A CRITICAL LOOK AT PASTEURIZATION STANDARDS[1]

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Since microorganisms differ in thermal resistance, the data for a single organism should not serve as a basis for establishing minimum pasteurization standards at all temperatures. Were data for all microorganisms that limit pasteurization available for plotting "safe" minimum-process graphs, the resulting curve would not be a straight line. Until additional thermal resistance data are available, approved "no-hold" and similar high temperature processes should require more severe treatments than those predicted from the limited criteria that have commonly been used to indicate safe treatment at temperatures in the range of vat and HTST pasteurization.

Considerable difficulty is involved in interpreting thermal resistance data in terms of requirements for pasteurization and sterilization. To simplify, let us speak in terms that apply specifically to pasteurization, although the same principles apply to sterilization, because basically pasteurization and sterilization are very nearly the same. In pasteurization we wish to destroy any disease-producing microorganisms that might be present in raw milk and those microorganisms that are most troublesome in causing off flavors and odors, particularly, at refrigeration temperatures. In sterilization we seek to destroy all microorganisms. This includes the very heat resistant spores produced by certain bacteria. The basic difference between the two processes is that the heat-treatments are "aimed" at different microorganisms.

The destruction of microorganisms by thermal processes has been studied over a period of many years. Most of the early studies of "thermal death point" and "thermal death time" were incomplete in certain fundamental aspects (3), and hence seem incomplete as a basis for establishing standards for such processes as "no-hold" pasteurization. However, they did serve in the establishment of our present-day pasteurization standards.

The temperature and time of heating influence the destruction of a microorganism. This is illustrated by the minimum temperature-time relationship for the vat method of pasteurization and that for the HTST method — 143° F. for 30 minutes, and 161° F. for 15 seconds, respectively.

Let us consider the way bacteria die when exposed to a destructive agent such as heat. Let us assume that species X is a pathogen or a microorganism that rapidly produces a defect during storage. We can add a large number of species X to milk at a constant temperature (e.g., 140° F.) and remove 1-ml. samples at various intervals — say 5-minute intervals. Each sample, immediately after it is taken, should be added to a dilution blank containing sterile, buffered ice-water. We then make plate counts to determine the number of species X in each sample. The whole procedure should be repeated several times and average values for the plate counts determined, because we do not want to be misled by errors that might occur in only one trial.

We will now plot the average numbers that we found after each 5-minute interval (Figure 1). For the plot we use a sheet of semi-logarithmic graph paper. Numbers of bacteria are plotted on the vertical, unevenly-spaced scale (the logarithmic scale), and time at 140° F. is plotted across the bottom, on the evenly-spaced arithmetic scale.

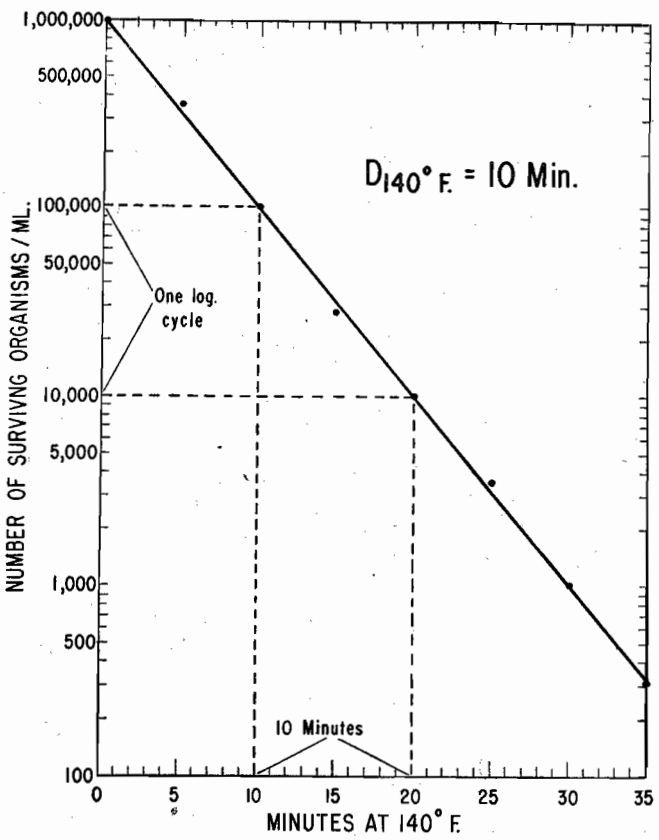

Figure 1. Rate of destruction curve for a hypothetical microorganism, species X.

Notice that a line connecting the average values is

[1]Presented at the Annual Dairy Industry Conference at the University of California, Davis, January 23, 1956.

a straight line. Although the line is straight, it is referred to as a "rate of destruction curve". Most bacteriologists agree that rate of destruction curves are straight lines, except for initial curvature in some cases (4). In our graph we have demonstrated the generally accepted view that, in the presence of a destructive agent such as heat, the order of death of bacteria is logarithmic in nature.

Notice on the curve that we had one million organisms per ml. at 0 minutes. After 10 minutes at 140° F. the plate count was only 100,000 per ml. The heat had destroyed 900,000 or 90 per cent of each original one million. A destruction of 90 per cent is referred to as one log. cycle of destruction. Notice that 90 per cent of the 100,000 were destroyed during the second 10 minutes. The same occurred during the third 10 minutes. Starting at any point on the curve, we find that a destruction of 90 per cent or one log. cycle occurred during 10 minutes. The number of minutes required for a destruction of one log. cycle is called the "D" value. (Schmidt (3) discusses the various symbols that have been used to designate the D value.) We may say that our rate of destruction curve shows that species X has a D_{143} of 10 minutes. Had the numbers of bacteria decreased more rapidly, the slope of the curve would have been steeper, and the D value lower.

The D value for an organism is not constant under all conditions. Several factors that influence rates of destruction are listed below.

Some factors that influence the resistance of a microorganism to treatment at a given temperature.

A. Inherent resistance
   1. Genus and species
   2. Different strains of one species.

B. Environmental influences active during the growth of bacteria that are later to be heat-treated
   1. Growth medium
   2. Growth phase of the bacteria
   3. Temperature of incubation

C. Environmental influences active during the period of heating
   1. pH of the suspension medium
   2. Composition of the suspension medium
      a. Sugar content
      b. Total solids

D. Subculture conditions
   1. pH of medium
   2. Composition of medium
   3. Incubation time

Bacteria vary in inherent resistance. Even the strains of one species may differ in heat-resistance.

Environment is important during the growth of the bacteria to be tested, during the heat-treatment, and during the growth of those organisms still living after heat-treatment. Some of these factors increase the heat-resistance of certain bacteria by as much as 200 to 300 per cent. Think of the difference in pasteurization standards for ice cream mix as compared to milk. A major reason for the difference is the protective action of the sugar in ice cream mix. Certain organisms are much more resistant when grown on an agar medium than when grown in milk. Which would you expect to find more resistant, (a)

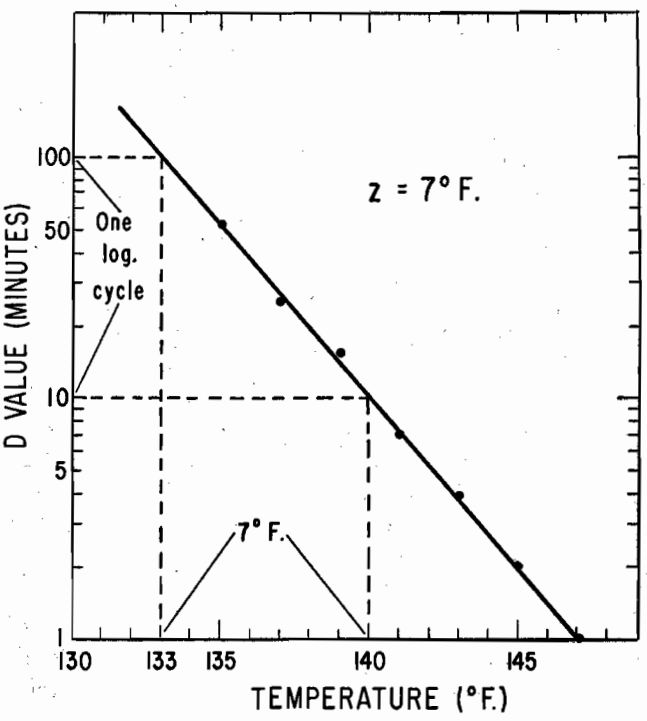

Figure 2. Thermal resistance curve for a hypothetical microorganism, species X.

an organism that is permitted to grow in improperly cooled milk, or (b) the same organism permitted to grow on unclean equipment and then permitted to get into cold milk by contamination? You likely would find the latter more heat resistant. Instances have been observed where supposedly "thermoduric" bacteria were isolated from pasteurized milk and then found not to be thermoduric after they had been permitted to grow in milk.

Let us consider what would be necessary to establish the minimum pasteurization requirements for our hypothetical organism, species X. In Figure 1 we found that the D value at 140° F. was 10 minutes. This one D value alone is not sufficient for our purpose. We must determine D values for species X at several other temperatures.

We will assume that this has been done and that the D values are those given in Table 1. It is not surprising that the D values decrease as the temperature increases, for each D value represents the same thing – the number of minutes required for a destruction of one log. cycle, or 90 per cent, at the given tempera-

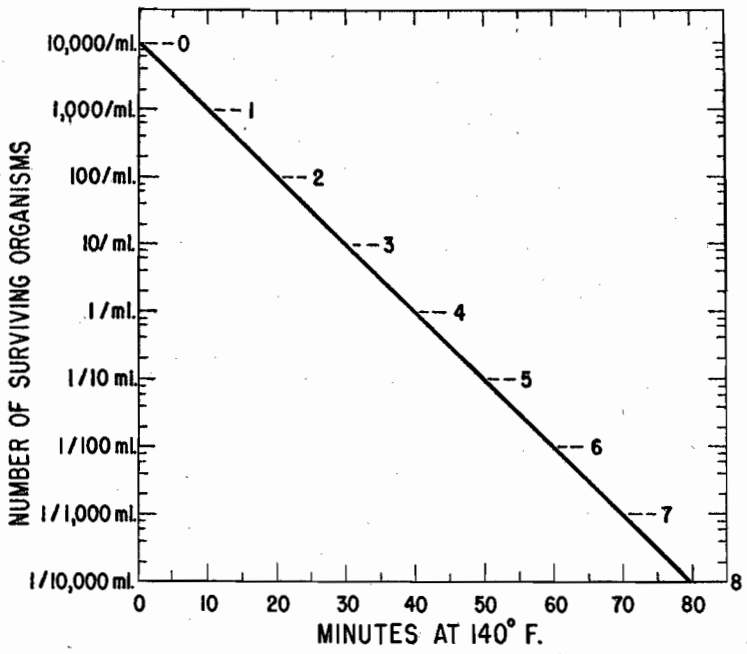

Figure 3. Extrapolated rate of destruction curve for a hypothetical microorganism, species X. If it is assumed that the maximum initial population is 10,000/ml., the arbitrary tolerance of survival is 1/10,000 ml., and the D value at 140° F. is 10 min., the minimum "safe" time at 140° F. can be calculated as follows:
D(log. 10,000 − log. 0.0001)=10 [4.0 − (− 4.0)]=80 min.

ture.

The D values may be plotted on a sheet of graph paper like that used for Figure 1. This time we plot increasing temperature across the bottom on the arithmetic scale, and time on the vertical logarithmic scale (Figure 2). When we draw a line joining the D values, we find that this line also is straight. It is called a thermal resistance curve (3).

Each point on this curve is a D value, representing a temperature and the number of minutes at that temperature required for a destruction of one log. cycle. Any point represents a time-temperature relationship equal in destructive effect on species X to that represented by any other point.

Notice that when the holding time is 100 minutes the temperature is 133° F. When we reduce the time to 10 minutes (e.g., by 90 per cent or one log. cycle), we must add 7 degrees to the temperature. The same is true for any place you start on the curve. If we reduce the time one log. cycle (10-fold) we must increase the temperature 7 degrees; if we increase the time one log. cycle the temperature is decreased 7 degrees. The amount of destruction is the same in all cases. When we express the slope of a thermal resistance curve as the number of degrees necessary to cause a 10-fold change in time, we have the "z" value. For species X the z value is 7 degrees.

For our hypothetical problem we want to know the minimum time-temperature relationships that we can use in pasteurization to destroy species X. We would like our pasteurization processes to destroy every single organism of species X. In the early days of experimental work, it was thought that every single organism could be destroyed. Now we know that it is theoretically impossible.

To demonstrate this point, the rate of destruction curve for species X has been reproduced on a larger sheet of graph paper (Figure 3). Notice that there are 10 organisms per ml. after heating for 30 minutes at 140° F. There is 1 per ml. after 40 minutes, 1 per 10 ml. after 50 minutes, 1 per 100 ml. after 60 minutes, etc. The curve could be extended indefinitely with the volume in which one organism is found getting larger and larger. Theoretically we would never destroy every single organism.

Certain undesirable microorganisms are more undesirable than others. Since "complete destruction" can not be achieved, the undesirability of each microorganism should be carefully considered. For certain pathogens the tolerance of survival might be as low as one organism in many gallons; with certain of the less important spoilage species, several bacteria per milliliter might be tolerated.

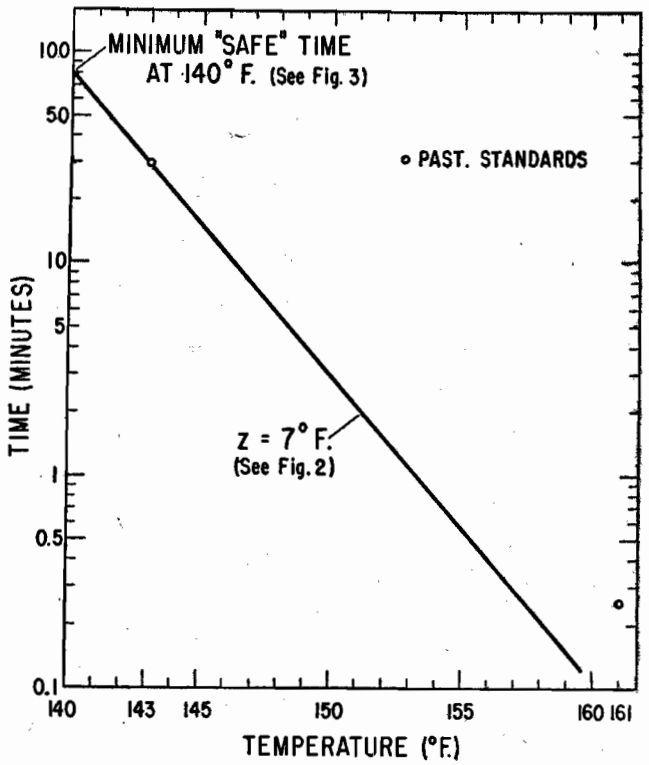

Figure 4. Minimum "safe" process graph for a hypothetical microorganism, species X.

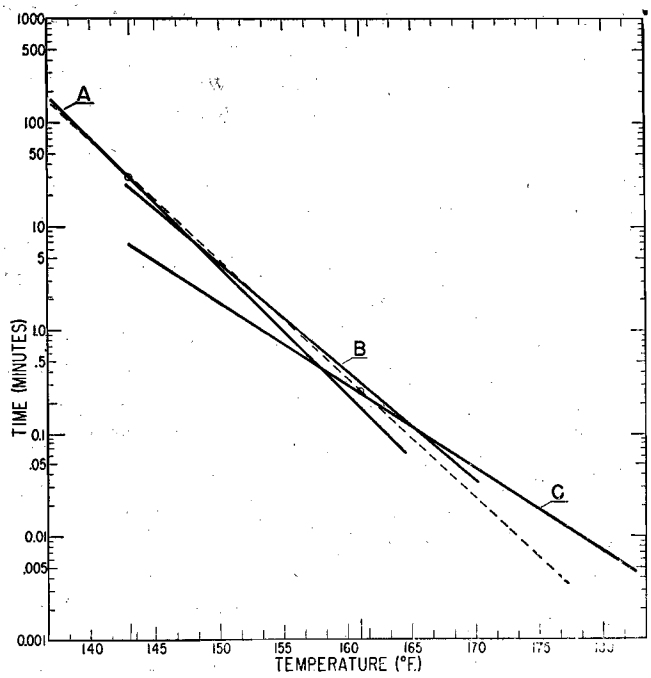

Figure 5. Minimum "safe" process curve limited by three hypothetical microorganisms. The broken line is drawn through the present pasteurization standards and extrapolated for comparison.

The number of species X we will wish to destroy by pasteurization will be determined by two values, (a) the tolerance of survival, and (b) the maximum initial number that might be found in raw milk. The tolerance of survival will be a decision; the maximum initial number that might be found will be an estimate or a determination.

Assume that for species X we decide that one organism per 10 quarts (approximately 10,000 ml.) will be permitted in the pasteurized product. Suppose experiments have shown that the maximum number we might encounter in raw milk is 10,000 per ml. We now can use the graph (Figure 3) for counting the number of log. cycles of destruction that we are going to require in pasteurization. The number is eight, which incidentally is a destruction of 99.999999 per cent of the initial population. It would be practically impossible to accurately determine this amount of destruction by the plate count. We have been able to arrive at the value by assuming that bacteria die according to the logarithmic law.

We found that species X has a D value of 10 minutes at 140° F. — that 10 minutes are required for one log. cycle of destruction. At this same temperature the arbitrarily chosen eight log. cycles of destruction will require 80 minutes. This value is the "safe" minimum time at 140° F.

The "safe" minimum time at 140° F. has been plotted in Figure 4 and a line drawn through it with a slope having a z value of 7 (7° F.). The resulting curve shows the time-temperature relationships that we have judiciously considered as "safe" pasteurization requirements for species X. We will call this curve a "safe" minimum-process graph. Any pasteurization process that employs a time-temperature relationship equal to those shown by this minimum-process graph — or greater — is "safe" with reference to species X. Notice that the present pasteurization standards are plotted on the graph and that they indicate "safe" processes. For species X the HTST method of pasteurization has a greater margin of safety than the vat method.

The minimum-process graph that we have established has significance only for species X. Several different pathogens and several different defect-producing species must be considered before one can safely propose pasteurization standards.

Unfortunately, known data do not permit the drawing of "safe" minimum-process graphs for many of the species that are important in pasteurization. The inadequacy of known data becomes acute when one attempts to use known data in evaluating pasteurization processes that use very short times and temperatures that are higher than those presently used.

It is known that different pathogens and defect-producing bacteria differ in resistance to treatment at low temperatures of about 143° F. This simply means that the D values for different species are not the same. We know that microorganisms react differently to increases in temperature, i.e., the z values are different. In fact, z values have been reported as ranging from about 6.0 or 7.0 up to about 18 or 20 (1). Further, maximum initial numbers are not the same for the many species that might be found in milk. Still further, microorganisms differ in their importance, and therefore the tolerances of survival for different species should not be the same.

The above considerations make it obvious that a single microorganism will not limit pasteurization processes at all temperatures. This is important because the organisms that are considered to limit vat pasteurization might not be the most important species at the temperature of HTST pasteurization and, particularly, at temperatures above those used at present.

Were sufficient data available, one could plot the "safe" minimum-process graphs for all microorganisms that limit pasteurization processes and one could establish a "safe" minimum-process curve. This curve likely would not be a straight line. The hypothetical graphs drawn in Figure 5 show a minimum-process curve limited by only three organisms. The curve is not straight; each organism limits the curve in a different temperature range. The present pasteurization

standards have been placed on the curve to show that a straight line drawn through them and extrapolated to higher temperatures would not appropriately designate "safe" pasteurization processes.

Several experiment stations are investigating standards for "no-hold" pasteurization. Theoretical considerations show that at high temperatures the destructive effects during heating and cooling can be sufficient to accomplish pasteurization without a holding period. Establishing minimum pasteurization requirements for no-hold processes is more difficult than establishing them for the conventional methods.

Practically all of the z values available for the microorganisms important in pasteurization were obtained at relatively low temperatures, about 140 to 150° F. The reason is that experimental difficulties limit the use of a "holding" procedure to low temperatures such as that which we imagined using for species X. Thus, the validity of extrapolating "safe" minimum-process graphs, as was done in Figure 5, has been proved for very few organisms (2).

Although sufficient data are not available at present to establish "safe" minimum treatments for no-hold processes by the procedure outlined above, investigators are aware of the need for additional thermal resistance data, and undoubtedly the needed information will be established.

Certain criteria have commonly been used to indicate safe treatment at temperatures in the range of vat and HTST pasteurization. These criteria are not adequate for the much higher temperatures used for no-hold processes. They include: (a) destruction of phosphatase (which has a z value approximately the same as that for a line drawn through our present pasteurization standards), (b) destruction of test organisms that have z values similar to that of a straight line drawn through the present pasteurization standards, and (c) extrapolation of a straight line drawn through the present pasteurization standards. Until additional thermal resistance data are available, approved no-hold and similar high temperature processes should require more severe treatments than those predicted from the above criteria.

References

1. Ball, C. O. Short time pasteurization of milk. Ind. and Eng. Chem., 35; 71-84. 1943.

2. Collins, E. B., Dunkley, W. L., Perry, R. L., and Edmondson, L. F. Thermal destruction of *Micrococcus freudenreichii* and *Streptococcus thermophilus* with particular reference to pasteurization without holding. J. Applied Microbiol., 4: 133-140. 1956.

3. Schmidt, C. F. Thermal resistance of microorganisms. Chapter 32, *Antiseptics, Disinfectants, Fungicides, and Sterilization.* Lea and Febiger, Philadelphia. 1954.

4. Wyss, O. Chemical factors affecting growth and death, Chapter 6, *Bacterial Physiology.* Academic Press, Inc., New York. 1951.