THERMAL DESTRUCTION OF MICROORGANISMS AND ENZYMES-I

Basic concepts and thermal resistance problems . . . by W. B. ESSELEN and I. J. PFLUG*

SUCCESSFUL preservation of food by thermal processing depends upon the application of sufficient heat to destroy microorganisms that might be of public health significance and microorganisms and enzymes that might cause subsequent spoilage. About 35 years ago, National Canners Association and associated companies initiated an extensive investigation on the thermal resistance of bacterial spores in relation to the processing of canned foods. These investigations have continued to the present day. The large volume of canned and glass-packed foods successfully packed every year attests to the successful application of the results of these investigations. The development of additional kinds of heat-processed foods and the advent of new canning methods such as aseptic canning have pointed out the need for more basic information on the thermal resistance of bacterial spores and factors that influence it. With the advent of quick freezing and the packing of certain heat-processed acid foods, increased attention has been devoted to the thermal destruction of enzymes in such products.

In the present discussion, some of the basic concepts relating to the thermal destruction of microorganisms and enzymes in food processing will be reviewed. Problems involved in the determination of the thermal resistance of bacterial spores will also be discussed.

Logarithmic Destruction Rate

A basic concept and general assumption made by workers in this field is that the death of bacteria and bacterial spores, when exposed to heat, follows a logarithmic course. That is, equal percentages of the surviving cells die in each successive unit of time as illustrated in Figs. 1 and 2. Chick (1910), Bigelow (1921), and others have pointed out that when the experimental data for thermal death times of bacterial spores are plotted on semilogarithmic paper, the resulting curve approximates a straight line. Bigelow, Bohart, Richardson, and Ball (1920) applied such data in the so-called, "General Method" of process calculation for canned foods. A comprehensive treatise of the logarithmic order of death has been presented by Rahn (1945, 1947). According to the logarithmic order, death must be caused by destruction of a single molecule. The bacteriologists define death of bacteria as the loss of power to reproduce. Rahn has defined such death as a lethal mutation, and the assumption was made that heat coagulation of a single essential gene prevents reproduction.

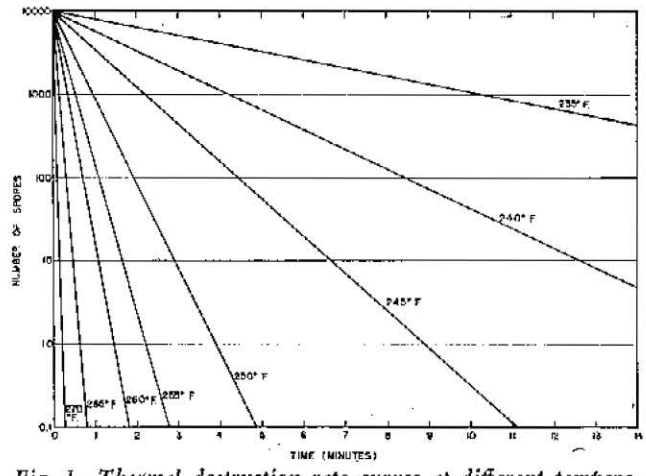

Fig. 1. Thermal destruction rate curves at different temperatures for spores of PA 3679 suspended in neutral phosphate M/15 buffer (10,000 per .01 ml). Average calculated D values.

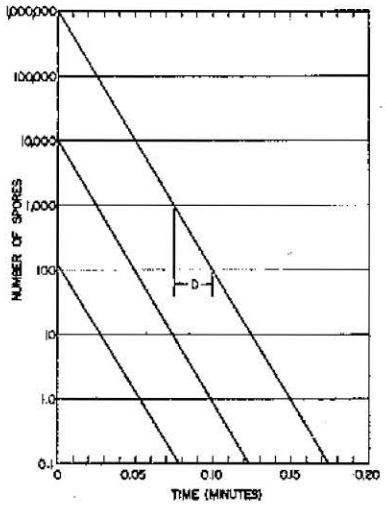

Fig. 2. Thermal destruction rate curves for concentrations of 120, 10,000 and 1,000,000 spores of PA 3679 per 0.01 ml in neutral M/15 phosphate buffer at 280 deg. F.

According to Rahn (1945), "Regardless of whether we accept one or the other explanation of the logarithmic order of death, its existence permits us to compute death rates and to draw conclusions from them which are independent of any explanation. Death rates make it possible to compare the heat resistance of different species at the same temperature, or the heat resistance of one species at different temperatures. It also enables us to describe in quantitative terms the effect of environmental factors, such as concentration of the medium or its pH, upon heat sterilization." The calculation of death rate constants and temperature coefficients of thermal destruction have been discussed in detail by the above author. The death rate was calculated by the following formula:

$$K = 1/t \log \frac{\text{initial number}}{\text{number of survivors}}$$

K represents the death rate constant and t the time in minutes. Rahn observed that the high temperature coefficient (Q_{10}) makes it almost certain that death by heat is a coagulation or denaturation process because such high coefficients are very rare except with protein coagulation.

Ball (1923, 1928) developed elaborate mathematical methods for relating the thermal resistance of microorganisms with the rate of heat penetration in the derivation of process times for canned foods. In these methods, he introduced the terms "F" and "z" for describing the thermal resistance of microorganisms in place of death

* Professors Esselen and Pflug are on the food technology faculty of the University of Massachusetts. This paper, Contribution 930 of the Massachusetts Agr. Exp. Sta., was delivered, in Boston, Dec. 29, 1953, before the American Association for the Advancement of Science.

rates and temperature coefficients. These symbols were defined as follows:

F value = the time in minutes required to destroy an organism at 250 deg. F.

z value = the slope of the thermal death time curve expressed as the number of deg. Fahrenheit on the temperature scale required for the curve to traverse one logarithmic cycle on the time scale.

In Fig. 3 the relationship of F and z values of a typical thermal death-time curve is shown. The z value is related to the temperature coefficient or Q_{10} value as follows (Rahn, 1945):

$$z = \frac{18}{\log Q_{10}}$$

Thus, the slope of the thermal death-time curve (z value) and a point designated by the F value serve to characterize the thermal resistance of bacterial spores at any temperature under a given set of conditions. In accordance with the concept that death of bacteria is logarithmic in order, Stumbo (1948a) suggested that the terms used in describing the thermal resistance of bacteria be redefined such as:

F = a symbol which represents the number of minutes required at 250 deg. F., assuming instantaneous heating and cooling, to reduce the number of organisms of a given species, present in a given quantity of a given food, to any given level.

To characterize the slope of a destruction rate curve, Katzin and Sandholzer (1942) (Rahn, 1947) pointed out that for a survival ratio of 10 per cent from the death rate constant formula

$$K = \frac{1}{t} \log \frac{\text{initial number}}{\text{survivors}}$$

$$Kt = \log \frac{a}{0.1a} = \log 10 = 1$$

and therefore

$$t = \frac{1}{K}$$

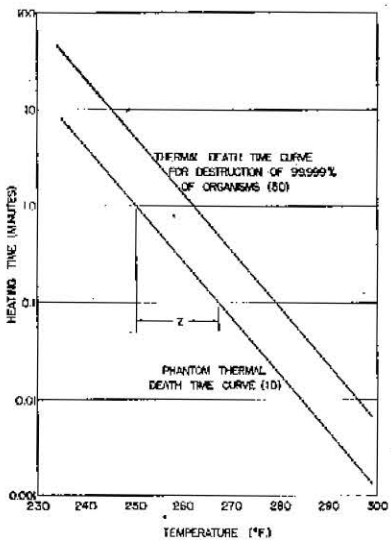

Fig. 3. Phantom thermal death time curve (1xD) and thermal death time curve based on destruction of 99.999 per cent of organisms or for time of 5xD. Spores of PA3679 (10,000 per .01 ml.) suspended in neutral M/15 phosphate buffer.

From this relationship, the term "decimal reduction time" indicates the time required to reduce the bacterial population to one-tenth of its original value. Thus, the decimal reduction time, Z(Zeta) or D value, is the reciprocal of the death rate constant. According to Ball (1943), the symbol Z(Zeta) for the slope value of the rate of destruction curve was suggested by Baselt. Z is expressed as the number of minutes required for the rate of destruction curve to traverse one logarithmic cycle and represents the time required under a given set of conditions for ninety per cent destruction of the organisms. In order to avoid confusion of this symbol (Z) with z, representing the slope of the thermal death-time curve, it has been agreed by workers in the field to use the symbol D instead of Z (Stumbo, Murphy and Cochran, 1950).

Ball (1943) discussed the evolution in methods of determining thermal death times. Because of the complexity of this problem, the trend is away from procedures that show absolute destruction points and toward those that reveal rates of destruction of bacteria in terms of numbers destroyed. He further pointed out that in most rate of destruction tests bacteria are counted, whereas in tests designed to give absolute destruction points, containers, as a rule, are the units counted. In considering the relationship of rate of destruction curves to thermal death-time curves, Ball (1943) observed that if each rate of destruction curve passed through the point representing 100 per cent survival in nil time, the slope value, z, of the thermal death-time curve could be obtained from a series of rate of destruction curves for an organism by plotting the slope values, D, of these curves against temperature. Such a curve has direction but not position. Without position with respect to destruction time or end point, the curve lacks a definite value in relation to the thermal death-time coordinate. Ball introduced the term, "phantom thermal death-time curve", for such a curve (Fig. 3). In the actual determination of thermal destruction rates sometimes there is no apparent reduction in the number of viable organisms for a period of time after the start of the heating. Ball introduced an equation for taking this factor into account in deriving the value of z. A thermal death-time curve with definite position with respect to destruction time is obtained by multiplying the plotted D values by the time in terms of D units required to give the desired destruction point or degree of reduction of the initial number of bacteria (Fig. 3).

Evaluation of Thermal Death Time Data

In most thermal destruction rate tests attempts are made to count the number of surviving organisms by means of appropriate plating techniques. Such procedures are accompanied by such problems as that of providing an adequate recovery medium, difficulties of obtaining growth when only small numbers of surviving cells are present, and initiating growth of cells that may have lost some of their capacity to grow and multiply after being subjected to a severe but sub-lethal exposure to heat.

The thermal death-time tube and can methods for determining thermal resistance have been used to show absolute destruction points. Ball (1943) has pointed out that, "The use of a container, whether it be tube or can, as the unit to be counted in establishing results has been criticized by some investigators because the last spores to remain viable in a run do not germinate readily under certain conditions of subculturing or direct culturing."

The shortest heating time at a given temperature that appeared to destroy all viable organisms has usually been considered as the destruction end-point. Stumbo (1948a, 1949) and Stumbo, Murphy, and Cochran (1950) have demonstrated that the magnitude of error may be reduced

if thermal death-time determinations are properly carried out and interpreted. The necessity for an adequate number of replicates and the proper spacing of time intervals was stressed. These factors were incorporated in a method they presented for treating such data that might be obtained by thermal death-time tube and can methods as well as the new thermoresistometer method of Stumbo (1948). According to this method, a logarithmic order of death is assumed, and the data are treated as follows:

$$D = \frac{U}{\log a - \log b} = \text{time in minutes to accomplish a 90 per cent reduction in number of spores.}$$

U = time of heating (minutes)

a = number of spores subjected to one time-temperature relationship (the number of spores initially present per sample) multiplied by the number of replicate samples

b = number of spores surviving at the end of heating time U

The value of b is derived by applying the equation of Halvorson and Ziegler (1932) to data obtained from samples subjected to time-temperature relationships that sterilized only a portion of the total number of samples as follows:

$\bar{x} = 2.3026 \log \text{of } \frac{n}{g}$ where

$\bar{x}$ = most probable number of spores surviving per sample

n = total number of samples

g = number of sterile samples as evidenced by lack of growth in subculture tubes

b = x times the number of samples

The average D values thus obtained for several temperatures may be plotted on semilog paper, and a phantom thermal death-time curve may then be fitted to the data.

As an alternative to Stumbo's procedures for calculating D values, Schmidt (1950a) applied Reed's (1936) method for treating bioassay data. By Schmidt's technique it was possible to calculate the D value based upon the total data acquired during the experiment and to eliminate the averaging of the different D values calculated for different time intervals at a given temperature. The probability of sterility is plotted against time upon arithmetic probability paper. The time corresponding to L.D. 50, or the point on the curve where the probability of a tube being viable or sterile is equal to 0.5, is assumed to represent the best estimate of the time at which there is on the average 0.5 spore surviving per tube. The D value is then calculated according to the formula:

$$D = \frac{\text{Time (L.D. 50)}}{\log \text{ initial number of spores per tube} - \log 0.5}$$

Good numerical agreement between an average D value calculated by Stumbo's method and the D value as calculated from L.D. 50 was reported. Reynolds, Kaplan, Spencer, and Lichtenstein (1952) compared these two methods of calculating D values and showed them to be in general agreement.

In a discussion of the thermal death-time relationships of bacterial spores Gillespy (1951) pointed out the value of using decimal reduction times to represent death rates. From reference to previous work on thermal resistance, he suggested that it may be that the death rate constant K is really related to temperature according to the Arrhenius equation:

$$\ln k = b' - \mu/RT$$

and, therefore, we may write $\log D = (M/T) - b$, when M and b are constants and T represents absolute temperature. M is defined as the slope of the thermal death-time curve with respect to the reciprocals of the absolute temperature, in deg. F. and logarithms to base 10. Thus, if D_{T_1} and D_{T_2} are the decimal reduction times at temperatures T_1 and T_2, respectively, then

$$M = \frac{\log D_{T_1}/D_{T_2}}{\frac{1}{T_1 + 460} - \frac{1}{T_2 + 460}}$$

These relationships are then shown by the equation:

$$D_{T_2} = D_{T_1} 10^{M(1/T_2 - 1/T_1)}$$

If this equation represents the true relationship, then M is constant and z (slope of thermal death-time curve) varies with temperature. Over small ranges of temperature the equation

$$D_{T_2} = D_{T_1} 10^{(T_1 - T_2)/z}$$

was reported to give good approximations to the former equation.

If it is assumed that M is constant and that z varies with the temperature, then the value of z at temperature T is then given by $z = T^2/M$ and the mean value over a range T_1 to T_2 by

$$z = T_1 T_2 / M,$$

temperature being measured from absolute zero (—460 deg. F.)

Equations for the thermal destruction of spores have also been formulated and discussed by Hicks (1951). He represented the course of the destruction of spores at a constant temperature by the equation: $c = c_o e^{-kt}$

When c is the concentration of spores surviving at time t, c_o is the concentration of spores at time t = o, and k is a constant for a particular organism, medium, and temperature. He pointed out that although this equation is supported by a great deal of experimental evidence, it is not quite certain that it is an adequate approximation under all conditions that might be encountered in canning practice. Hicks (1952) observed that the basic principles of thermal destruction of spores as set forth by Stumbo (1949), Gillespy (1951), and himself are almost, if not quite, identical. The concepts as set forth by these three authors are at variance from the classical concepts primarily as relates to the interpretation of values for the number of surviving spores, which are less than 1.0. It was pointed out that the equations used are obviously statistical laws so that survivor values of less than 1.0 should be interpreted as probabilities of a spore surviving. He further suggested that a better understanding of the thermal destruction of spores might lead to a modification of the present equation and would give greater confidence in extrapolating to very small values of surviving spores.

Amaha (1953) reported that in the case of spores of aerobic bacilli and Putrefactive Anaerobe 3679 the relationship between spore concentration (N) and survival time (t) can be expressed by the following linear equation:

$$\log t = a + b \cdot \log N$$

in which a and b are variables depending on strain, temperature, and nature of the suspending medium. He observed that although the fundamental cause of these linear relationships cannot be explained as yet, similar relationships have been found in work with disinfectants. Temperature coefficients of the thermal destruction of PA 3679 spores decreased slightly with rising temperature, thus the thermal-death-time curve was not essentially a straight line, but was very slightly concave downward. He concluded that though the curves can be safely assumed to be straight in the narrow range of temperatures now employed in commercial processing of foods, if the resistance values of an organism at higher temperatures were determined by extrapolation of the thermal-death-time curve of lower temperature ranges, the values obtained would become somewhat smaller than the real resistance values. Amaha also evaluated the activation energy (E) of the thermal destruction of spores by the Arrhenius equation. For $Q_{5\ deg.\ C}$ values of a 1×10^4 sport concentration of PA 3679 the following values of E were obtained:

Between 105 deg. and 110 deg. C., $Q_{5\ deg.\ C} = 2.50$—$E = 53{,}030$ calories.

Between 110 deg. and 115 deg. C., $Q_{5\ deg.\ C} = 2.40$—$E = 52{,}010$ calories.

This magnitude of activation energy supports the theory that the thermal destruction of spores by moist heat is the denaturation of a protein molecule in the cells.

LITERATURE CITED

Amaha, M., 1953. Heat resistance of Cameron's Putrefactive Anaerobe 3679 in phosphate buffer (*Clostridium sporogenes*). *Food Research 18*:411-420.

Ball, C. O., 1923. Thermal process time for canned foods. Bull. Natl. Research Council 7, Part 1, No. 37, 76 pp.

Ball, C. O., 1928. Mathematical solution of problems on thermal processing of canned food. Univ. of Calif. Pubs. in Public Health 1, 245 pp.

Ball, C. O., 1943. Short-time pasteurization of Milk. *Ind. Eng. Chem. 35*:71-84.

Bigelow, W. D., 1921. The logarithmic nature of thermal death time curves. *J. Infect. Dis. 29*:528-536.

Bigelow, W. D., Bohart, G. S., Richardson, A. C. and Ball, C. O., 1920. Heat penetration in processing canned foods. Natl. Canners Association Bulletin 16-L, 128 pp.

Chick, H., 1910. The process of disinfection by chemical agencies and hot water. *J. Hyg. 10*:237-286.

Gillespy, T. G., 1951. Estimation of sterilizing values of processes as applied to canned foods. I. Packs heating by conduction. *J. Sci. Food Agr. 2*:107-125.

Halvorson, H. O. and Ziegler, N. R., 1932. Application of statistics in bacteriology. *J. Bact. 25*:101-121.

Hicks, E. W., 1951. On the evaluation of canning processes. *Food Technol. 5*:134-142.

Hicks, E. W., 1952. Some implications of recent theoretical work on canning processes. *Food Technol. 6*:175-178.

Katzin, L. I. and Sandholzer, L. A., 1942. Bacterial death rates and decimal reduction time. *J. Bact. 44*:722.

Rahn, O., 1945. Physical methods of sterilization of microorganisms. *Bact. Revs. 9*:1-47.

Rahn, O., 1947. Injury and death of bacteria by chemical agents. Biodynamica, Normandy, Mo. 183 pp.

Reed, L. J., 1936. Statistical treatment of biological effects of radiation. In Duggar, B. M., Editor, *Biological Effects of Radiation*, I, 227-251. McGraw-Hill Book Co., Inc., New York, N. Y.

Reynolds, H., Kaplan, A. M., Spencer, F. B. and Lichtenstein, H., 1952. Thermal destruction of Cameron's Putrefactive Anaerobe 3679 in food substrates. *Food Research 17*:153-167.

Schmidt, C. F., 1950 a. Continental Can Company, Inc., Chicago, Ill. Personal Communication.

Schmidt, C. F., 1950 b. A method for the determination of the thermal resistance of bacterial spores. *J. Bact. 59*:433-437.

Stumbo, C. R., 1948 a. Bacteriological considerations relating to process evaluation. *Food Technol. 2*:115-132.

Stumbo, C. R., 1948 b. A technique for studying resistance of bacterial spores to temperatures in the higher range. *Food Technol. 2*:228-240.

Stumbo, C. R., 1949. Thermobacteriology as applied to food processing. *Advances in Food Research*. Vol. II. Academic Press, Inc., New York, N. Y., pp. 47-115.

Stumbo, C. R., Murphy, J. R. and Cochran, J., 1950. Nature of thermal death time curves for P.A. 3679 and *Clostridium botulinum*. *Food technol. 4*:321-326.

THERMAL DESTRUCTION OF MICROORGANISMS AND ENZYMES-II

Despite wide use of thermal death-time data new processing techniques point to need for still more experience with high temperatures. . . .

by W. B. ESSELEN and I. J. PFLUG*

• *In Part I of this paper the authors reviewed some basic concepts of thermal destruction of microorganisms and enzymes in food processing, including the logarithmic destruction rate and the evaluation of thermal death time data.*

Use of Thermal Death-Time Data In Calculation of Process Times

By correlating thermal death time with rate of heat penetration, the length of time theoretically required to destroy any specific bacterial spores in a container of food at a given process temperature may be calculated. In practice the pertinent thermal death-time data must be converted into its so-called lethality rate for use in the actual process time calculation. The rate of destruction of an organism at any given temperature in a process is the reciprocal of the time in minutes (t) required to destroy (or reduce to a given level) the organism at the temperature (T).

From the thermal death-time curve (Fig. 4), a simple geometric relationship exists between the sides of similar right triangles, and may be expressed by the equation:

$$\frac{\log t - \log F}{\log 10} = \frac{250 - T}{z}$$

from which $\log \frac{t}{F} = \frac{250 - T}{z}$ or $\frac{t}{F} = \log^{-1} \frac{250 - T}{z}$

when t/F is the time to destroy the organism at temperature (T), if F = 1.

F/t, the reciprocal of t/F, represents the lethal rate at any given temperature.

z = the slope of the thermal death-time curve in degrees F.

F = minutes at 250 deg. F. to reduce the number of organisms under consideration to a given low level.

T = temperature under consideration (deg. F.).

t = minutes to reduce number of organisms under consideration to a given low level at temperature T.

From the thermal death-time curve, F and z are known. At any temperature (T), it is possible to solve the above equation for t, from which the reciprocal F/t can be determined. The lethal rate of any temperature can be calculated once the F and z values are determined. In the processing of low acid foods, the base temperature for F is 250 deg. F., as shown in the above equation. However, these same relationships can be employed in the calculation of similar relationships at lower temperatures, such as are encountered in acid foods, vegetative cells, and enzymes. In such cases, because the heat resistance is much lower, base temperatures, such as 212 deg., 190 deg., 160 deg., or 140 deg. F., might be employed and the corresponding F values would be designated as F_{212}, F_{190}, F_{160}, and F_{140}, respectively.

Methods of Determining Thermal Resistance

Several methods have been described and employed for the determination of the heat resistance of bacterial spores. These methods include: (1) the thermal death-time tube method (Bigelow and Esty, 1920); (2) the thermal death-time can method (American Can Co., 1947); (3) the rate of destruction method (Williams *et al.*, 1937); (4) the "Thermoresistometer" method (Stumbo, 1948b); (5) the miniature retort method (Schmidt, 1950b); and (6) the thermoresistometer method of Pflug and Esselen (1953). The former methods were applicable for temperatures below 250 deg. F., because of the heating lag of the thermal death-time tubes or cans and their contents. The methods of Schmidt and of Stumbo were particularly designed to study the heat resistance of spores in the general temperature range of 250 deg. to 270 deg. F., whereas the apparatus of Pflug and Esselen has been used at temperatures as high as 300 deg. F. An excellent discussion of the problems of accurately determining the heat resistance of bacterial spores and the characteristics of some of the methods that have been used has been presented by Stumbo (1949).

The selection of the method to be employed in the determination of thermal death times and thermal destruction rates is contingent upon a number af factors. Consideration must be given to such conditions as the length of the exposure times required, the nature of the organisms, the necessity for keeping the heating and cooling lag factors of the sample being heated to a minimum; the method of subculture desired (in the original heated sample or in enriched subculture media), and the method of evaluating the num-

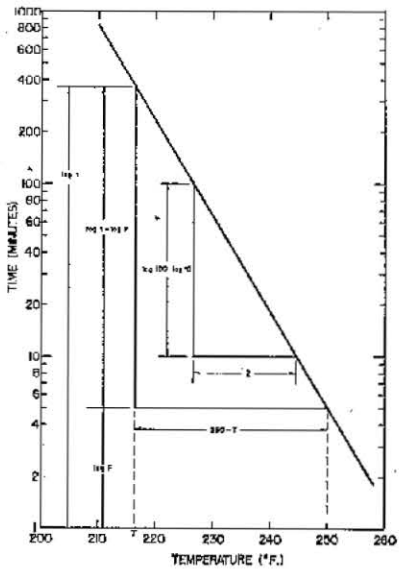

Fig. 4. Thermal death time curve showing geometrical relationships as related to destruction times and lethal rates.

* Professors Esselen and Pflug are on the food technology faculty of the University of Massachusetts. This paper, Contribution 930 of the Massachusetts Agr. Exp. Sta., was delivered, in Boston, Dec. 29, 1953, before the American Association for the Advancement of Science.

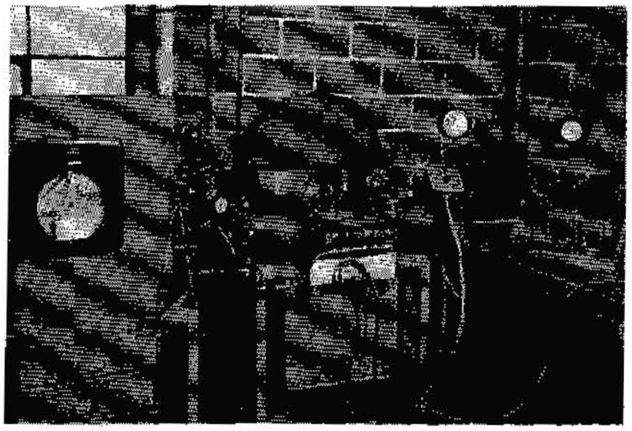

Fig. 5. Apparatus for determining thermal resistance of bacterial spores in temperature range of 250 to 300 deg. F.

ber of surviving organisms. There are also a number of problems, some as yet unresolved or only partially resolved that must be considered regardless of the actual method followed in such studies. Some of these factors may be enumerated as follows:

1. Past history and growth, preparation, and standardization of microorganisms to be employed.
2. Obtaining an accurate initial count of the spore or vegetative cell concentration in the original suspension.
3. Degree of thermal activation required to obtain maximum germination of spores.
4. Effect of clumping.
5. Effect of cold shock on microorganisms after thermal activation or exposure to heat.
6. Nutritive requirements of organisms in relation to spore germination and growth in recovery media.
7. A basic concept of the logarithmic destruction theory is that it refers to organisms of uniform heat resistance. How close can this condition be approached in practice?
8. Statistical evaluation of accuracy of data in relation to number of samples to be run at each time and temperature interval and over-all accuracy and reproducibility of results both in one laboratory and between different laboratories.

Investigators in this field should be well aware of the above factors and other problems encountered in research work on the thermal resistance of microorganisms. On a basis of the actual experimental data reported with their many deviations, it would perhaps be bold to make a blanket statement that microorganisms and spores exhibit a logarithmic destruction rate. It might be more appropriate to say that thermal destruction rates and death-time curves tend to approximate straight lines in many cases. However, in carrying on such work and in reviewing the work of others, one cannot help but be impressed with the proximity to logarithmic conditions usually achieved, especially in view of the many variables that are encountered.

The advent of high temperature, short-time, and aseptic canning methods has pointed to a need for basic information on the thermal resistance of microorganisms in the temperature range of 250 deg. to 300 deg. F. Most of our information to date is from data obtained at temperatures below 250 deg. F.

In working at temperatures above 250 deg. F., the speed with which spores are destroyed requires that heating and cooling lags of the heated samples be reduced to a minimum, that small size samples be used, and that the timing of the heating periods be carried out with increased accuracy. The development of equipment or so-called "thermoresistometers" to achieve these ends has been reported by Stumbo (1948b) and Pflug and Esselen (1953). The latter apparatus is illustrated in Figures 5 and 6. This apparatus has been operated at temperatures up to 305 deg. F. with exposure times as short as 0.01 minutes (0.6 second).

Sublethal Heat Treatment

It is well known that spores that have re[illegible]sublethal exposure to heat may exhibit delayed [illegible]ion or dormancy, even in ordinarily favorable cult[illegible]dia, for long periods of time. This behavior [illegible]ificance in relation to heat processed foods. [illegible]mancy is still unknown, although a numb[illegible]ve been proposed to explain it. It has [illegible]t in a sublethal exposure to heat, spores [illegible] to the extent of interfering with [illegible] processes. Such heat-injured [illegible]e more fastidious in their [illegible]rements for germination and [illegible]1937). Wynne and Foster [illegible] of 0.1 per cent soluble st[illegible]marked effect in stimulat[illegible]. They reported that th[illegible]ve substances present [illegible]se tend to inhibit or re[illegible]

Recent wo[illegible]ores has shown that [illegible]on the phenomenon [illegible] determining the [illegible] suspension. Ev[illegible]d Evans (1945, [illegible] certain thermo[illegible] a preliminar[illegible] in such culture[illegible]quire heat activation[illegible]fter they have [illegible]owed that the sp[illegible]ilic, heat-acti[illegible]pid alteration[illegible]nder conditions [illegible]on. They postu[illegible] are stimulate[illegible]bsence of an [illegible] contained supp[illegible]pidly exhaus[illegible]es and factors [illegible]viewed by C[illegible]

[illegible]ported that the[illegible] for 0.233 minu[illegible]3679 and *Clo*[illegible] treatments, though[illegible] were appare[illegible] spores to germ[illegible] may still have [illegible] activation, even [illegible] in lower conc[illegible]equired to

activate some spores than to kill others. They further indicated that this is probably the case and that maximum spore counts may be only rarely, if ever, obtained by spore counting techniques employing pasteurization. Youland and Stumbo (1953) obtained maximum spore counts for *Bacillus coagulens* by a thermal activation treatment of 0.14 minutes at 220 deg. F.

Reed, Bohrer, and Cameron (1951) obtained evidence that boiling spore suspensions for five minutes might not provide sufficient thermal activation to give maximum spore counts.

Deviations from Logarithmic Destruction Rate

Rahn (1947) has pointed out that although the available evidence was overwhelmingly in favor of logarithmic order of death in bacteria, there were reports of exceptions that should be considered. He has emphasized the fact that uniformity of experimental conditions and of the suspension of microorganisms being tested is essential for a demonstration of a logarithmic order of death. Such factors as lack of uniformity of heat resistance in the cells of a test suspension, clumping, age, and protection of living cells by dead cells have been emphasized as having an influence in altering the shape of the death-rate curve.

Reed, Bohrer, and Cameron (1951) presented extensive data on thermal destruction rates of spores. Many of their survivor curves could not be represented as single straight lines passing through the origin. These deviations from the logarithmic order of death were taken as evidence that the spores in the suspensions tested were not of uniform resistance to heat. When D values are calculated from thermal destruction data by the method of Stumbo, Murphy, and Cochran (1950), there is generally an apparent trend for the D value to increase with heating time at a given temperature. This trend is especially obvious when extensive data are available, such as presented by Pflug and Esselen (1953). Reynolds and Lichtenstein (1952) studied this observed increase in D value with heating time through the analysis of data reported by others as well as by themselves. They proposed methods for statistical evaluation of variation in such data and concluded that data presented are not consistent with the assumption that thermal death-rate curves for PA 3679 spores are exponential. Increasing rates of death during the first intervals of exposure of spores to lethal heat and the regular observance of increasing D values in the neighborhood of complete spore destruction indicate the sigmoid type of survivor curve characteristic of populations made up of individuals of varying resistance. In a continuation of this work, Kaplan, Lichtenstein, and Reynolds (1953) reported that the initial deviation from linearity of the thermal death-rate curve of PA 3679 is real and not an artifact of experimental methods.

Anderson, Esselen, and Fellers (1949) found that a number of destruction rate curves for *Bacillus thermoacidurans* were not straight lines. Youland and Stumbo (1953) were of the opinion that the nature of the above curves might have been due to factors associated with the experimental methods employed. These authors determined the order of death of *Bacillus coagulans* (*Bacillus thermoacidurans*, Berry) by the newer technique developed by Stumbo (1948b) using four different spore concentrations at four different temperatures. No significance was found for differences in "D" values representing rates of destruction of spores subjected to any of the four temperatures. It was concluded that the logarithmic order of death was indicated by all the data obtained.

Fig. 6. Placing samples inoculated with bacterial spores and subculture tubes in thermal resistance apparatus prior to making thermal death time test.

Pflug and Esselen (1954) reported that D values obtained for three different concentrations of PA 3679 spores at 280 deg. F. (Figure 2) were in good agreement, thus indicating a logarithmic order of death. In an analysis of thermal death-time data for spores of PA 3679, the apparent increase in D value with heating time when calculated by the method of Stumbo, Murphy, and Cochran (1950) was considered. When large numbers of samples were tested and the calculated probable numbers of surviving spores (b) were plotted on a destruction rate curve, they aligned themselves around a line that required more time to traverse one log cycle than the lines connecting any of these points with the initial point. It was suggested that in the use of this method of calculating D values, the calculated number of surviving spores (b), for a high percentage of positive tubes is somewhat low, especially when they are compared to a destruction rate curve having an average calculated D value. Preliminary data obtained from quantitative counts of surviving spores indicated that this might well be the case. If the calculated number of surviving spores (b) were in agreement with the straight line destruction rate curve based on the average D value, a uniform calculated D value would be obtained. Because of the difference in slope of the calculated destruction rate curve as compared to the curve through the number of spores (b) calculated from the number of positive tubes, there could not help but be an

increasing D value with heating time (U). The effect of the number of samples on the experimental variation to be expected was also discussed. The increase in accuracy of any test that might be achieved by increasing the number of samples was demonstrated.

It would thus appear that at least many of the apparent deviations from the logarithmic order of death that have been indicated on a basis of reported experimental data may be due in large part to limitations in lack of uniformity of heat resistance in spore suspensions used, experimental techniques and methods of evaluating such data. In spite of the limitations and as yet unresolved problems encountered in thermal destruction work, the preponderance of available data indicates that the thermal destruction of bacterial spores closely approximates a logarithmic order of death. The successful application of this concept in the derivation of process times for canned foods provides a massive backlog of experience in support of this theory.

Thermal Destruction of Enzymes

Information relating to the thermal destruction of enzymes in acid foods, such as fruits, fruit juices, and pickles, was reviewed by Esselen (1950). It was pointed out that some of the enzymes concerned have a thermal stability comparable to or greater than many of the spoilage organisms encountered in such products. Failure to inactivate such enzymes during thermal processing may result in deleterious changes in the product during subsequent storage. Thus, consideration should also be given to the destruction of enzymes as well as microorganisms in the development of processing procedures for acid fruits and vegetable products.

Kaplan, Esselen, and Fellers (1949) showed that through the application of procedures commonly used for determining processing conditions necessary to sterilize canned foods, the effect of heat preservation upon their enzyme systems could be observed and the nature of the thermal inactivation of the enzymes could be studied. Processing times necessary to inactivate enzymes of canned foods at specific temperatures could then be predicted. Thermal destruction curves obtained for ascorbic acid oxidase and peroxidase approximated a straight line, within certain temperature limits, when plotted on semilogarithmic paper, and indicated that such enzyme systems are relatively heat labile. Since then, further work on the thermal resistance of peroxidase and pectolytic enzymes in pickles and fruit juices has been carried on at this laboratory as reported by Nebesky, Esselen, Kaplan, and Fellers (1950), Anderson Ruder, Esselen, Nebesky, and Labbee (1951), Esselen and Anderson (1952), Kohn Esselen, and Fellers (1953), Labbee and Esselen (1954), and Esselen and Anderson (1954). The thermal inactivation of phosphatase has been widely used as a measure of the adequacy of pasteurization of fresh fluid milk. The thermal inactivation characteristics of this enzyme in milk have been discussed by Ball (1943), Hetrick and Tracy (1948), and others. Typical destruction time curves for peroxidase and pectolytic enzymes as determined in this laboratory and for phosphatase in milk (Hetrick and Tracy, 1948) are shown in Fig. 7 together with a destruction time curve for 3679 spores. The relatively low heat resistance of enzymes as compared with

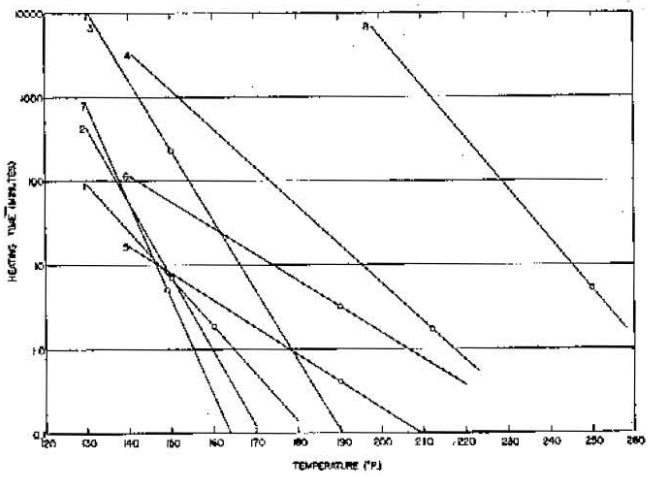

Fig. 7. Thermal destruction time curves for enzymes and bacterial spores.

Curve 1.—Pectin polygalacturonase in dill pickle brine. $F_{180} = 1.85;\ z = 17.7$

Curve 2.—Pectin esterase (Pectinol A) in apple juice. $F_{150} = 7.2;\ z = 11.3$

Curve 3.—Pectin polygalacturonase (Pectinol A) in apple juice. $F_{150} = 255;\ z = 11.5$

Curve 4.—Peroxidase in fresh pack pickles. $F_{212} = 1.7;\ z = 22$

Curve 5.—Peroxidase in peaches. $F_{190} = 0.41;\ z = 31$

Curve 6.—Perovidase in apple cider. $F_{180} = 3.2;\ z = 32.5$

Curve 7.—Phosphatase in milk. $F_{145} = 5.0;\ z = 8.8$

Curve 8.—PA 3679 spores in neutral phosphate. $F_{250} = 5.3;\ z = 16.8$

bacterial spores is apparent. On a basis of available information, peroxidase would appear to be the most heat resistant of the enzymes that might be encountered in food processing. As is the case with microorganisms, the thermal resistance of enzymes appears to be affected by such factors as concentration, substrate, and acidity. These factors should be considered in the design of heat treatments to inactivate enzymes in foods.

Although the present discussion is concerned primarily with heat processed foods, it should be pointed out that the thermal inactivation of enzyme by heat (blanching) is an important step in the successful production of many dehydrated and frozen foods.

Summary

Evidence relating to the thermal destruction of microorganisms and enzymes in food products is indicative of the logarithmic order of destruction, since thermal destruction time and rate curves tend to approximate straight lines when plotted on semilogarithmic paper. The successful and widespread application of this concept in the thermal processing of foods provides further convincing evidence of its validity. Refinements in methods and techniques of determining thermal resistance and a better understanding of the nature of death should ultimately lead to a more complete understanding of the thermal destruction rates of microorganisms and factors that influence them. Developments in the field of high temperature and aseptic canning methods point to the need for some fundamental knowledge regarding the behavior of bacterial spores at temperatures above 250 deg. F.

LITERATURE CITED

American Can Company, 1947. Canned Food Reference Manual. Third Ed. American Can Company, New York, N. Y. 595 pp.

Anderson, E. E., Esselen, W. B., Jr. and Fellers, C. R., 1949. Effect of acids, salt, sugar and other food ingredients on thermal resistance of *Bacillus thermoacidurans*. *Food Research 14*:499-510.

Anderson, E. E., Ruder, L. F., Esselen, W. B., Jr., Nebesky, E. A. and Labbee, M., 1951. Pasteurized fresh whole pickles. II Thermal resistance of microorganisms and peroxidase. *Food Technol.* 5:364-368.

Ball, C. O., 1943. Short-time pasteurization of milk. *Ind. Eng. Chem. 35*:71-84.

Bigelow, W. D. and Esty, J. R., 1920. The thermal death point in relation to time of typical thermophilic organisms. *J. Infect. Dis. 27*:602-617.

Curran, H. R., 1952. Resistance in bacterial spores. *Bact. Revs. 16*:111-117.

Curran, H. R. and Evans, F. R., 1937. The importance of enrichments in the cultivation of bacterial spores previously exposed to lethal agencies. *J. Bact. 34*:179-189.

Curran, H. R. and Evans, F. R., 1945. Heat activation inducing germination in the spores of thermotolerant and thermophilic aerobic bacteria. *J. Bact. 49*:335-346.

Curran, H. R. and Evans, F. R., 1947. The viability of heat activatable spores in nutrient and non-nutrient substrate as influenced by prestorage and poststorage heating and other factors. *J. Bact. 53*:103-113.

Esselen, W. B., Jr., 1950. Thermal inactivation of fruit enzymes in relation to processed products. *The Glass Packer 29*:483-485, 496, 598, 600.

Esselen, W. B. and Anderson, E. A., 1952. Pasteurization of genuine dill pickles. *The Glass Packer, 31*:600, 603.

Esselen, W. B. and Anderson, E. E., 1954. Thermal inactivation of pectolytic enzymes in genuine dill pickles. *Food Technol.* in press).

Evans, F. R. and Curran, H. R., 1943. The accelerating effect of sublethal heat on spore germination in mesophilic aerobic bacteria. *J. Bact. 46*:513-523.

Hetrick, J. H. and Tracy, P. H., 1948. Effect of high-temperature short-time heat treatments on some properties of milk. I. Inactivation of the phosphatase enzyme. *J. Diary Sci. 31*:867-879.

Kaplan, A. M., Esselen, W. B., Jr., and Fellers, C. R., 1949. Enzyme systems of canned acid foods. Effect of processing conditions of time and temperature. *Ind. Eng. Chem. 41*:2017-2020.

Kaplan, A. M., Lichtenstein, H. and Reynolds, H., 1953. The initial deviation from linearity of the thermal death rate curve of a putrefactive anaerobe. *J. Bact. 66*:245-246.

Kohn, G. G., Esselen, W. B., Jr. and Fellers, C. R., 1953. Thermal inactivation of pectolytic enzymes used in the clarification of apple juice. *Food Research 18*:511-515.

Labbee, M. D. and Esselen, W. B., 1954. Effect of peroxidase concentration, acidity and storage temperature on the development of off-flavors in fresh pack pickles. *Food Technol.* (in press).

Nebesky, E. A., Esselen, W. B., Jr., Kaplan, A. M. and Fellers, C. R., 1950. Thermal destruction and stability of peroxidase in acid foods. *Food Research 15*:114-124.

Pflug, I. J. and Esselen, W. B., 1953. Development and application of an apparatus for study of thermal resistance of bacterial spores and thiamine at temperatures above 250° F. *Food Technol.* 7:237-241.

Pflug, I. J. and Esselen, W. B., 1954. Observations on the thermal resistance of Putrefactive Anaerobe No. 3679 spores in the temperature range of 250 to 300° F. *Food Research* (in press).

Rahn, O., 1947. Injury and death of bacteria by chemical agents. Biodynamica, Normandy, Mo. 183 pp.

Reed, J. M., Bohrer, C. W. and Cameron, E. J., 1951. Spore destruction rate studies on organisms of significance in the processing of canned foods. *Food Research 16*:383-408.

Reynolds, H. and Lichtenstein, H., 1952. Evaluation of heat resistance data for bacterial spores. *Bact. Revs. 16*:126-135.

Stumbo, C. R., 1948 b. A technique for studying resistance of bacterial spores to temperatures in the higher range. *Food Technol. 2*:228-240.

Stumbo, C. R., Murphy, J. R. and Cochran, J., 1950. Nature of thermal death time curves for P.A. 3679 and *Clostridium botulinum*. *Food Technol. 4*:321-326.

Williams, C. C., Merrill, C. M. and Cameron, C. J., 1937. Apparatus for determining spore destruction rates. *Food Research 2*:369-375.

Wynne, E. S. and Foster, J. W., 1948. Physiological studies on spore germination with special reference to *Clostridium botulinum*. *J. Bact. 55*:61-68.

Youland, G. C. and Stumbo, C. R., 1953. Resistance values reflecting the order of death of spores of *Bacillus coagulens* subjected to moist heat. *Food Technol.* 7:286-288.

REPRINTED FROM

THE GLASS PACKER

JANUARY - FEBRUARY, 1954