STERILIZATION

- ✓ The term "sterilization" refers to the achievement of commercial sterility.
- ✓ It is defined as a condition where microorganisms that cause illness, and those capable of growing in the food under normal non-refrigerated storage and distribution, are eliminated.
- ✓ A sterile product is one in which no viable microorganisms are present.
    - a viable organisms being one that is able to reproduce when exposed to conditions optimum for its growth.
- ✓ Temperatures slightly above the maximum for bacterial growth result in the death of vegetative bacterial cells, whereas bacterial spores can survive much higher temperatures.
- ✓ Since bacterial spores are far more heat resistant than are vegetative cells, they are of primary concern in most sterilization processes.
- ✓ There could be some dormant nonpathogenic microorganisms in the food (any pathogenic microorganisms are dead) but that environmental conditions are such that the organisms do not reproduce.
- ✓ The thermal conditions needed to produce commercial sterility depend on many factors include,
    - nature of the food (e.g., pH),
    - storage conditions of the food following the thermal process,
    - heat resistance of the microorganisms or spore,
    - heat transfer characteristics of the food, its container, and the heating medium
    - initial load of microorganisms.
- ✓ The food being heated affects not only the type of organisms on which the process should be based but also the resistance of the organism to heat energy.
- ✓ Food that is commercially sterile is generally present in a hermetically (gas-tight) sealed container to prevent recontamination.
- ✓ Since low oxygen levels are purposely achieved in most of these containers, microorganisms that require oxygen (obligate aerobes;which cannot use oxygen for growth and are even harmed by it) are unable to grow sufficiently to pose spoilage or health problems.
- ✓ Also spores of most obligate aerobes are less heat resistant than are spores of organisms that are capable of growing under anaerobic conditions(facultative anaerobes, which can grow without oxygen but can utilize oxygen if it is present. or obligate anaerobes)

- ✓ However, in canned, cured, meat products in conjunction with other preservative methods (curing agents and/or refrigeration), such aerobes as *Bacillus subtilis* and *Bacillus mycoides* have been found to cause spoilage.
- ✓ For processes based on inactivation of facultative or obligate anaerobes, pH of the food is a critical factor.
- ✓ There are some extremely heat-resistant spores that might survive a commercial process, but due to the low pH of the food they do not constitute a health or spoilage hazard.
- ✓ For thermal process design, foods are divided into three pH groups:
- ✓ high-acids foods with pH < 3.7,
- ✓ acid foods with 3.7 < pH < 4.5, and
- ✓ low-acid foods with pH > 4.5.
- ✓ Since spore-forming bacteria do not grow at pH values less than 3.7, heat processes for high-acid foods are generally based on inactivation of yeasts or molds.
- ✓ The pH value 4.5, which represents the dividing line between acid and low-acid foods was carefully chosen to be slightly lower than the pH at which strains of *Clostridium botulinum* can grow and produce toxin.
- ✓ *Clostridium botulinium* is an obligate anaerobe which is widely distributed in nature and is assumed to be present on all products intended for canning.
- ✓ For low-acid canned foods the anerobic conditions that prevail are ideal for growth of and toxin production by *C. botulinum*. This organism is also the most heat-resitant, anerobic, spore-forming pathogen that can grow in low-acid canned foods, and consequently its destruction is the criterion for successful heat processing of this type of product.
- ✓ There are several important strains of *C. botulinum* which produce toxin, is extremely potent (one millionth of a gram will kill man) but can be destroyed by exposing it to moist heat for 10 min at 100°C.
- ✓ There exists a nontoxic obligate anaerobic spore former that is significantly more resistant to heat than *C. botulinum*, and it is used to determine safe thermal processes for low-acid foods. This mesophilic organism, identified only as putrefactive(causing rot) anaerobe (PA) 3679, resembles *Clostridium sporogenes*. Fortunately, contamination of food with the more heat-resistant strains of PA 3679 is minimal. PA 3679 instead of *C. botulinum* is used to determine process adequacy for low acid foods because it is nontoxic, is easy to assay, and has suitable heat resistance.

- ✓ In low-acids foods, processing could be based on inactivation of spores more heat resistant than those of *C. botulinum* since such spores do exist. Of greatest importance are facultative anaerobes such as *Bacillus stearothermophilus*. Spores of this organism are extremely heat resistant (up to 20 times more resistant than *C. botulinum* spores). Growth of these spores result in "flat sour" spoilage because acid is produced but little or no gas. *Bacillus stearothermophilus* is sometimes referred to as FS (flat sour) 1518. Optimum growth temperatures for these flat sour thermophiles vary from about 49 to 55°C. Fortunately, most do not grow at temperatures below about 38°C. In the commercial processing of canned foods these types of microorganisms can be ignored provided the product is quickly cooled and stored below 43°C. This causes the organisms to remain in a dormant state.
- ✓ For acid foods, thermal processes are usually base on facultative anaerobes, such as *Bacillus coagulans (B. thermoacidurans), B. mascerans,* and *B. polymixa*. There are several obligate anaerobes that are important in food spoilage, but they are less heat resistant than the *Bacillus* organisms. For tomatoes and tomato products, destruction of *B. coagulans* is the basis of the thermal process.
- ✓ There are two basic methods used to obtain commercial sterility in foods:
  1) heating the food after it has been placed in the container and
  2) heating and cooling the food and then packaging it aseptically.
- ✓ The heating in container is the conventional canning method and is, in principle, the same method that was used by Appert, the Frenchman who invented canning (hence canning is also called as *Appertizing*). Heating, cooling and then packaging is known as aseptic canning.

D value

The preservative effect of heat processing is due to the denaturation of proteins, which destroys enzyme activity and enzyme-controlled metabolism in micro-organisms.

The rate of destruction is a first-order reaction; when food is heated to a temperature that is high enough to destroy contaminating micro-organisms, the same percentage die in a given time interval regardless of the numbers present initially.

This is known as the logarithmic order of death and is described by a death rate curve. The time needed to destroy 90% of the micro-organisms (to reduce their numbers by a factor of 10) is referred to as the decimal reduction time or D value.

D value differs for different microbial species and a higher D value indicates greater heat resistance.

Higher the number of micro-organisms present in a raw material, the longer time it takes to reduce the numbers to a specific level.

Commercially, each batch has different quantity and it is difficult to recalculate process times for each batch.

Hence, a specific temperature- time combination is used to process every batch of a particular product.

Microbial destruction takes place logarithmically; it is theoretically possible to destroy all cells only after heating for an infinite time.

The destruction of micro-organisms is temperature dependent; cells die more rapidly at higher temperatures. By bring together D values at different temperatures; a thermal death time (TDT) curve is constructed.

Z value

The slope of the TDT curve is termed the z value and is defined as the number of degrees Celsius required to bring about a ten-fold change in decimal reduction time.

The D value and z value are used to characterize the heat resistance of an enzyme, a micro-organism or a chemical component of a food.

F value

The F value is used as a basis for comparing heat sterilization procedures. It represents the total time-temperature combination received by a food and is quoted with suffixes indicating the retort temperature and the z value of the target micro-organism. For example, a process operating at 115^{o}C based on a micro-organism with a z value of 10 ^{o}C would be expressed as

$$F_{115}^{10}$$

The F value may also be thought of as the time needed to reduce microbial numbers by a multiple of the D value. It is found using

$$F = D(\log n_1 - \log n_2)$$

Where n_1 is the initial number of micro-organisms and n_2 the final number of micro-organisms.

Inactivation of microrganisms and enzymes

The heat resistance of microorgansims runs from high (spore-forming bacteria) to relatively low (vegetative cells). Some enzymes can be as heat resistant as the spore-forming bacteria.

The kinetics of thermal inactivation usually follows a first-order chemical reaction, although the mechanism may be more complex. At a given temperature the rate of inactivation of a population (N) of microorganisms is given by

$$-\frac{dN}{dt} = kN$$

k is the first-order rate constant for microbial inactivation. Rate constant has units of reciprocal time and is the slope of a plot of ln(C) against time. C is the concentration of reactant. Integrating above equation and using the initial condition, $N = N_0$ at $t = 0$

$$\ln\left(\frac{N}{N_0}\right) = -kt$$

Equation suggests a linear semi-logarithmic plot of N against t. It expressed in common logarithms is

$$2.303\log\left(\frac{N}{N_0}\right) = -kt$$

$$\log\left(\frac{N}{N_0}\right) = \frac{-kt}{2.303}$$

or

$$\log\left(\frac{N}{N_0}\right) = -\frac{1}{D}$$

Above equation defines D-value, the decimal reduction time expressed usually in minutes.

The D value is the time (min) required for per cent reduction (one log cycle) of a population. It is determined as the inverse of the negative slope of a semi log survivor plot of $\log(N/N_0)$ versus time (t).

The inactivation of microorganisms is determined by measuring the survivors, i.e., the microorganisms that can grow in a standard microbiological medium. The D value characterizes the heat resistance of a microorganism at a given temperature. Equation can be used for the heat inactivation of spoilage enzymes and the determination of the corresponding D values at a given temperature.

Commercial sterility implies the inactivation of all microorganisms that endanger public health to a very low probability of survival. For canned foods, the critical organism is

Clostridium botulinum. The 12D concept as a minimal process for inactivation of *C. botulinum* in canned foods is accepted in principle by regulatory agencies and the food industry. However, its interpretation has undergone a process of evolution, from a literal 12 decimal reduction, to what is now generally accepted as a probability of survival of 10^{-12}.

The latter interpretation signifies a dependence of minimum processes according to the 12D concept on initial spore loads. Thus, packaging materials that have very low spore loads will not require as severe a process as products such as mushrooms which may have very high spore levels.

Spoilage from microorganisms that pose no danger to public health is called *economic spoilage*.

If t_1 and t_2 are the heating time and N_1 and N_2 are the respective number of survivors, then using the partial sterilization technique, the D value is determined by :

$$D = \frac{t_2 - t_1}{\log(N_1) - \log(N_2)}$$

The z value had its origins in thermobacteriology and was used to represent the temperature dependence of microbial inactivation rate. z was defined as the temperature change needed to change microbial inactivation rate by a factor of 10. The z value has also been used to express the temperature dependence of degradative reactions occuring in foods during processing and storage. The z value expressed in terms of the raction rate constant is as follows:

$$k_2 = k_1(10)^{(T_2 - T_1)}$$

Taking the logarithm:

$$\ln\left[\frac{k_2}{k_1}\right] = \frac{T_2 - T_1}{z}\ln(10)$$

The Arrhenius model and thermal death time (TDT) model can be used to develop mathematical models for thermal process calculations.

The activated complex theory for chemical reaction rates is the basis for the Arrhenius equation which relates reaction rate constants to the absolute temperture. The Arrhenius equation is

$$k = A_0 e^{-E_a/RT}$$

Where,

k is the reaction rate constant (1/min)

A_0 is the rate constant, the frequency factor (1/min)

E_a = the activation energy (cal/mol)

R = the gas constant (1.987 cal/K mol)

T = the absolute temperature (K)

The frequency factors can be evaluated by letting the reaction rate constant k_1 at temperature T_1. Then,

$$A_0 = k_1 e^{-E_a/RT_1}$$

Substituting the value of A_0 in equation for k and taking the logarithm yields

$$\log\frac{k}{k_1} = \frac{-E_a}{2.303R}\left(\frac{1}{T} - \frac{1}{T_1}\right) = \frac{-E_a}{2.303R}\left(\frac{T_1 - T}{T_1 T}\right)$$

The thermal death time (TDT) is required to obtain the specified inactivation (reduction of the microorganism population) is a multiple of the decimal reduction time (D), e.g., TDT=12D for the inactivation of spores of toxic anaerobic bacterium *C. botulinum*. For microbial inactivation the thermal deat time is given the symbol F. Since F value is dependent on temperature and is specific for one organism, it is usually identified with superscropt denoting the z value of the organism and a subscript denoting the temperature (^{o}F). In the thermal processing of low-acid foods (pH>4.5), a reference temperature of T_0 = 121 ^{o}C is normally used. The F value (usually minute) at a given temperature is converted to the equivalent F_0 at the reference temperature by the TDT equation

$$\log\left(\frac{F_0}{F}\right) = \frac{(T - T_0)}{z}$$

The Q_{10} value of a reaction is often used for reporting temperature dependence of biological reactions. It is defined as the number of times a reaction rate changes with a 10°C change in temperature. If a reaction rate doubles with a 10°C change in temperature, the Q_{10} = 2. In terms of D value it can be presented as,

$$Q_{10} = \frac{D_T}{D_{T+10}}$$

In terms of z value it can be presented as

$$\log Q_{10} = \frac{10}{z}$$

Thus, for the usual value of z = 10, the ratio Q_{10} = 10, i.e., the decimal reduction time (D) decreases 10 –fold when the temperature is increased by 10 °C.