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Inactivation of Salmonellae in Autoclaved Ground Beef Exposed to Constantly Rising Temperatures[1]

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ABSTRACT

Inactivation of a composite of five serotypes of salmonellae was monitored in autoclaved ground beef exposed to constantly rising temperatures increased at rates similar to those used in beef cookery. Rising temperature rates of 6.0 C/h, 8.5 C/h and 12.5 C/h and constant temperatures of 55, 57, 61 and 63 C were examined. Survival of *Salmonella typhimurium* TM-1 was compared to survival of the composite. D and z values were compared for constant and rising temperature rates. The $D_{50\,C}$ for constant temperature data was 30.2 min, and the $D_{50\,C}$ for changing temperature data was 78.6 min (6.0 C/h), 82.4 min (8.5 C/h), and 49.8 min (12.5 C/h). Neither serotype nor heat treatment of ground beef had a major influence on apparent heat resistance of salmonellae. A comparison of these results to previous rising temperature work with *Clostridium perfringens* suggested that controlling *C. perfringens* will result in control of salmonellae. On the basis of these results, the July 18, 1978, USDA processing ruling appears adequate to control salmonellae in precooked beef roasts.

The increased incidence of human salmonellosis contracted from precooked roasts of beef in the last three years has been of major public health concern (*6*). In the 1975 outbreak caused by *Salmonella saint paul* (*11*), the beef had been injected with a spice mix in preparation for cooking. Subsequent cases have involved intact roasts where no such procedures were used, yet salmonellae were isolated from external and internal surfaces of the roasts (*13*). Indications were that the roasts were not being cooked to internal temperatures greater than 54.4 C (130 F) (*13*). For example, in one outbreak the roasts were removed from the oven and the internal temperature was allowed to rise to only 51.7 C (125 F) (*12*). Ten outbreaks with 110 cases of salmonellosis were attributed to roast beef in 1976 and 1977 (*1*). Several of the *Salmonella* serotypes involved in these outbreaks, including *Salmonella newport, Salmonella chester, Salmonella typhimurium, Salmonella waycross,* and *Salmonella bovis-morbificans*, were isolated from unopened precooked roast beef (*14*).

In an effort to eliminate this public health hazard, the USDA passed a ruling on September 2, 1977, requiring processed roasts to be cooked to a minimum internal temperature of 63 C (145 F) in all parts of the roast (*1*). This ruling was intended to control potential salmonellosis due to roast beef. However, many consumers prefer rare roast beef which is difficult to produce at this temperature. A new ruling was proposed on May 2, 1978 (*2*), to provide alternative times and temperatures to the first ruling, and the final version of this ruling was published on July 18, 1978 (*3*).

Much of the work which was the basis for these USDA proposals dealt with inactivation of salmonellae during timed exposures to constant temperatures (*7,16*). However, these constant temperatures may not represent the actual heat treatment incurred in precooked roasts of beef.

The objectives of this research were to study inactivation of representative serotypes of salmonellae while increasing the temperature at rates which are representative of beef cookery over a range of cooking temperatures, to compare the results with previous work on *Clostridium perfringens* by Willardsen et al. (*22*), and to use the data to evaluate the recent USDA proposals.

MATERIALS AND METHODS

Test organisms

Salmonella typhimurium Tm-1 was obtained from J. A. Garibaldi, USDA, Western Regional Research Laboratories, Albany, CA. *Salmonella saint paul, Salmonella newport,* and *Salmonella waycross* were obtained from the Center for Disease Control, Atlanta, GA. *Salmonella heidelberg* was obtained from the food microbiology culture collection, Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN. The five serotypes selected for this study were intended to be representative of strains implicated in salmonellosis. The first four serotypes have been implicated in outbreaks from roast beef.

Culture preparation

The stock culture was prepared by transferring one loopful of inoculum into 10 ml of brain heart infusion (BHI; BBL). This was incubated at 37 C for 24 h. From this culture, 1% inoculum was transferred into 10 ml of BHI and stored at - 20 C.

Test cultures were prepared according to the method of Bayne et al. (*9*). The stock culture was thawed as needed and incubated 24 h at 37 C. From this culture, 1% inoculum was placed in 100 ml of Trypticase Soy Broth (BBL) with 2% yeast extract (BBL). This was incubated with shaking at 150 rpm and 37 C (New Brunswick Scientific, Gyrotory Water Bath Shaker, Model G76) for 72 h. After 72 h, the cells were centrifuged at 3000 × *g* for 10 min and were then washed twice with sterile 1% NaCl. After washing and resuspension in 1% NaCl, a composite of the five strains was prepared by combining 2 ml of each culture into a 25 × 150-mm screw-capped test tube and mixing for 10 sec with a Maxi-Mix. (Maxi-Mix, Model No. M16715, Thermodyne, Dubuque, IA).

Media

The ground beef was prepared in the laboratory according to the

[1]*Paper No. 10.505, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul, MN 55108.*

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method of Willardsen et al. (*22*) from beef chucks using 0.5-mm and 3.2-mm grinder plates in succession and was frozen at - 30 C for a maximum of 8 months. Three batches (fat content 23.9, 17.0 and 20.5%) were used during this research. A 15-g portion of ground beef was rolled into a cylindrical shape, tamped with a glass rod (8-mm diameter) into 25 × 150-mm screw-capped test tubes (Pyrex No. 9825) and autoclaved at 121 C for 15 min. After cooling overnight, the tubes were inoculated with 0.1 ml of inoculum using a 15-gauge needle 13 cm in length.

Xylose lysine agar (XL) and xylose lysine deoxycholate agar (XLD) (Difco) were used as enumeration media. Plates were poured 2 days before use and held at room temperature to ensure dryness.

Apparatus

A constant-temperature water bath (Blue M Model MW-1140E-1) was supplemented with a variable wattage heater-circulator (Haake Model E-52) to allow control of rising temperature rates. Water bath temperatures were monitored on a strip chart temperature recorder (Honeywell Model 153X64P16-X-40) and an electronic probe thermometer (Fluke 2100A Digital Thermometer) was used to measure all temperatures of the samples reported in this study.

Temperature treatments and determination of constant

Rising temperature rates of 6.0, 8.5, and 12.5 C/h were selected to simulate cooking rates used in the home or in the meat processing and foodservice industries. Samplings were taken frequently between 47 C and 60 C where the most inactivation effects were expected to occur. Because *S. typhimurium* Tm-1 has been reported to be one of the most heat resistant serotypes (personal communication, J. A. Garibaldi, Western Regional Research Laboratory, Albany, CA 94710), it was studied separately at the same rising temperature rates to determine its contribution to the composite's response.

Constant temperature inactivation of *S. typhimurium* Tm-1 at 55, 57, 61 and 63 C also was measured. D and z values were compared between constant and changing temperature data. Data were calculated and plotted by computer to present curves of best fit (*19*).

Enumeration procedure

Constant-temperature samples were placed in an ice bath immediately after removal from the heating bath. Changing-temperature samples were not chilled but immediately transferred as follows. A sterile 23-cm chrome letter opener was used to transfer the sample into a 18 × 30-cm, 3 ½-mil polyethylene bag. The sample was diluted to 100 g with sterile distilled water (85 ml added), and macerated for 30 sec in a Colworth 400 Stomacher (*21*). Serial dilutions were made into 9.9 ml of 0.1% peptone (Difco), spread plated on XL or XLD agar and incubated for 48 h at 37 C.

RESULTS AND DISCUSSION

Previous work has indicated that a time-temperature relationship exists for inactivation of salmonellae (*4,5*). Much of the work that was the basis for the USDA proposals was based on inactivation of salmonellae exposed to various constant temperatures for several periods (*7,16*). However, as discussed by Willardsen et al. (*22*), food does not remain at any single constant temperature in most home, industrial, and institutional food handling, preparation, processing and storage procedures. Pre-cooked beef roasts are an excellent example of a changing temperature process.

Although studies have indicated that surface contamination of salmonellae can result in survival more frequently than that observed with internal contamination (*8,10*), the effect of surface contamination was not considered in this study.

A representative temperature - based inactivation curve for salmonellae exposed to constantly-rising temperatures is presented in Fig. 1. The data show inactivation of a composite of five serotypes of salmonellae in autoclaved ground beef at temperatures rising at a rate of 6.0 C/h. The N_0, or initial population, was developed using an average of colony forming units (CFU) in samples from 25 to 46 C. This temperature range was used because no inactivation effects were observed in this interval. N was the CFU at each sampling time and N/N_0 is plotted vs. temperature (C). No inactivation occurred until a temperature of approximately 51 C was attained and at 60 C less than 0.0001% survival was observed. This was the most inactivation observable within the sensitivity of this experiment.

Figure 2 shows salmonellae inactivation at 8.5 C/h and is similar to the curve in Fig. 1. Inactivation appears to begin at 51 C and is below the measurement threshold at 60 C. The solid line was computer-drawn based on kinetic parameter values determined by the least squares method. The square of the vertical distance between the line and the data points has been minimized in fitting the curve. This method, first order kinetics and least squares, results in some variation between the computer

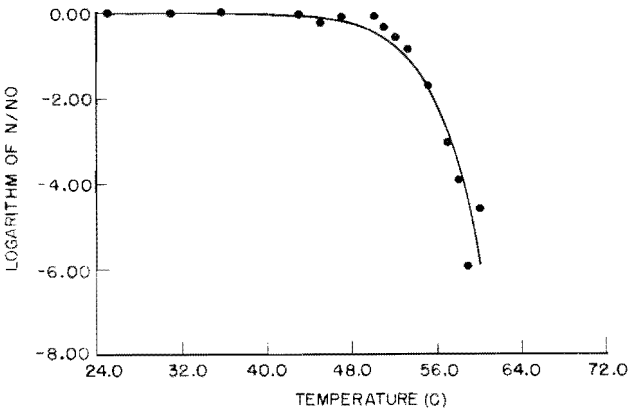

Figure 1. *Survival of five-strain composite of salmonellae exposed to constantly rising temperatures at 6.0 C/h in autoclaved ground beef. N/N_0 determined from CFU/g data. Curve plotted with computer assistance.*

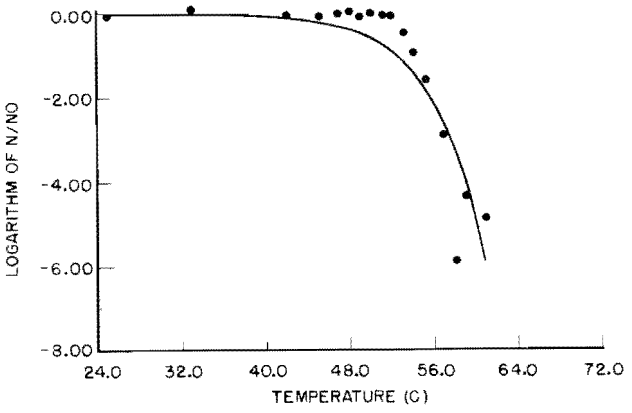

Figure 2. *Survival of five-strain composite of salmonellae exposed to constantly rising temperatures at 8.5 C/h in autoclaved ground beef. N/N_0 determined from CFU/g data. Curve plotted with computer assistance.*

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line and actual data points.

Figure 3 demonstrates inactivation at 12.5 C/h. This curve also compares well with the previous curves, with inactivation beginning at 51 C and being beyond minimal levels at 60 C.

All trials were done in duplicate. Figure 4 shows trial reproducibility with duplicate curves from the 8.5 C/h temperature rate trials. The variation between curves was not unexpected for the small number of trials.

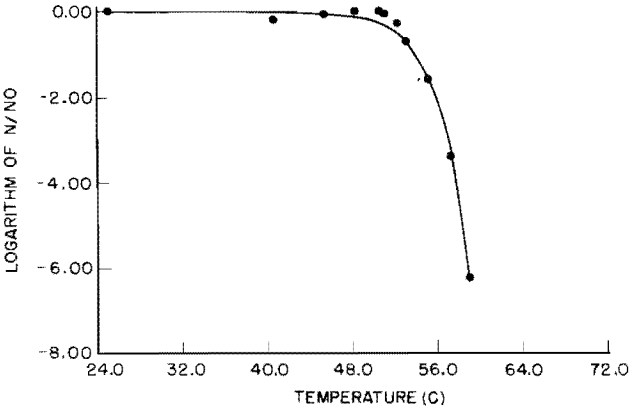

Figure 3. *Survival of five-strain composite of salmonellae exposed to constantly rising temperatures at 12.5 C/h in autoclaved ground beef. N/N_0 determined from CFU/g data. Curve plotted with computer assistance.*

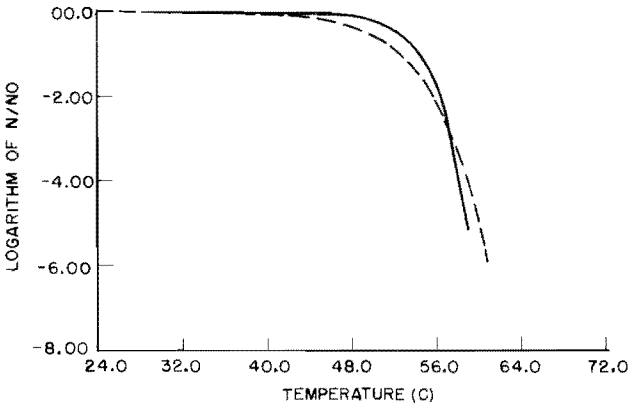

Figure 4. *Survival pattern in duplicate trials of five-strain composite of salmonellae exposed to constantly rising temperatures at 8.5 C/h in autoclaved ground beef. Curves generated with computer assistance from CFU/g data.*

S. typhimurium Tm-1 has been the subject of many thermal inactivation studies (*18*). Its heat resistance when grown under the appropriate conditions is greater than the other strains used in this study (personal communication, J. A. Garibaldi, Western Regional Research Laboratory, Albany, CA 94710). It was studied separately at all three rising temperature rates to determine its contribution to the composite's response. A comparison (Fig. 5) reveals little difference between the two curves, since inactivation began at approximately the same temperature and continued at similar rates. This seems to indicate that all five of the composite organisms had similar heat resistance, which could be due to growing the organisms to the late stationary phase through the 72-h incubation. Late stationary cells are the most heat resistant cells (*17,20*).

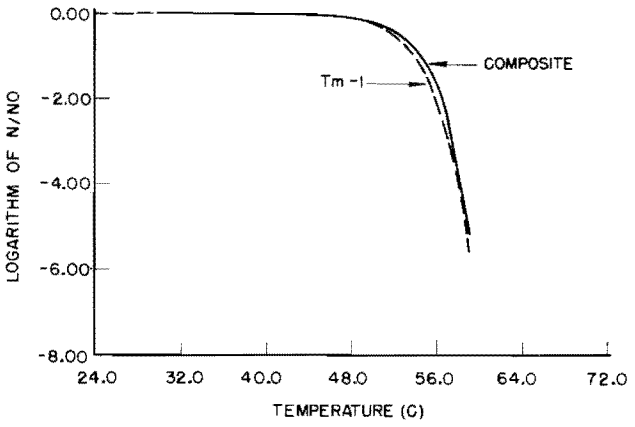

Figure 5. *Survival of* Salmonella typhimurium *Tm-1 compared to survival of five-strain composite of salmonellae exposed to constantly rising temperatures at 8.5 C/h in autoclaved ground beef. Curves generated with computer assistance from CFU/g data.*

To determine the influence of autoclaving, raw ground beef was compared to autoclaved ground beef. In both constant and changing data, heat resistance of *S. typhimurium* Tm-1 in raw ground beef, was consistently equal to or less than that in autoclaved ground beef (Fig. 6). This indicates that the D-values found in autoclaved beef should be an adequate prediction for raw beef products.

Constant-temperature inactivation was studied in duplicate at 55, 57, 61 and 63 C with examples of trials at 57 and 63 C shown in Fig. 7. The time axis for the 57-C curve is 10 times the scale of the time axis for the 63-C curve. All constant temperature survivor curves showed this definite tail. A culture produced from a survivor isolated at the end of one of these tails was used in a special separate study. No mutation or heat resistant substrain was observed because data from this survivor isolate were identical to those obtained originally,

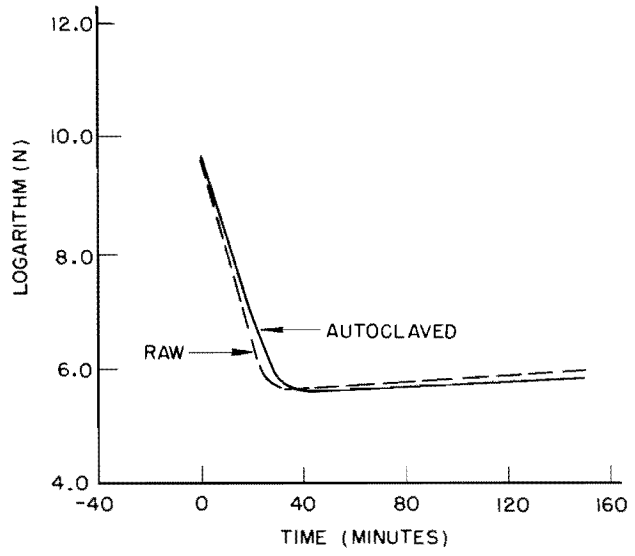

Figure 6. *Survival of* Salmonella typhimurium *Tm-1 exposed to 55 C in raw ground beef (broken line) or autoclaved ground beef (solid line). Curves generated with computer assistance from CFU/g data; natural logarithm of N represents conversion of CFU/g data.*

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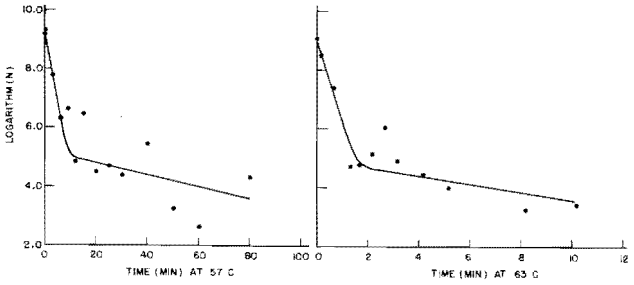

Figure 7. *Comparison of survival of* Salmonella typhimurium *Tm-1 exposed to 57 C (left curve) vs. 63 C (right curve). Curves generated with computer assistance from CFU/g data; natural logarithm of N represents conversion of CFU/g data.*

including the tail. This tail could be due to heterogeneity of heat resistance resulting from growing the cells over 72 h (*15*).

Table 1 presents D, k and R^2 values for both temperatures (57 and 63 C). D is the time of heating at a specific temperature to result in 10% survival, k is the rate constant for inactivation in min^{-1}. D values of 2.130 and 2.667 min were observed at 57 C and these corresponded to rate constants (k) of 1.081 and 0.863 min^{-1}. For 63 C the D was 0.359 min and the rate constant was 6.413 min^{-1}. Only values from the first segment of each curve are presented because of the small R^2 values calculated for the second segment or tail portion of the curve.

D, z, k (rate constant), and E (activation energy) values were developed as averages from all the constant temperature curves (Table 2) by computer analyses. The z value represents the degrees Celsius required to change the D value tenfold. The first and second segments of the curves were considered separately because of the large difference between the two segments (Table 2). For example, the first segment has a $D_{50\ C}$ value of 30.2 min, and the tail segment has a $D_{50\ C}$ value of 818.6 min.

Table 3 shows D, z, the rate constant (k) and activation energy (E) for each changing temperature trial. The differences between replicate trials did not appear to be significant, especially considering the small number of

TABLE 1. Salmonella typhimurium *Tm-1 inactivation kinetics at constant temperatures.*

Temperature	D[a] (min)	k[b] (min^{-1})	R^2
57 C	2.130	1.081	0.996
	2.667	0.863	0.949
63 C	0.359	6.413	0.967

[a]D = the time of heating at a specific temperature to result in ten percent survival.
[b]k = the rate constant in $min.^{-1}$

TABLE 2. Salmonella typhimurium *Tm-1 inactivation kinetics at constant temperatures from 55 to 63 C.*

Segment	$D^a_{50\ C}$ (min)	z[b] (C)	k[c] (min^{-1})	(J/mol)E[d]
1	30.2	6.5	0.1404	326,671.2
2	818.6	5.4	0.0058	388,914.3

[a]D = the time of heating at a specific temperature to result in ten percent survival.
[b]z = the degrees Celsius required to change the D-value tenfold.
[c]k = the rate constant in $min.^{-1}$
[d]E = activation energy in J/mol.

TABLE 3. Salmonella typhimurium *Tm-1 inactivation kinetics at changing temperatures.*

Temperature rise, Rate (C/h)	$D^a_{50\ C}$ (min)	z[b] (C)	k[c] (min^{-1})	(J/mol)E[d]
6.0	78.6	9.9	0.045	202,395
8.5	82.4	6.7	0.053	301,938
12.5	49.8	9.9	0.071	202,395

[a]D = the time of heating at a specific temperature to result in ten percent survival.
[b]z = the degrees Celcius required to change the D-value tenfold.
[c]k = the rate constant in $min.^{-1}$
[d]E = activation energy in J/mol.

trials. $D_{50\ C}$ values did not vary significantly between the composite trials and *S. typhimurium* Tm-1. Average $D_{50\ C}$ values were 78.6 min for 6.0 C/h, 82.4 min for 8.5 C/h and 49.8 min for 12.5 C/h. The k and E values are also listed. Although mean values of z and E appear to be different among the three heating rates, they could not be statistically differentiated because each value is the average of duplicate determinants.

The constant-temperature data were used to generate or predict changing-temperature curves with computer assistance (Fig. 8). The curve from data on the composite cultures indicates that the rate constants for constant-temperature could not be used to predict the changing-temperature curves. The predicted curve consistently fell short of the actual data. The large difference between the two curves is on the ordinate (N/N_0). The break at the end of the computer-generated curve is due to the effect of the constant-temperature tails.

A comparison of $D_{50\ C}$ values between the changing and constant temperature data shows a considerable difference (Tables 2 and 3). The $D_{50\ C}$ value of 30.2 min at constant temperatures is smaller than any of the $D_{50\ C}$ values for the changing temperature data.

Table 4 shows a comparison of D values observed in four different inactivation media. Evaluation of the results shows that autoclaved ground beef resulted in a higher D value than any of the other three inactivation media. No explanation for this observation was readily evident; however, it did support the earlier statement on

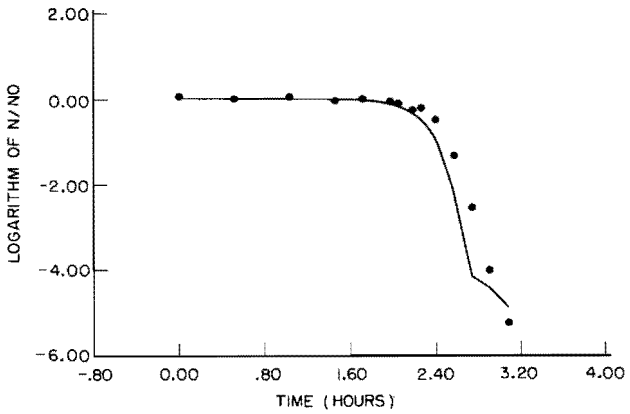

Figure 8. *Theoretical survival curve generated by computer from constant temperature data used to predict survival of* Salmonella typhimurium *Tm-1 exposed to constantly rising temperatures at 8.5 C/h in autoclaved ground beef. Circles are actual data points. N/N_0 determined from CFU/g data.*

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TABLE 4. *Comparison of inactivation suspending media on survival of* Salmonella typhimurium *Tm-1*.

Inactivation medium	$D^a_{55\,C}$ (min)	$D^a_{57\,C}$ (min)
Autoclaved ground beef	8.151	—
Typticase Soy Broth with 2% Yeast Extract	8.039	1.432
Brain Heart Infusion	6.618	1.290
Phosphate buffer (pH = 7.0)	—	1.133

[a]D = the time of heating at a specific temperature to result in ten percent survival.

Fig. 6 that data obtained in autoclaved ground beef were useful in evaluating a raw beef system.

A comparison of the heat resistance of cultures incubated for 24, 48 and 72 h with shaking under constant conditions was also made (data not presented). D values showed little difference between the 48-h ($D_{57\,C}$ = 4.095 min) and 72-h ($D_{57\,C}$ = 3.897 min) culture. The $D_{57\,C}$ value for 24 h ($D_{57\,C}$ = 3.149 min) was significantly lower than the other two D values.

A comparison of these *Salmonella* data was made to work done previously with changing temperatures (Fig. 9). The data on growth and survival of *C. perfringens*, an organism also linked with foodborne illness, were reported for changing temperatures by Willardsen et al. (*22*). A comparison of these two curves indicates that if *C. perfringens* is controlled, the *Salmonella* cells are also inactivated. *C. perfringens* growth, which was initiated at about 40 C in this study, ceased at about 55 C, and a large number of *C. perfringens* cells survived above 60 C when exposed to rising temperatures at 8.5 C/h.

Thermal inactivation data from several studies are summarized in Fig. 10. The USDA values are taken from the July 18, 1978, processing ruling (*3*). The USDA values are based on 7D levels of inactivation. The ABC data are D values from the report on fate of salmonella inoculated into beef for cooking cited in the USDA proposal (*7,16*). The changing-temperature curves are based on the D and z values presented above and the changing temperature rate is indicated on each curve. D values

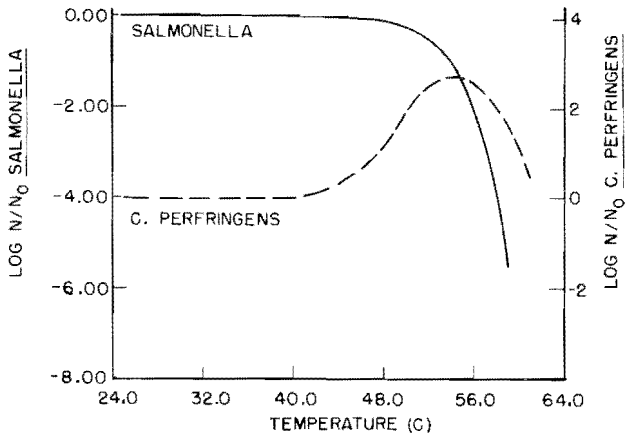

Figure 9. *Comparison of survival of* Salmonella typhimurium *Tm-1 to growth and survival of* Clostridium perfringens *exposed to constantly rising temperatures at 8.5 C/h in autoclaved ground beef. Data on* C. perfringens *from Willardsen et al. (22).*

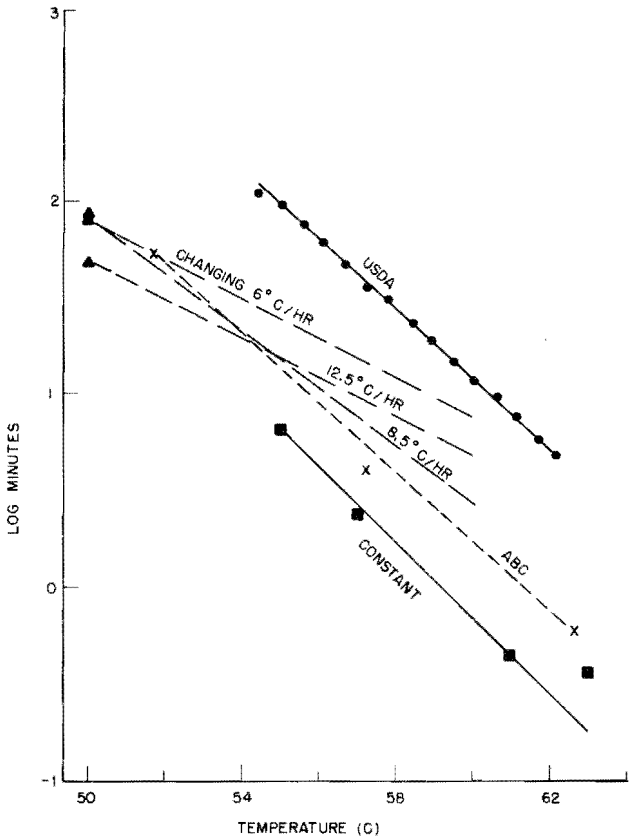

Figure 10. *Comparison of inactivation constants and relationships: (a)* CONSTANT *curve from D values for Salmonella typhimurium Tm-1 exposed to constant temperatures, (b) CHANGING 6 C/h, 8.5 C/h, 12.5 C/h curves from D values for* Salmonella typhimurium *Tm-1 exposed to changing temperatures, (c) ABC curve from D values on salmonellae survival by American Bacteriological and Chemical Research Corporation (7,16), (d) USDA curve from minimum times at indicated temperatures published as cooking requirements by USDA* (3).

from the constant-temperature data are plotted as indicated. Evaluation of these data indicates that the USDA values should be sufficient to inactivate *Salmonella* cells in beef, although our data would suggest that a 7D process might not always be attained with changing temperatures.

On the basis of this work, several observations can be made. By computer simulation, constant-temperature data do not generate curves identical to those observed with changing temperatures. Neither serotype nor heat treatment of ground beef had a major influence on apparent heat resistance of salmonellae. The information used to develop the USDA ruling appears adequate and the prescribed treatments should inactivate *Salmonella* cells in precooked beef roasts. If the growth and survival of *C. perfringens* are controlled, the salmonellae are inactivated as well.

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How CPR training helps business.

CPR—cardiopulmonary resuscitation training is one of the valuable assets an employee can have.

If even one employee has this training, (which is available from Red Cross), every other co-worker benefits. Everybody can breathe easier knowing that in the event of a cardiac arrest, help is immediately available.

Why not set up a goal for your company... so many employees with CPR training per floor—or area?

It's easy to do. Call your Red Cross Chapter... they'll be glad to help you do it.

CPR training from Red Cross is one way you can help your company be ready. And a way you can help keep Red Cross ready, too.

Keep Red Cross ready.

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