Thermal Resistance of Salmonellae and Staphylococci in Foods

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Received for publication 11 May 1966

ABSTRACT

Thomas, Constance T. (Cornell University, Ithaca, N.Y.), J. C. White, and Karla Longréé. Thermal resistance of salmonellae and staphylococci in foods. Appl. Microbiol. 14:815–820. 1966.—The heat-resistant Salmonella senftenberg 775W and two strains of Staphylococcus aureus were tested at temperatures up to 68.3 °C (71.1 °C for S. senftenberg) in four different media. From the survival data, decimal reduction times (D values) were calculated for each set of conditions, and decimal reduction time curves were constructed for each bacterial strain in each medium. Slopes of decimal reduction time curves (ZD) ranged from 4.52 to 6.38 °C with a single exception. There was no statistical heterogeneity among the remaining values. Results were in close agreement with published results of similar studies conducted at somewhat lower temperatures and support the practice of using a slope value (ZD) of 5.56 °C for establishing time-temperature relationships for food processing. It is recommended that such a decimal reduction time curve not be extrapolated to temperatures more than 5.56 °C higher than those actually tested.

Modern technology has made available many new food products, frozen, canned, or dried, both cooked and uncooked. The wide distribution of many of these products, with their increasing use for quantity service in cafeterias and large institutions, makes desirable the establishment of standards to assure that they will be bacteriologically safe, while still remaining palatable and attractive. Recent outbreaks of food poisoning have accentuated the need for proper processing, but there is a dearth of information on the thermal killing times of food-poisoning organisms at higher temperatures.

Recent thermal death time studies (1, 2, 4, 17) have been conducted to determine the time necessary for destruction of salmonellae and staphylococci over the temperature range from 48.9 to 65.6 °C, and the results have been expressed in terms of the slope of the decimal reduction time curve for a particular organism and suspension medium. There are data derived from tubular heat exchangers for thermal destruction of several bacteria in milk at temperatures up to 81.1 °C (5, 6, 14).

The value of the decimal reduction time curve to the food processor is that, theoretically: (i) it is independent of initial inoculum concentration; (ii) its slope is assumed to be a constant value regardless of the suspending medium (10); and (iii) it may be extrapolated to temperatures higher than those actually tested. Thus, once such a curve has been established for a given organism in one medium, it is necessary only to determine the decimal reduction time at one temperature in any other medium and, using the known slope, to determine the time-temperature relationship for safe processing at other temperatures by simple extrapolation. This, of course, assumes that there is some prior knowledge of the number of contaminating organisms present initially so that a margin of safety is allowed in the process calculations.

Construction of a decimal reduction time, or thermal resistance, curve consists of establishing D values (number of minutes of heating at a given temperature required to reduce the inoculum to 10% of its initial concentration) at several temperatures in a particular suspending medium. These D values are then plotted on the ordinate of semilogarithmic graph paper against temperature on the abscissa, and the slope is calculated. ZD, the reciprocal of the slope, expresses the number of degrees increase in temperature necessary to effect the passage of the decimal reduction
time curve over 1 log cycle. For vegetative bacteria, this curve is most frequently described in terms of the $D_{60}$ value and $Z_D$, usually found to be about 5.6 ± 1.1 C (15). Although the logarithmic nature of thermal killing is widely accepted, some workers, such as Kaplan et al. (9) and Humphrey and Nickerson (8), have found significant deviations from this ideal with bacterial spores. White (18) and Elliker and Frazier (7) noted similar findings with nonsporeforming bacteria. In addition, Rahn (13) and Wang et al. (16) caution against the extrapolation of thermal resistance data far beyond those temperatures which have actually been tested.

The present study was undertaken in an attempt to confirm the existing work relating to certain of the nonsporeforming bacteria, and to determine whether time-temperature relationships already established at temperatures up to 62.8 C or 65.6 C are in fact maintained at somewhat higher temperatures. Such information should be of value, especially to those concerned in the preparation of milk- or egg-containing foods where a very short process time may be of major importance in the production of acceptable menu items.

**Materials and Methods**

**Cultures.** The bacteria employed were *Salmonella senftenberg* 775W, an exceptionally heat-resistant strain, and *Staphylococcus aureus* strains MS149 and 196E. Cultures of these organisms were obtained from Robert Angelotti. Stocks were maintained on agar slants stored at 4 C and transferred approximately every 60 days, with the following exception: initial experiments with *S. senftenberg* 775W yielded results which indicated that the population might consist of a mixture of cells of high and low heat resistance. A number of single colonies were isolated and tested individually, and two were finally selected, a rough form (775W-R) exhibiting a resistance level similar to that reported by Angelotti et al. (1) and a smooth type, designated 775W-S, which was less resistant. To maintain the rough form, plates containing well-isolated colonies from one experiment were saved as stocks to provide an inoculum for the next experiment.

**Preparation of inocula.** Cultures were grown for 18 to 20 hr at 37 C in 45 ml of Penassay Broth (Difco) in 125-ml flasks, centrifuged, and resuspended in 1 to 2 ml of sterile 0.5% NaCl.

**Test media.** Thermal resistance tests were conducted in 0.5% NaCl, green pea soup (Campbell’s), beef bouillon (Herb-Ox or Wyler’s granulated), and pasteurized skim milk. The skim milk was diluted with an equal amount of sterile distilled water, and the soups were made up with distilled water to one-half the strength indicated in the producers’ instructions. The pH of all four media was between 6.0 and 6.5.

**Experimental procedure.** Erlenmeyer flasks (500-ml) containing 300 ml of test medium were immersed to a depth about 1.3 cm above the level of the medium and allowed to equilibrate in a thermostatically controlled water bath at the desired temperature. When equilibrium had been achieved, approximately 0.5 ml of the inoculum suspension was added to the flask (giving a concentration of 10^7 to 10^8 cells per milliliter) and mixed by swirling. Flasks were agitated by hand periodically throughout the course of an experiment. At various time intervals, 1-ml samples were removed with a pipette to cold dilution blanks or to empty tubes in a cold-water bath. Appropriate dilutions were made in 0.5% NaCl, and duplicate samples were spread on plates of Penassay Broth solidified with 1.5% agar. Colony counts were made after 48 hr of incubation at 37 C. Zero-time counts were not routinely made, since these were not essential for determining decimal reduction times.

**Treatment of data.** A typical survival curve was constructed from the data obtained in each experiment, and the slope was determined by the regression method. The data from three replicate experiments at 60 C and no fewer than five at each of the higher temperatures were then pooled for the calculation of an overall slope, and for an analysis of variance test for heterogeneity among the individual slope determinations. The reciprocal of the regression coefficient obtained from the pooled data was used as the $D$ value, or decimal reduction time, for that particular bacterial strain, test medium, and temperature. (The negative sign of the regression coefficient was ignored here, and also below.)

From these pooled $D$ values, decimal reduction time curves were constructed for each bacterial strain in each of the test media. A regression coefficient for each curve was calculated, and its reciprocal was taken as the $Z_D$ value.

**Results**

Results of thermal resistance tests are summarized in Table 1. The rough strain of *S. senftenberg* possessed the highest resistance, in all media, among the bacteria tested, giving results similar to those reported by Angelotti et al. (1) for the same strain at temperatures where a direct comparison could be made. The smooth form, although giving $D$ values only about half as great, was still somewhat more heat-tolerant than most salmonellae (3, 12). Strain 775W-R in broth grew in dense clumps and underwent spontaneous agglutination in saline suspension. This characteristic could conceivably account for the extreme resistance to thermal destruction observed, although the high degree of consistency between experiments and in simple plate count dilutions makes this seem unlikely.

Even after the initial separation of *S. senftenberg* 775W into rough and smooth cultures as described under Materials and Methods, a high degree of instability of each remained, with interme-
diate forms appearing regularly. Limited tests of some of these variants indicated that their resistance to thermal killing was closer to that of the smooth strain than the rough, although there was considerable variation among those examined, with the results resembling those reported among various lactobacilli by Niven et al. (11). Two derivatives of strain 775W-R which formed apparently smooth colonies on an agar surface were submitted for serotyping. Both agglutinated in appropriate S. senftenberg O-typing sera, but the reactions were considerably weaker than the smooth 775W-S control. Both also underwent rapid and complete agglutination in acriflavine dye, indicating that they were still "rough" cultures even though enough change had occurred to render them typable. Heat-tolerance levels of the two strains used throughout the experimentation to establish decimal reduction time curves remained uniform within the limits of experimental error for over 1 year. This fact would seem to indicate that the high resistance exhibited by strain 775W-R may be at a maximum, since the method of maintaining the stock, already described, apparently did not lead to selection of cells of increased resistance.

It was noted that fairly wide day-to-day fluctuations in total numbers of survivors occurred with all strains of bacteria and media tested, and that increasing heat treatment led to variations in the lag period of individual cells as judged by time required for colony development after plating of samples. However, the survival curves generally adhered closely to a logarithmic plot, even though there was a slight indication in some salmonella experiments of a short initial period of faster kill. The use of cells from cultures at the stationary phase of development for inocula was adopted in view of reports indicating that such a population, incubated at its temperature optimum, should be at a uniform level of high thermal resistance (7, 18).

Analysis of variance tests for homogeneity of $D$ values calculated for the different sets of conditions in nearly all instances led to the conclusion that variations were not significant and that use of the pooled $D$ values from similar sets of experiments was justified in constructing decimal reduction time curves. Those few pooled $D$ values where significant heterogeneity was found were included in the final plots, since the values seemed to fit with the corresponding ones for the other temperatures tested, resulting in a straight line relationship. It also appeared that the very small deviations within individual experiments, rather than large variations among the regression coeffi-

Table 1. Results of thermal resistance experiments with salmonellae and staphylococci

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Test medium</th>
<th>Range of temp tested</th>
<th>$D_{10},$ experimental</th>
<th>$D_{10},$ calculated</th>
<th>$D_{10},$ experimental</th>
<th>$D_{10},$ calculated</th>
<th>$Z_0$^d</th>
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<tr>
<td><strong>Salmonella senftenberg 775W-R</strong></td>
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<tr>
<td>0.5% NaCl</td>
<td>Skim milk</td>
<td>140-160</td>
<td>7.519</td>
<td>6.252</td>
<td>0.837</td>
<td>0.843</td>
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<td></td>
<td>Green pea soup</td>
<td>140-160</td>
<td>10.020</td>
<td>10.640</td>
<td>1.001</td>
<td>1.114</td>
<td>10.204</td>
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<td>145-165</td>
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<td>10.790</td>
<td>1.208</td>
<td>1.327</td>
<td>10.989</td>
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<td><strong>S. senftenberg 775W-S</strong></td>
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<tr>
<td>0.5% NaCl</td>
<td>Skim milk</td>
<td>140-155</td>
<td>3.650</td>
<td>3.358</td>
<td>0.519</td>
<td>0.394</td>
<td>10.753</td>
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<td></td>
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<td>140-155</td>
<td>5.181</td>
<td>5.420</td>
<td>0.584</td>
<td>0.637</td>
<td>10.753</td>
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<td>6.039</td>
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<td>0.662</td>
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<td><strong>Staphylococcus aureus MS149</strong></td>
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<tr>
<td>0.5% NaCl</td>
<td>Skim milk</td>
<td>140-155</td>
<td>2.016</td>
<td>2.042</td>
<td>0.365</td>
<td>0.355</td>
<td>13.158</td>
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<td>140-155</td>
<td>3.289</td>
<td>3.281</td>
<td>0.287</td>
<td>0.394</td>
<td>10.870</td>
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<td>3.289</td>
<td>3.281</td>
<td>0.287</td>
<td>0.394</td>
<td>10.870</td>
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<tr>
<td><strong>S. aureus 196E</strong></td>
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<tr>
<td>0.5% NaCl</td>
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<td>140-155</td>
<td>2.247</td>
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<td>0.264</td>
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<td>140-155</td>
<td>6.667</td>
<td>6.871</td>
<td>0.276</td>
<td>0.405</td>
<td>8.130</td>
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<td>140-155</td>
<td>3.135</td>
<td>3.444</td>
<td>0.230</td>
<td>0.280</td>
<td>9.174</td>
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^a Tests were conducted at 5 F intervals over the indicated range.

^b Time, in minutes, at the indicated temperature required to reduce the inoculum by 90%. Figures given are results of pooling data from similar experiments.

^c $D$ values calculated from the regression equation for each decimal reduction time curve.

^d Number of degrees F required for the passage of the decimal reduction time curve through one log cycle.
coefficients, or slopes, were responsible for the significant $F$ ratios. For several sets of experimental data, the regression method of determining the slope was compared with the formula $K = 1/T$ \((\log_{10}N_0 - \log_{10}N_T)\) (15) where: $K$ = the slope, a constant; $T$ = exposure time in minutes; $N_0$ = number of organisms present at beginning of time interval; and $N_T$ = number of organisms remaining at end of time interval. There was excellent agreement between slope determinations obtained by the two methods. However, the regression method was chosen, in spite of the somewhat more laborious calculations involved, since it utilized all of the information obtained in each experiment.

As can be seen in Table 1, the $D$ values obtained experimentally agree closely with the values calculated from the regression equations for each decimal reduction time curve. $Z_D$ values (reciprocal of $b$, the regression coefficient), with the exception of that for $S. aureus$ MS149 tested in $0.5\%$ NaCl, fell within the $10 \pm 2\%$ ($5.6 \pm 1.1\,\text{C}$) range. No significant heterogeneity of $Z_D$ values was found, either among the media tested or between the two bacterial genera. For the salmonellae, an overall $Z_D$ of 10.753 was obtained and for the staphylococci, 9.901; the combined $Z_D$ was 10.309 ($F = 1.56$, degrees of freedom $= 3$, 43). Those test substances containing particulate materials in uniform suspension (milk and pea soup) generally gave somewhat higher $D$ values. Thermal protection, in terms of $Z_D$, was greater in several instances in the other media, though the differences, as has been noted, were not statistically significant. Collins et al. (5), using a strain of Micrococcus, and Read et al. (14), working with $Escherichia coli$, found good agreement between $Z_D$ values established at temperatures below 65.6 C and results obtained above 76.7 C. This study, covering intermediate temperatures, also adds evidence to support the hypothesis that, for practical purposes, thermal destruction curves are of a logarithmic nature, at least over the temperature range from 54.4 to about 76.7 C.

No corrections were made in this work for heating or cooling lag, the assumption being that the 0.5 ml of inoculating suspension would be heated essentially instantaneously when added to 300 ml of medium. Sample time intervals at the lower temperatures were long enough that the short cooling time involved would be insignificant. Sampling intervals at the highest temperatures covered were as short as 6 sec; however, good reproducibility between experiments and close fit to calculated curves would seem to indicate that the values obtained reflect no great error introduced by cooling lag.

Since 140 F (60 C) is a reference temperature frequently employed in reports of thermal death time studies, $D_{140}$ values for the various sets of conditions are included in Table 1 for comparative purposes. Figure 1 illustrates, though, that smaller errors due to extrapolating to higher temperatures result when $D_{140}$ is used as a reference value for positioning a decimal reduction time curve; hence, these values have also been included in Table 1. The central line in Fig. 1 represents the hypothetical slope of 10, and the upper and lower pairs of lines are based upon the two extreme $Z_D$ values, 13 and 8, respectively, obtained in these experiments. If the slope for a given organism in a particular substrate actually is 13, extrapolating from a $D_{140}$, assuming $Z_D = 10$, to obtain a process time at 160 F will lead to an error about twice as great as when $D_{140}$ is used, and the probability of surviving bacteria is correspondingly greater. Where the nature of the food to be processed makes time a critical factor, obviously the errors involved with slopes less than 10 become important as well, although it is to be hoped that a margin of safety in de-

![Fig. 1. Illustration of possible errors in process time calculations resulting from extrapolating a decimal reduction time curve with $Z$ greater or less than 10 F. Symbols: extrapolation based on (O) $D_{140} = 10\,\text{min}; (\bullet) D_{150} = 1\,\text{min}.$]
The findings of S. senftenberg 775W make it a good reference strain for establishing D values, because the heat treatments necessary to eliminate it are almost certain to kill other salmonellae and staphylococci present, thus providing a “built-in” safety factor. The food processor must have a good estimate of the numbers of organisms which are to be destroyed as well as a reference D value in the type of food to be treated before a valid heating time can be calculated. A D<sub>160</sub> of 1 min for S. senftenberg 775W, for instance, would lead to the adoption of 0.1 min at 160°F for a 90% reduction in numbers. In 0.2 min, 99% would be killed, and so on to the desired degree of destruction.

Where the food to be processed contains relatively large solid particles, or incorporated air bubbles, extra caution should be exercised in establishing thermal process times, since such factors may alter the ideal upon which calculations are normally based. It should be emphasized, too, that the most careful and elaborate attempts to obtain safe time-temperature relationships for destroying bacterial contaminants in food must be accompanied by high sanitary standards at all stages of preparation. It is of little use to prepare a safe item for consumption, only to have it recontaminated before it reaches the consumer.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research grant EF-00245 from the Division of Environmental Engineering and Food Protection, and by Hatch project No. 264.

LITERATURE CITED


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