dehydration or concentration effect. Intracellular ice crystals due to the freezing of water may rupture cell membrane and alter the permeability of cell membrane. Intracellular ice crystals are more injurious than the extracellular ice crystals to the microbial cells.

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### 5.10 CONTROL OF MICROORGANISMS BY VARIOUS MEANS

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Most foods are either of plants or animal origin. Their spoilage is prevented by controlling microbial growth on them by using various methods. Some important methods are listed below:

1. Asepsis or preventing contacts with microorganisms.
2. Killing the microorganisms by heat treatment.
3. Keeping away from the microorganism e.g. maintenance of anaerobic conditions in sealed or evacuated container.
4. Storage at low or ultra low temperature
5. Drying.
6. Increasing osmotic concentration in foods.
7. Mixing with preservative.
8. Change of pH
9. Mechanical destruction in industry: grinding or high pressure.

Usually a combination of more than one method is used to control the microbial spoilage of food. For example: canned foods are preserved by heat treatment followed by evacuation and sealing of can. Similarly, many processed foods involve heating, mixing with preservative, evacuation of air and sealing.

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### 5.11 PRINCIPLES INVOLVED IN VARIOUS METHODS TO CONTROL MICROBIAL SPOILAGE OF FOOD

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The basic principle involved in various methods to control microbial spoilage of foods are prevention or delay of microbial decomposition. These are achieved by followings methods:

## Controlling Organisms

- a) By keeping out microorganisms. For example: Aseptic condition during processing of food.
- b) By the removal of microorganisms: For example
  - By removing the microbially infected portion of food, covering, skin etc.
  - Washing of raw food.
  - Filtration.
- c) By hindering the growth and activity of microorganisms by low temperature, drying, anaerobic condition or chemical.
- d) By killing the microorganisms: sterilization by heat or radiation.



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## 5.12 LET US SUM UP

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Human foods are excellent substrate for various microorganisms, which are present in air, water, soil, raw foods, on the body of living and non-living organisms. These microorganisms may harm the body if enter in the body/tissue due to rupture or injury of outer covering layer. Many of these microorganisms may enter the food during the processing and remain either dormant for long time till get the right conditions for multiplication, if consumed, cause unpleasant odour or taste making the food unfit for consumption. Such spoilage is called microbial spoilage of food.

Prevention of microbial spoilage of foods depend upon the kind of foods. The acidity or pH of the food influence heat resistance of microorganisms. Microbial cells are more resistance to heat at pH near to neutrality and therefore, low acid foods are heated under pressure to kill the microorganisms, where as the high acid foods are heated upto 100°C for short duration to make free from organisms.

The thermal death time (TDT) which is defined as the time required to kill a known population of microorganisms at a certain temperature by heat. This value differ for different microorganisms and also on various environmental factors. TDT values are of great significance in heat control of microorganisms.

The death of microbial cells is at constant rate and is expressed in *D*-value i.e. decimal reduction time. *D*-values of a microbial species at several temperatures are estimated and plotted against temperature to construct curve, which are used to determine the actual time required for killing microbes in a food at a particular temperature. In practice 12*D* concept is used in canning industry. This is based on the reduction of 10<sup>12</sup> spores ml<sup>-1</sup> of *Clostridium botulinum* in low acid food to 1 spore ml<sup>-1</sup> by heat treatment.

Low temperature under freezing and refrigeration reduce microbial growth and at 0°C most of the microorganisms stop the growth. However a few exceptions of molds, yeast and bacteria, which may grow up to -6 to -38°C have been encountered.

Freezing may have lethal or sub-lethal effect on microbial cell. In sub-lethal effect the freezing injury makes the microbial cell unable to multiply, however under favourable conditions, such cells may get repaired and grow.

The various method for controlling microorganisms are asepsis, heat treatment, anaerobic conditions, storage at low temperature, drying, increasing osmotic concentration, mixing with preservatives, change of pH, irradiation and mechanical destruction by grinding or high pressure. The basic principle involving in these methods are; prevention or delay of microbial decomposition by keeping out or removing the microorganisms or creating suboptimal conditions for survival and growth of microorganisms or by killing the microorganisms.

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**Check Your Progress Exercise 1**



**Note:** a) Use the space below for your answer.  
b) Compare your answers with those given at the end of the unit.

1. What are acid foods?

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2. Based on acidic reaction of food, which foods require more heating time to kill the microorganisms?

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3. Which of the two is more resistant to heat and why? *Escherichia coli* or *Clostridium botulinum*.

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**Controlling Organisms**

4. Define thermal death time.

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5. Define thermal death point.

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6. What do you understand by decimal reduction time?

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7. Why TDT curves are constructed?

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8. What is 12D concept?

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9. Thermal processing of food is affected by the ..... of food.

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10. Which method is most common and simple to determine thermal processes?

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11. Will all microorganisms stop growing, if the food is stored at 0°C?

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12. What is cryoinjury?

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13. Should the food be cooled rapidly to 0°C to kill microbial cells?

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**Controlling Organisms**

14. Intracellular ice cause .....

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15. Microorganisms in food can be controlled by .....

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16. Spores are more resistant to heat than .....

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17. Which of the following microorganisms is insensitive to freezing:

- i) *Escherichia coli*
- ii) *Vibrio cholerae*
- iii) *Clostridium botulinum*

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18. Freezing of food is one of the important method to prevent their spoilage on what principle it is based?

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19. Most microorganisms should be killed but the enzyme should not be destroyed. What method of heat processing should be applied?

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20. If heat treated food is cooled slowly, what kind of microorganisms during storage may grow.

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### 5.13 KEY WORDS

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- TDP** : Thermal death point is the lowest temperature at which all microorganisms in a liquid suspension are killed in 10 minutes.
- TDT** : Thermal death time is defined as the time required, at a given temperature, for heat killing of a population of a single species of microorganism in aqueous suspension.
- D Value** : Decimal reduction time is defined as the time of heating at a temperature to cause 90% reduction in the population of viable cells or spores.
- 12D Concept** : A process in which enough heat is provided to reduce  $10^{12}$  spores of *clostridium botulinum* to 1 spores per ml in canned food.

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### 5.14 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

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#### Check Your Progress Exercise 1

1. Your answer should include the following points:  
Food which have pH between 3.7 and 4.5. Example: pear, pineapple and tomatoes.
2. Your answer should include the following points:  
Low acid foods.

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3. Your answer should include the following points:  
*Clostridium botulinum* as it is spore former.
4. Your answer should include the following points:  
Thermal death time is the minimum time required to kill a population of microorganisms in liquid suspension at certain temperature.
5. Your answer should include the following points:  
Thermal death point is the minimum temperature required to kill population of microorganism in liquid in 10 min.
6. Your answer should include the following points:  
By any killing agent, the death of microorganisms is at a constant rate. Suppose a population of  $10^6$  cells are subjected to heat treatment, 90% of them will die in first minute. Of the remaining population, 90% will die in next one minute and so on until all the population die.
7. Your answer should include the following points:  
TDT curves are constructed to determine the thermal process time at different temperature to kill the microorganisms in a food.
8. Your answer should include the following points:  
It is a concept of time required to reduce  $10^{12}$  spores of *Clostridium botulinum* in 1 ml suspension to 1 spore by heating to certain temperature. It is used in canning industry.
9. Your answer should include the following points:  
Thermal processing is affected by the **consistency** of food.
10. Your answer should include the following points:  
Graphic method.
11. Your answer should include the following points:  
No; psychrophiles will continue growing.
12. Your answer should include the following points:  
Injury to microbial cells during freezing.
13. Your answer should include the following points:  
Yes.
14. Your answer should include the following points:  
Rupture of cell membrane leading to the leakage of cell constituent.
15. Your answer should include the following points:  
Sterilization

16. Your answer should include the following points:  
Vegetative cells
17. Your answer should include the following points:  
*Clostridium botulinum*
18. Your answer should include the following points:  
Reducing or suspending the metabolic activity and growth.
19. Your answer should include the following points:  
Pasteurization
20. Your answer should include the following points:  
Thermophiles

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### **5.15 SOME USEFUL BOOKS**

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1. Adams, M.R. and Moss, M.O. (1995) Food Microbiology. Low cost Indian edition published by New Agro International (P) Limited Publishers, New Delhi 398p.
2. Frazier W.C. and Westhofl D.C. (1967) Food Microbiology. Tata McGraw Hill Publishing Co., New Delhi 540p.
3. Jay, J.M. (1970) Modern Food Microbiology. Van Nostrand Reinhold Co., London. 328p.