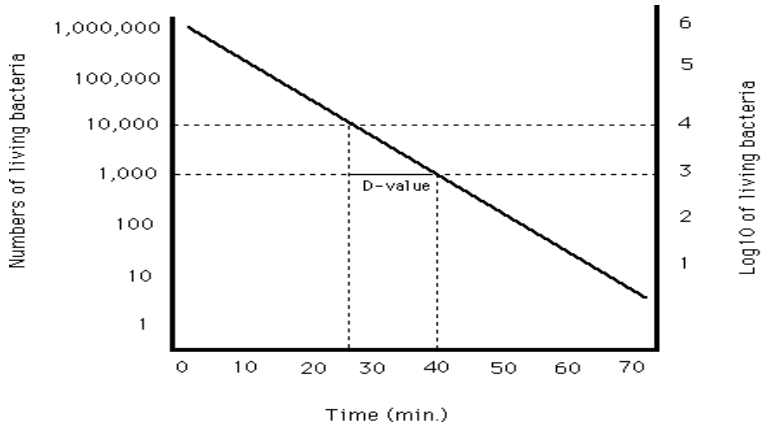
Thermal Destruction of Microorganisms

Heat is lethal to microorganisms, but each species has its own particular heat tolerance. During a thermal destruction process, such as pasteurization, the rate of destruction is logarithmic, as is their rate of growth. Thus bacteria subjected to heat are killed at a rate that is proportional to the number of organisms present. The process is dependent both on the temperature of exposure and the time required at this temperature to accomplish to desired rate of destruction. Thermal calculations thus involve the need for knowledge of the concentration of microorganisms to be destroyed, the acceptable concentration of microorganisms that can remain behind (spoilage organisms, for example, but not pathogens), the thermal resistance of the target microorganisms (the most heat tolerant ones), and the temperature time relationship required for destruction of the target organisms.

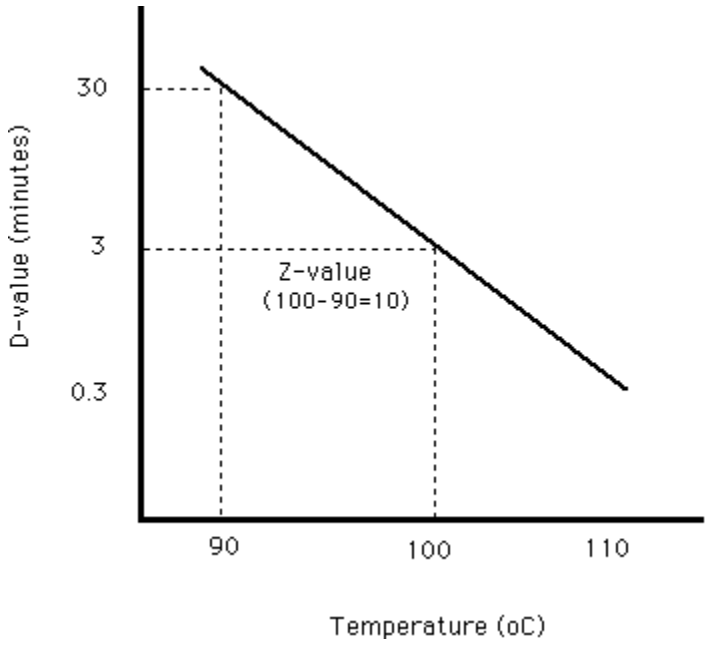
The extent of the pasteurization treatment required is determined by the heat resistance of the most heat-resistant enzyme or microorganism in the food. For example, milk pasteurization historically was based on ***Mycobacterium tuberculosis*** and ***Coxiella burnetti***, but with the recognition of each new pathogen, the required time temperature relationships are continuously being examined.

A thermal death curve for this process is shown below. It is a logarithmic process, meaning that in a given time interval and at a given temperature, the same percentage of the bacterial population will be destroyed regardless of the population present. For example, if the time required to destroy one log cycle or 90% is known, and the desired thermal reduction has been decided (for example, 12 log cycles), then the time required can be calculated. If the number of microorganisms in the food increases, the heating time required to process the product will also be increased to bring the population down to an acceptable level. The heat process for pasteurization is usually based on a 12 D concept, or a 12 log cycle reduction in the numbers of this organism.

Several parameters help us to do thermal calculations and define the rate of thermal lethality. The D value is a measure of the heat resistance of a microorganism. It is the time in minutes at a given temperature required to destroy 1 log cycle (90%) of the target microorganism. (Of course, in an actual process, all others that are less heat tolerant are destroyed to a greater extent). For example, a D value at 72°C of 1 minute means that for each minute of processing at 72°C the bacteria population of the target microorganism will be reduced by 90%. In the illustration below, the D value is 14 minutes (40-26) and would be representative of a process at 72°C.



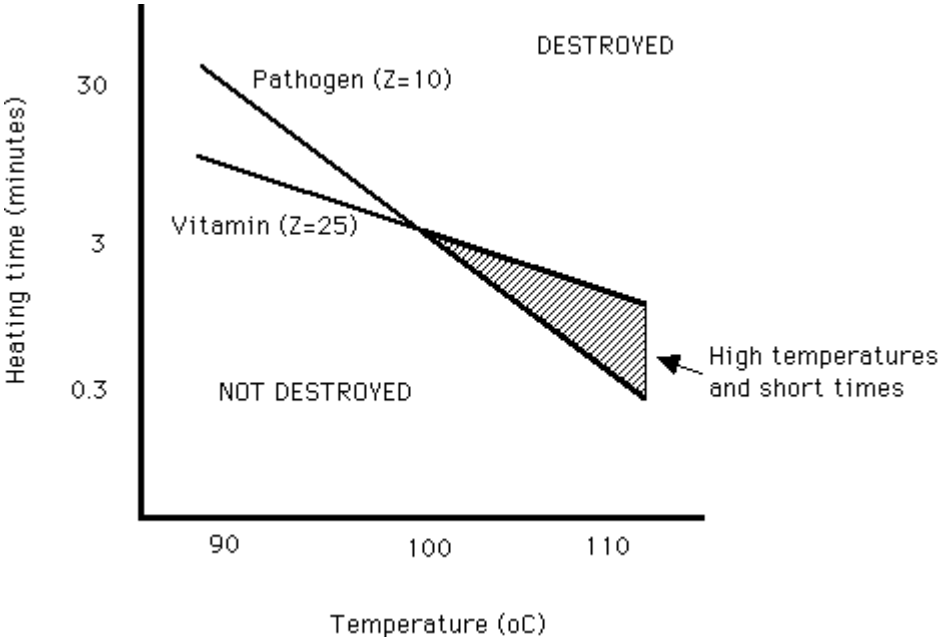
The Z value reflects the temperature dependence of the reaction. It is defined as the temperature change required to change the D value by a factor of 10. In the illustration below the Z value is 10°C.



Reactions that have small Z values are highly temperature dependent, whereas those with large Z values require larger changes in temperature to reduce the time. A Z value of 10°C is typical for a spore forming bacterium. Heat induced chemical changes have much larger Z values that microorganisms, as shown below.

	Z (°C)	D121 (min)
bacteria	5-10	1-5
enzymes	30-40	1-5
vitamins	20-25	150-200
pigments	40-70	15-50

The figure below illustrates the relative changes in time temperature profiles for the destruction of microorganisms. Above and to the right of each line the microorganisms or quality factors would be destroyed, whereas below and to the left of each line, the microorganisms or quality factors would not be destroyed. Due to the differences in Z values, it is apparent that at higher temperatures for shorter times, a region exists (shaded area) where pathogens can be destroyed while vitamins can be maintained. The same holds true for other quality factors such as colour and flavour components. Thus in milk processing the higher temperature, shorter time (HTST) process (72°C/16 sec) is favored compared to a lower temperature longer time (batch or vat) process since it results in a slightly lower loss of vitamins and better sensory quality.



Alkaline phosphatase is a naturally-occurring enzyme in raw milk which has a similar Z value to heat-resistant pathogens. Since the direct estimation of pathogen numbers by microbial methods is expensive and time consuming, a simple test for phosphatase activity is routinely used. If activity is found, it is assumed that either the heat treatment was inadequate or that unpasteurized milk has contaminated the pasteurized product.

A working example of how to use D and Z values in pasteurization calculations:

Pooled raw milk at the processing plant has bacterial population of 4x10exp5/mL. It is to be processed at 79°C for 21 seconds. The average D value at 65°C for the mixed population is 7 min. The Z value is 7°C. How many organisms will be left after pasteurization? What time would be required at 65°C to accomplish the same degree of lethality?

Answer:

At 79°C, the D value has been reduced by two log cycles from that at 65°C since the Z value is 7°C. Hence it is now 0.07 min. The milk is processed for 21/60=0.35 min, so that would accomplish 5 log cycle reductions to 4 organisms/mL. At 65°C, you would need 35 minutes to accomplish a 5D reduction.

STERILIZING DEFINITION and SYMBOLS (D, z, F)

Pasteurization

Pasteurization is one type of preservation by heat that most people are familiar with. This process involves heating a particular food to a certain temperature and keeping that temperature over a specific amount of time to kill the organisms ***Mycobacterium tuberculosis*** and ***Coxiella burnetii***. These two organisms are the most heat resistant of pathogens that are not spore forming. Milk is a product that most people know is pasteurized. There are many different time/temperature combinations that can be used in the pasteurization of milk. The LTLT (low-temperature/long-time) process involves brining the milk to a temperature of 145°F (63°C) for 30 minutes. Conversely, the HTST (high-temperature/short-time) method brings the milk to a temperature of 161°F (72°C) for 15 seconds. Both of these processes accomplish the same thing: the destruction of *Mycobacterium tuberculosis* and *Coxiella burnetii*. So, you can see that not only is temperature important, but the time at that temperature is also important.

Organisms that can survive pasteurization temperature belong to the groups of organisms referred to as thermodurics and thermophiles. Thermoduric organisms are those that can survive high temperature, but dot necessarily grow and reproduce at those temperatures. Thermophiles are organisms that can grow and reproduce at high temperatures. Remember psychrotrophs and psychrophiles.

Sterilization

Some products are referred to as commercially sterile. This means that no viable organisms can be grown from traditional culture methods. In other words, the product should have been subjected to a heat treatment having a sufficiently high lethal effect so that - after incubation at 30°C or 35°C for 5 days - no spoilage occurs and the changes in flavor, odor, color and nutritional value are minimized. In addition to ensuring the destruction of microorganisms, the heat treatment of milk also results in a number of other reactions and changes occurring.

The main changes are:

- Inactivation of enzymes
- Denaturation and complex formation
- Maillard browning reactions
- Losses of vitamins
- Losses of amino acids

Many canned products are referred to in this manner. Time/temperature relationships are different for different products, depending on the types of microbes that are commonly found in the fresh product.

2. DEFINITION OF "STERILE" AND "STERILIZATION"

Sterile

Free from viable micro-organisms

Sterilization

Any physical or chemical process which destroys all life forms, with special regard to microorganisms (including bacteria and sporogenous forms), and inactivates viruses.

Therefore the terms "sterile" and "sterilization", in a strictly biological sense, describe the absence or destruction of all viable micro-organisms. In other words, they are absolute terms: an object or system is either "sterile" or "non-sterile". The destruction of a microbial population subjected to a sterilization process follows a logarithmic progression. Therefore only a treatment of infinite duration provides the absolute certainty that the entire microbial population has been destroyed and that the system is sterile.

Making the characteristics of the sterilization treatment more drastic (i.e. increasing time and/or temperature) usually entails a decay of the qualities of the product and certainly increases process costs. It is therefore agreed that the product is acceptable as sterile when the probability of finding a non-sterile unit in a sterilized batch entails a risk which is lower than the other risks associated with the use of the product itself.

More properly, in the pharmaceutical industry, in order to define a unit as sterile we must be able to certify, on a statistical basis related to the conditions of preparation and sterilization of that specific product and of that specific batch, that less than one unit in a million is exposed to the risk of not being sterile.

The probability of finding a non-sterile unit (PNSU = Probability of Non Sterile Unit) must therefore be lower than 10^{-6}.

UHT Aseptic Technology -- Ultra High Temperature Sterilization

A sterilization process is defined as a UHT (Ultra High Temperature) process, if the product is heat-treated in a continuous flow at a temperature of not less than 135℃ for a very short time, aseptically packaged in sterile containers, and has undergone minimum chemical, physical and Organoleptic changes in relation to the severity of the heat treatment required for sterilization.

Thermal Death Time (TDT)

Thermal death time is the amount of time that is necessary to kill a specific number of microbes at a specific temperature. This value is obtained by keeping temperature constant and measuring the time necessary to kill the amount of cells specified.

Decimal reduction time (D-value)

The D-value, which denotes the decimal reduction time, is the time required at a specific temperature and under specified conditions to reduce a microbial population by one decimal. The decimal reduction time is dependent on the temperature, the type of microorganism and the composition of the medium containing the microorganism.

The term D-value refers to decimal reduction time. This is the amount of time that it takes at a certain temperature to kill 90% of the organisms being studied. Thus after an organism is reduced by 1 D, only 10% of the original organisms remain. The population number has been reduced by one decimal place in the counting scheme. When referring to D values it is proper to give the temperature as a subscript to the D. For example, a hypothetical organism is reduced by 90% after exposure to temperatures of 300F for 2 minutes, Thus the D-value would be written as D_{300F} = 2 minutes.

It is often more convenient to use the D-value as a measure of rate of microbial inactivation. The D-value is the exposure time required for the number of survivors to change by a factor of 10 or the time required to achieve a decrease of one log cycle in the survivor curve [in other words the temperature or radiation dosed required to reduced the initial population by 90% . The D-value may be estimated graphically see graph or mathematically from the equation

$$D = \frac{t}{\log N_0 - \log N_t}$$

The D-value and K are specific for each set of microorganisms and each sterilization process. Thus with data for heat inactivation of microbes the temp is shown D121 ℃. For radiation inactivation the d-value is stated in the terms absorbed dose (kGy).

D-value is the time required to kill 90% of the spores or vegetative cells of a given microorganism at a specific temperature in a specific medium. D-values can be determined from survivor curves when the log of

population is platted against time (Figure TD-1 for a microorganism having a D_{185} = 1.0 minutes), or by the formula:

$D_{\text{reference temperature}} = \text{Time}/(\text{Log}_a - \text{Log}_b)$

Where a = the initial population, and b = the survivors after a time interval

The 12-D Process

Canned foods are susceptible to the spores of the organism *Clostridium botulinum*. This is the organism that causes botulism. These bacterial spores can survive many heat treatment processes. However, in modern food production, canned foods are subjected to a time/temperature process that will reduce the probability of the survival by the most heat-resistant C. botulinum spores by 12 logs or 12-D at 250°F (the temperature used in the calculation of most commercial 12-D processes is 250°F, and the D-value for this organism at 250°F is 0.21 minutes). This process is based on the assumption of the number of surviving spores in one can. If we assume that there are 10 surviving spores in one can, then we can calculate the time for a 12-D process to occur by using the following formula:

- $F_0 = D_{250°F}(\log a - \log b)$, where *a* = initial population and *b* = final population.
- So $F_0 = (0.21\text{min.})(\log 10^1 - \log 10^{-11})$, we move down 12 log values (1 - (-11)) = 12
- So, $F_0 = (0.21\text{min.})(1 - (-11))$, or 0.21 x 12 = 2.52 minutes.

Simply put, (D-value at 250°F) x (12) results in a 12-D process.

The Z-value.

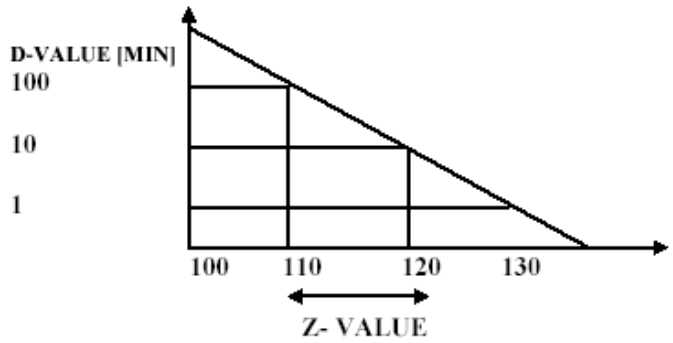
The Z-value is the increase or decrease in temperature required to reduce or increase the decimal reduction time by one decimal. It is a measure of the change in death rate with a change in temperature.

The number of degrees Fahrenheit or Centigrade required for a thermal death time curve to traverse 1 log cycle. This is the temperature increase required to reduce the thermal death time by a factor of 10. The z-value gives an indication of the relative impact of different temperatures on a microorganism, with smaller values indicating greater sensitivity to increasing heat. The z-value is obtained by plotting the logarithms of at least 2 D-values against temperature or by the formula:

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

Where T = temperature and D = D-value

THERMAL RESISTANCE CURVE



$$Z = \frac{T_1 \, . \, T_2}{\log D_1 - \log D_2}$$

The z-value of an organism is the temperature, in degrees Fahrenheit, that is required for the thermal destruction curve to move one log cycle. While the D-value gives us the time needed at a certain temperature to kill an organism, the z-value relates the resistance of an organism to differing temperatures. So, the z-value allows us to calculate a thermal process of equivalency, if we have one D-value and the z-value. So, if it takes an increase of 10°F to move the curve one log, then our z-value is 10. So then, if we have a D-value of 4.5 minutes at 150°F, we can calculate D-values for 160°F by reducing the time by 1 log. So, our new D-value for 160°F is 0.45 minutes. This means that each 10°F increase in temperature will reduce our D-value by 1 log. Conversely, a 10°F decrease in temperature will increase our D-value by 1 log. So, the D-value for a temperature of 140°F would be 45 minutes.

Sterilizing effect or lethality

The sterilizing effect, which is also called lethality or death rate, indicates the effect of a heat treatment, expressed as the number of decimal reductions in the number of microorganisms.

F-value

The F value for a process is the number of minutes required to kill a known population of microorganisms in a given food under specified conditions. This F value is usually set at 12 D values to give a theoretical 12 log cycle reduction of the most heat-resistant species of mesophilic spores in a can of food. For example, if there were 10,000 spores of a species of spore in a can of food and a 12 D process was given, the initial 10,000 spores (10^4 spores) would be reduced to a theoretical 10^{-8} living spores per can, or again in theory, one living spore per 10^8 cans of product (one spore per one hundred million cans). To refer back to the original example where the D 240 was 1 min., the F value for the process would be 12 min. or F 240 = 12 min.

When F is used without a subscript indicating temperature, 250°F is assumed. When the symbol F is used, a z value of 18°F is assumed with an exposure temperature of 250°F. The actual processing time a can of food is given in a retort is always greater than the F value due to heat penetration requirements. Industry makes extensive use of F values in maintaining processes and in developing new schedules. Optimally the old and new processes are equated to acceptable F values. Two different processes are considered equivalent when the processes are equally effective with respect to destruction of a given microorganism.

Marker enzyme:

The degree and rate of inactivation of gamma-glutamyltransferase in raw cow's milk by heating at 50, 60, 70, and 80 degrees C for 1, 2, 3, 5, 10, 15, 20, 25, and 30 min were measured to evaluate the suitability of this enzyme as a marker for the pasteurization of milk. The enzymes alkaline phosphatase and lactate dehydrogenase were also measured under similar conditions for comparison. The patterns of heat inactivation of gamma-glutamyltransferase and alkaline phosphatase were similar, with only a minimal inactivation of the enzymes at 50 degrees C. The rate of inactivation increased as a result of increasing temperatures and time. A complete inactivation of both enzymes was seen at 70 degrees C after 10 min and at 80 degrees C after 1 min. Lactate dehydrogenase showed a higher heat resistance with almost complete inactivation at 70 degrees C for 30 min, and compete inactivation at 80 degrees C for 3 min. No activities of these enzymes were found in commercially pasteurized or heat-treated milk. The levels of gamma-glutamyltransferase in raw milk were between 8 and 10% higher than those of alkaline phosphatase and lactate dehydrogenase, making it more sensitive and accurate as a testing marker. It seems that gamma-glutamyltransferase may serve as a good pasteurization marker. Furthermore, the simplicity of testing and the availability of commercial kits for testing by both wet and dry chemistry make it an attractive choice, especially because dry chemistry procedures overcome the difficulties originating from the turbidity of milk, which interferes with spectrophotometric procedures.