HEAT RESISTANCE OF MICROORGANISMS

Heat Resistance of Microorganisms

- Bacteria or its spores subjected to moist heat at lethal temperatures show exponential decrease in their numbers i.e. they exhibit logarithmic order of death.
- The higher the temperature of sterilization, the greater is the rate of thermal destruction.
- For an example the killing of bacteria by exposing them for 10 minutes at 121.1°C is approximately equivalent to 100 minutes at 101°C.

Measures of Heat resistance of Microorganisms

- "Thermal death point" which is the lowest temperature beyond which when heated, the organism ceases to grow or multiply.
- This found limited use in thermal process calculations because killing of bacteria is dependent on time, temperature and number of bacteria.
- Later the concept of "Thermal Death Time(TDT)" was introduced. TDT is defined as the minimum time in minutes required to destroy a given bacterial population at a specified lethal temperature.

Decimal reduction time

- D value which is the time required in minutes to destroy 90% of a given population of bacteria or its spores at a specified temperature.
- Death of vegetative cells or its spores occur exponentially when heated at specified temperature.





- The equation of this graph is t= (log a log b) x D a= Initial number of survivor b= number of survivor at the end of heating for 't' minutes D is the D value
- In the above example a=104 spore i.e., log a=4, log b = 3 duration of heating t=12.5-7.5 min = 5 min. therefore D value is 5=(4-3)xD, i.e. D = 5 minutes.
- The curve is also known as logarithmic survivor curve or simply "Survivor curve" or "Death rate curve"

- The D value of an organism is temperature dependent
- The higher the temperature, the lower is the D value.
- The value is independent on the initial number of bacteria present.
- D value is written with a subscript which denotes the temperature to which D value is estimated e.g. D250 denotes death rate was measured at 250oF.
- D Value (Decimal reduction time at a particular temperature) of organism is defined as time in minutes require to bring down the microbial population to 1/10th of its original number when heated at a particular temperature.

Decimal reduction times (D values) of bacteria

Organism

Bacillus stearothermophilus Clostridium thermosaccharolyticum Cl. nigrificans Cl. botulinum (Type A&B) Cl. sporogenes (P.A.3679) Bacillus coagulans

D Value (minutes at 121.1°C) 4.0 - 5.0 3.0 - 4.02.0 - 3.00.1 - 0.20.1 -1.5 0.01 - 0.07

Time temperature relationship in bacterial destruction and "thermal death time" of bacteria

- The higher the lethal temperature of heating, the shorter is the time required to destroy any given number of bacteria or spores.
- D value gives a good representation of the proportion of number of cells killed in unit time during heating process but the effect of change in temperature of heating cannot ascertained.
- Therefore, to obtain the combined effect of time and temperature on destruction of bacteria/spore, thermal destruction curve or often referred as thermal death time curve is required.

Thermal Death Time (TDT)

- TDT is defined as number of minutes of heating required to destroy a specified number of organisms at a given temperature.
- The thermal death time follows a logarithimic relationship with the temperature of heating.
- Increase or decrease the temperature of heating, the thermal death time for a bacteria will increase or decrease in a logarithimic scale.

Thermal Death Time Data

- Thermal death times are plotted on a log scale against corresponding temperature in linear scale. Alternately, corresponding temperature, both in linear scale.
- The result in either case is a straight line graph called the 'Thermal death time curve' (TDT curve).
- Thermal death time for any temperature not listed in the graph also can be obtained from the curve or an extrapolation of the same.
- A typical TDT curve is presented in Fig.



- When TDT plotted on the log scale and temperature of heating on linear scale, a straight line of best fit is known as thermal destruction curve or thermal death time (TDT) curve. The TDT curve constructed in this manner is called end point curve.
- This curve can be used for finding out the thermal death time for a fixed number of organisms at an unknown temperature.
- TDT values obtained in this manner are valid only for the number and type of organisms used in that particular experiment.

- TDT curves for the same organism present at higher density would be different.
- Instead of using semilog paper, TDT curve can also be plotted on a linear graph paper by taking log of the TDT values on "Y" axis and heating temperature on X axis.
- While D values represent killing of 90% of a population and TDT represent destruction of 100% of the population they are always proportional.

Thermal destruction/ thermal death time (TDT) curve on a semilog paper



• The slope of a TDT curve is indicative of the thermal tolerance of a bacteria. The steeper a TDT curve is, the faster the destruction of the bacteria indicating lower tolerance.

The slope of the TDT curve is defined as the 'z' value.

- Z value is the number of degrees (°F or °C) required for the TDT curve to pass through one log cycle.
- From the curve it can be seen that z is equal to the number of degrees on the temperature scale when the curve traverses one log cycle.
- In other words, z is the change in temperature necessary to cause a ten-fold change in the D value.

Since an increase in the heating temperature causes a decrease in the D value, the z value will be equal to the increase in temperature necessary to effect a ten-fold or decimal decrease in the D value.

• The value of z for *C*. *botulinum* is 10°C by which it is meant that for every 10°C change in the temperature there is a tenfold change in its death rate.

- *F* value, originally called the sterilizing value, is used to denote the time in minutes required to kill an organism in a specific medium at 121.1°C, i.e., its thermal death time.
- It is the number of minutes required for the specified number of bacteria to get destroyed at a given temperature (The definition is same as for TDT)
- When the z value of the process is 10°C, *F* is denoted as F_o . F_o is equal to the integrated lethal value of heat received by all points in a container.

- The total sterilizing effect of a thermal process can be considered as the sum of all sterilizing effects achieved by all the time-temperature combinations throughout the entire thermal process at all points inside the can.
- By convention, sterilization effect is expressed in standard units at 121.1°C so that the entire processing cycle is expressed as being equivalent to holding the product at 121.1°C for a given time.
- The unit of sterilization is F_o, which can be defined as the integrated lethal value of heat received by all points inside a continer during processing.
- *F*_o value of 1 is equivalent to holding the product at 121.1°C for one minute assuming instantaneous heating and cooling.

- F_o value of Clostridium botulinum is 2.4 minutes.
- If the sterilizing value of thermal process in terms of F_o value is
 2.4 min, it means the most feared organism i.e. *Cl. botulinum* is destroyed.
- This thermal process is known as minimum botulinum cook which is essential for a low acid food can.
- While minimum botulinum cook indicates safety from botulinum food poisoning, in most cases, minimum, it is not sufficient to destroy spoilage organisms especially in low acid foods.
- A much higher process say $F_o = 5$ to 6 is required for fish cans to destroy the spoilage organisms.

'12 D' Concept

- '12 D' concept is a method of expressing process lethality requirements.
- To determine the extent to which the canned foods must be processed, the probability of survival of bacterial spores is taken into account.
- The bacteria of significance in low acid foods which cover most of canned fish products, as well as medium acid foods, is *C. botulinum* because of its ability to produce a deadly lethal toxin in the food.

- Therefore, the modern canning practice demands a reduction in the *C. botulinum* spores by a factor of 10¹² in such foods.
- This means that the probability of survival of spores of *C. botulinum* must be reduced to one can in 10¹² i.e., one in million million cans.
- D value of *C. botulinum* spores as measured from the survival curve obtained on heating at 121.1°C is 0.21.
- Considering an initial loading of one spore of *C*. *botulinum* per can and that this has to be reduced by a factor of 10^{12} , the heating time required at 121.1° C is $0.21 \times 12 = 2.52$ min.