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Influence of Heat Transfer with Tube Methods on Measured Thermal Inactivation Parameters for *Escherichia coli*

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ABSTRACT

The purpose of this study was to investigate the influence of heat transfer on measured thermal inactivation kinetic parameters of bacteria in solid foods when using tube methods. The bacterial strain selected for this study, *Escherichia coli* K-12, had demonstrated typical first-order inactivation characteristics under isothermal test conditions. Three tubes of different sizes (3, 13, and 20 mm outer diameter) were used in the heat treatments at 57, 60, and 63°C with mashed potato as the test food. A computer model was developed to evaluate the effect of transit heat transfer behavior on microbial inactivation in the test tubes. The results confirmed that the survival curves of *E. coli* K-12 obtained in 3-mm capillary tubes were log linear at the three tested temperatures. The survival curves observed under nonisothermal conditions in larger tubes were no longer log linear. Slow heat transfer alone could only partially account for the large departures from log-linear behavior. Tests with the same bacterial strain after 5 min of preconditioning at a sublethal temperature of 45°C revealed significantly enhanced heat resistance. Confirmative tests revealed that the increased heat resistance of the test bacterium in the center of the large tubes during the warming-up periods resulted in significantly larger *D*-values than those obtained with capillary tube methods.

Thermal processing is one of the most effective methods of reducing or eliminating food spoilage and pathogenic bacteria in foods *(35, 38)*. Reliable thermal processing calculations require an understanding of the thermal inactivation kinetics of microorganisms. Thermal inactivation has conventionally been assumed to follow first-order reaction kinetics when developing industrial thermal processes *(14, 34, 35)*. First-order thermal inactivation of microorganisms is characterized by semi–log-linear behaviors observed in microbial survival curves under isothermal conditions. *D*-values (decimal reduction times at constant temperatures) and *z*-values (changes in *D*-values with temperature) are used to describe the impact of lethal temperatures on a microbial population. However, numerous articles report non–log-linear behaviors of microorganisms *(4, 15, 16, 29)*. Even in studies in which *D*-values were used to characterize heat resistances of microorganisms, often only the linear portion of the survival curves were analyzed because of a certain degree of nonlinearity in the overall curves. Some of those non–log-linear behaviors may reflect the intrinsic thermal inactivation characteristics of the microorganisms in consideration, but others are merely an artifact of the test methods.

Results from different studies concerning the heat resistance data of the same microorganisms are sometimes conflicting and indicate differences in thermal death kinetics of the same bacteria under different test conditions *(3, 10, 32, 37)*. For example, Sorqvist *(32)* reported that the *D*-values obtained in 9-mm test tubes were about 8 to 29 times larger than those obtained with a capillary tube method for *Yersinia enterocolitica* strains at 60°C in physiological saline. The large variations in measured heat resistance of bacteria can be caused by nonisothermal test conditions. Different tests methods have been developed to eliminate possible artifacts caused by those conditions. Those methods include multiport flasks, thermoresistometers, and capillary tubing for liquid media *(1, 2, 19, 20, 26, 28)*. However, tube methods are commonly used for solid media *(6, 8)*.

Slow conductive heat transfer in a solid medium may cause a significant delay in heating the sample core in large test tubes, resulting in severe nonisothermal conditions during the warm-up period. Surface heating of the tubes also causes a sharp drop in microbial population close to the tube walls, rendering the distribution of the microorganisms nonuniform when the same sample core reaches the desired test temperature. The effect of heat transfer on thermal inactivation behavior may be studied with a mathematical model that combines the calculation of transit heat transfer in a solid medium with bacterial inactivation kinetic information obtained under isothermal conditions.

Failure to fully explain the non–log-linear behavior by computer simulations as presented later in this article inspired us to examine the influence of preconditioning on the bacterial population reduction in large tubes. This was based on reports that microorganisms exposed to a slowly changing environment develop an increased thermotolerance similar to the induction of the heat-shock response *(24, 33)*. Researchers have reported increased thermotolerance of microorganisms with heating rates below or equal to 0.7°C min^{-1} *(33)* and by preconditioning at 45 to 50°C for 5 to 60 min *(7, 28, 40)*.

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TABLE 1. *Geometry of tubes and samples*

Tube type	Outside diam (mm)	Tube height (mm)	Tube wall thickness (mm)	Sample height (mm)
Capillary (3 mm)	3	150	1.00	130
13 mm	13	100	1.15	60
20 mm	20	125	1.25	80

The goal of this study was to investigate the influence of heat transfer on heat resistance characteristics of the selected bacterium, *Escherichia coli* K-12, which has demonstrated log-linear inactivation kinetics under isothermal conditions. Specific objectives were to (i) determine *D*- and *z*-values of *E. coli* using three tubes of different sizes, (ii) conduct computer simulations to provide insight into the influence of heat transfer on *D*- and *z*-values determined with the three tubes of different sizes, and (iii) provide an explanation for non–log-linear survival curves using the results from preconditioned *E. coli* samples and confirmative tests with capillary tubes in the center of 20-mm tubes.

MATERIALS AND METHODS

Microbial experiment: cultures and cell suspension. The *E. coli* K-12 strain was obtained from the Food Science Department of Washington State University (Pullman). In our preliminary tests, this strain demonstrated clear first-order inactivation kinetics under isothermal heat treatment conditions and therefore was selected for the study. Stock cultures were made in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.) with 15% (vol/vol) glycerol and stored at −10°C. Active cultures for experiments were prepared by streaking material from the frozen cultures onto tryptic soy agar (TSA; Difco, Becton Dickinson) plates. Single colonies were transferred to TSB and incubated at 37°C for 24 h. Because microorganisms are more heat resistant in the stationary phase than in other phases *(25),* the *E. coli* K-12 strain in stationary phase was chosen. Following overnight incubation at 32°C, a homogeneous 1-ml aliquot was removed from the broth culture. The cells were harvested by centrifugation (3 min at 13,000 × *g*) and washed in an equal volume of sterile 0.1% (vol/vol) buffered peptone water (Difco, Becton Dickinson). After a second washing, the cell fractions of *E. coli* K-12 were resuspended to approximately 10^9 cells per ml in sterile 0.1% buffered peptone water. Mashed potato was chosen as a model semisolid food to eliminate heat convection and facilitate placement into small capillary tubes. One milliliter of the culture was mixed with 99.0 ± 0.2 g of mashed potato, which was prepared with 15.38% (wt/wt) potato flakes (Washington Potato Co., Warden) with sterile water.

Microbial experiment: reference method. Glass capillary tubes (Rupe Zellner Science Kit Inc., Tonawanda, N.Y.) were heat sealed with flame at one end and sterilized before the samples were added. The dimensions of the test tubes are listed in Table 1. Glass capillary tubes commonly used to test heat resistance of culture suspensions are microhematocrit tubes with an inside diameter range of 0.9 to 1.8 mm (Fisher Scientific, Pittsburgh, Pa.). We selected medium-size capillary tubes with an inside diameter of 1 mm and an outside diameter of 3 mm. Mashed potato samples (0.25 ± 0.2 g each) inoculated with *E. coli* K-12 was carefully injected into the tubes using a sterilized syringe with an 18-gauge, 15.2-cm pipetting needle (Popper & Sons, Inc., New Hyde Park, N.J.). The open ends of the tubes were heat sealed about 20 mm from the sample. Sets of capillary tubes were heated in a water bath at 57, 60, and 63°C and removed after 0.5, 1.5, 3, 4, and 5 min at 57°C; 0.5, 1, 1.5, and 2 min at 60°C; and 20, 30, 40, and 50 s at 63°C. After removal from the water bath, the capillary tubes were cooled immediately in ice water and then washed in 70% ethyl alcohol. Both ends of the capillary tubes were cut aseptically, and mashed potato was flushed out with 3 ml of 0.1% peptone water. Heat-treated samples were serially diluted in 0.1% peptone water. As controls, sets of tubes containing unheated mashed potato were handled with the same procedure. Tenfold serial dilutions were made in 0.1% peptone water, and 1 ml of each dilution was pour plated onto TSA in duplicate. The plates were incubated at 32°C for 2 h to allow injured microorganisms to repair and resuscitate *(18).* The plates were then overlaid with 7 ml of a selective medium for *E. coli,* violet red bile agar (Difco, Becton Dickinson). After solidification, plates were incubated for an additional 22 h at 32°C. Following incubation, typical red *E. coli* colonies were enumerated.

Microbial experiment: test tube methods. Thermal inactivation experiments were performed in accordance with previously reported procedures for evaluation of the thermal resistance of bacteria *(5, 12, 21).* Test tubes of two different sizes (outside diameter, 13 and 20 mm) were used to investigate the effect of tube size on the thermotolerance of *E. coli.* The 13- and 20-mm test tubes were filled with 6 and 18 ± 0.2 g of mashed potato inoculated with *E. coli* K-12, respectively (Table 1), using a sterile syringe to avoid coating the inside wall of the tube with mashed potato. Tubes were tightly sealed with lids and immersed in a water bath at 57, 60, or 63°C. Sets of tubes were removed from the water bath at 1- to 2-min intervals. The posttreatment procedures for these larger tubes were the same as those used for the capillary tubes.

The mean viable colony counts for each heating time were log transformed and plotted against time in minutes. *D*-values were obtained by taking the negative reciprocal of the slope from the linear portion of the curve after the come-up time at 57, 60, and 63°C. The *z*-values were determined from the thermal death time curve obtained by plotting log *D*-values against the corresponding heating temperatures. Each experiment was performed twice. The means and standard deviations of *D*- and *z*-values were obtained for the three different sized tubes and treatment temperatures.

Microbial experiment: preconditioning. Capillary tubes with a 3-mm outside diameter were used to reduce the thermal lag effect due to tube size. Two parallel sets of capillary tubes containing mashed potato with the same *E. coli* K-12 culture were prepared following the same procedures described above. One set of capillary tubes was heat shocked by immersion into a water bath at 45°C for 5 min and then cooled immediately in ice water. The pretreatment condition was chosen based on the measured average temperature of internal layers in 13- and 20-mm tubes during the come-up time and the possible optimal development of the heat shock response of mesophilic bacteria reported in the literature *(7, 28, 40).* After preconditioning, the capillary tubes were heat treated at 57, 60, and 63°C as described previously. *D*-values at each temperature were calculated from the survival curves. As controls, samples in the other set of capillary tubes were treated in the same way except that they were not heat shocked at 45°C.

To further investigate the effect of come-up time in large test tubes on the thermal resistance of *E. coli,* additional paired tests were conducted. In those tests, inoculated mashed potato (~10^7

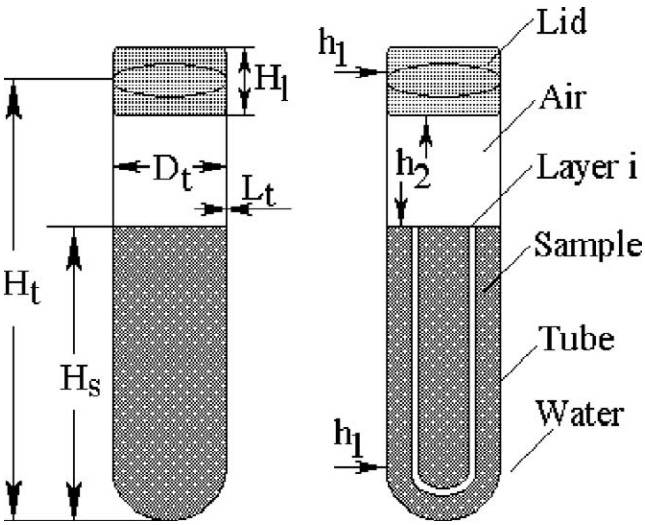

FIGURE 1. *Geometry and heat transfer model in thermal death time tube heating. Abbreviations are defined in the text.*

CFU/g) was placed in capillary tubes as described above. One set of the capillary tubes was used as a control and was directly heated in a water bath at 60°C. For the other set of capillary tubes, each tube was placed individually in the center of 20-mm tubes filled with plain mashed potato (~17.5 ± 0.2 g). The 20-mm tubes were then heated in a water bath at 60°C. After predetermined time intervals, the capillary tubes containing the control samples and the capillary tubes heated inside the 20-mm tubes were removed from the water bath and immediately cooled in ice water. Each experiment was conducted twice.

Computer simulation: heat transfer in test samples. The computer model consisted of heat balance equations for tube wall, sample, head space, and lid (Fig. 1). It included convective heat transfer from hot water to the tube surface, conductive heat transfer through the tube wall into the sample, and heat loss from the head space to the lid. For heat conduction in the radial and axial directions in the cylindrical-shaped samples, the following governing equation was used:

$$\frac{1}{\alpha}\frac{\partial T}{\partial t} = \left(\frac{\partial^2 T}{\partial r^2} + \frac{1}{r}\frac{\partial T}{\partial r} + \frac{\partial^2 T}{\partial y^2}\right) \quad (1)$$

where α is thermal diffusivity ($m^2\ s^{-1}$) and $\alpha = k/\rho C$, where k is the thermal conductivity (W m^{-1} °C^{-1}), ρ is the density (kg m^{-3}), and C is the specific heat (J kg^{-1} °C^{-1}); T is the temperature (°C); t is the time (s); r is the radius (m); and y is the distance along the axial direction (m). The boundary conditions were represented by the following heat convection from the heating medium (water):

$$-k\left.\frac{\partial T_t}{\partial r}\right|_{r=r_0} = h_1\left[T_t(t) - T_w\right] \quad (2)$$

Heat flow at tube surface — Heat flow from heating medium (*Convection to heat tube surface*)

where r_0 is the inner radius of the test tube (m). The heat balances for the lid (l) and head space (a) are described as

TABLE 2. *Thermal properties of different materials in tube heating*

Material	Density, ρ (kg m^{-3})	Specific heat, C (J kg^{-1} °C^{-1})	Thermal conductivity, k (W m^{-1} °C^{-1})	Thermal diffusivity, α ($m^2\ s^{-1}$) × 10^{-7}	Reference
Glass tube	2,483	770	1.090	5.70	*31*
Rubber lid	1,200	2,000	0.156	0.65	*27*
Mashed potato	1,050	3,640	0.563	1.47	*31*
Water	1,000	4,179	0.613	1.47	*31*
Air	1.16	1,007	0.026	222.6	*31*

$$\rho_l C_l V_l \frac{dT_l}{dt} = h_1 A_l (T_w - T_l) + h_2 A_l (T_a - T_l) \quad (3)$$

$$\rho_a C_a V_a \frac{dT_a}{dt} = h_2 A_t (T_t - T_a) + h_2 A_l (T_l - T_a) + h_2 A_s (T_s - T_a) \quad (4)$$

where A is the surface area (m^2); V is the volume (m^3); w, t, l, a, and s represent water, tube wall, lid, head space, and sample, respectively; and h_1 and h_2 are the external and internal surface heat transfer coefficients (W m^{-2} °C^{-1}), respectively. The height of the lid was 0.002 m. Dimensions of the tubes are listed in Table 1, and the thermal properties of the model elements are given in Table 2. We considered a natural convection and laminar flow for both the external heat convection outside the test tubes and the internal heat convection in the head space. The value of h_2 was selected to be 10 W m^{-2} °C^{-1} between the interior air and the sample, and the values of h_1 were selected to be 2,800 W m^{-2} °C^{-1} for both 20- and 13-mm tubes based on published information *(9).* The partial differential equation (equation 1) was reduced to algebraic equations through the finite difference method *(39).* In the simulation, the tubes were divided into 3 and 10 concentric sublayers for the straight portion of the capillary tube and larger test tubes (13 and 20 mm), respectively, and divided into semispherical shells for the bottom portion of the tubes (Fig. 1). The sample in each sublayer was assumed to be at the same temperature. Temperatures of each layer during heat treatments and cooling processes were calculated at a time interval of 1 s with the Gauss-Seidel numerical method.

Computer simulation: heat transfer model validation. The finite difference simulation model was validated by comparing temperature predictions with measured values. The central temperature of uninoculated mashed potato samples at heights of 20, 20, and 30 mm in 3-, 13-, and 20-mm tubes was measured with thin precalibrated Type-T thermocouples (THQSS-020U-6, Omega Engineering Inc., Stamford, Conn.) with an accuracy of ±0.5°C and an 0.8-s response time. Samples in the tubes were heated in a circulating hot water bath (model ZD, Grant, Cambridge, UK) at 60°C until the sample core reached the bath temperature; the tubes were then cooled in ice water. All data were recorded with a data logger (DL2e, Delta-T Devices Ltd., Cambridge, UK) at a time interval of 5 s.

To confirm the sample temperature profiles obtained by the finite difference simulation model, commercial software based on the finite element method (FEMLAB version 3.0, COMSOL AB, Inc., Stockholm, Sweden) also was used to predict the sample temperature profiles in the test tubes. After convergence tests, fine mesh containing 5,124 elements was set for half of the symmetric

tubes. All the parameters including the geometry, boundary, and initial conditions in the software were exactly the same as those used in the finite difference simulation model.

Only the finite difference simulation model validated by FEMLAB and experiments was used to predict the microbial reduction because the FEMLAB software could not be used readily in combination with microbial reduction kinetic information to predict localized microbial reductions.

Computer simulation: prediction of microbial reduction in tubes. The first-order thermal inactivation kinetic model can be expressed as

$$\frac{dN}{dt} = -kN \tag{5}$$

where N represents microbial population, t is the heating time under isothermal conditions (min), and k is the rate constant (min^{-1}). This equation can be integrated into a form more familiar to thermal processing specialists *(14, 34)*:

$$\log N = \log N_0 - \frac{t}{D} \quad \text{or} \tag{6}$$

$$N = N_0 \times 10^{-t/D} \tag{7}$$

where $D = 2.303/k$. The susceptibility of bacteria to heat at a specific temperature is characterized by the value of D, which varies with temperature. Plotting log D-values against temperature often reveals a linear relationship, commonly referred to as the thermal death time curve. A z-value was obtained as the temperature increase needed to result in a 1-log reduction in D-value from the thermal death time curve:

$$z = \frac{T_2 - T_1}{\log D_{T_1} - \log D_{T_2}} \tag{8}$$

where D_T is the value of D measured at temperature T, and T_1 and T_2 are two different temperatures. The z-value is also obtained by the -1/slope of the regression equation of the log D-value against temperature.

Combining equations 7 and 8 yields

$$N(t) = N_0 10^{\{(-t)/[D_{\text{ref}}\, 10^{[T_{\text{ref}} - T(t)]/z}]\}} \tag{9}$$

where D_{ref} is the D-value at a reference temperature T_{ref}. Temperature history $T(t)$ at each sublayer in a test tube for different treatment conditions was calculated with the finite difference computer simulation model based on the heat transfer and balance equations described above. Using D- and z-values determined with capillary tubes that represented close-to-isothermal conditions, changes in microbial population in each time increment (Δt) in each sublayer (i) for a sample in large tubes that experienced nonisothermal conditions were calculated as follows:

$$N(t, i) = N_0(i) 10^{\{(-\Delta t)/[D_{\text{ref}}\, 10^{[T_{\text{ref}} - T(t,i)]/z}]\}} \tag{10}$$

where $N_0(i)$ is estimated from the initial population over the volume of layer i. The total microbial population in the tube at heating time t was obtained by

$$N(t) = \sum_{i=1}^{n} N_0(i) 10^{\{(-\Delta t)/[D_{\text{ref}}\, 10^{[T_{\text{ref}} - T(t,i)]/z}]\}} \tag{11}$$

Statistical analysis. D-values from three tubes subjected to heat treatment with two independent replicated experiments were analyzed. Diluted samples were plated in duplicate. Data were analyzed using the Statistical Analysis System (SAS Institute, Cary, N.C.). Analysis of variance of D-values was then conducted (PROC GLM procedure of SAS) with least square means used to determine significant differences ($P < 0.05$).

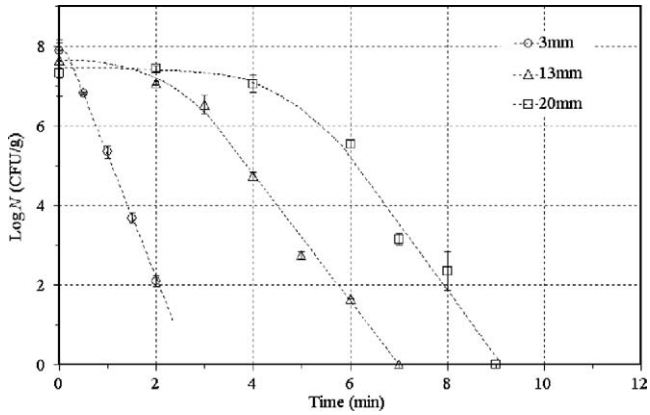

FIGURE 2. *Survival curves at 60°C for* Escherichia coli *K-12 in mashed potato in tubes of three different sizes.*

RESULTS AND DISCUSSION

Heat resistance of *E. coli* for the different methods. Initial populations of *E. coli* in mashed potato analyzed immediately after inoculation were approximately 7.9 ± 0.22, 7.6 ± 0.24, and 7.4 ± 0.21 log CFU/g for the capillary, 13-mm, and 20-mm test tubes, respectively. Figure 2 shows survival curves of *E. coli* in three tubes at 60°C. Similar curves were obtained at 57 and 63°C (data not shown). The capillary tubes took only 0.27 min for the sample core to reach within 0.5°C of the water bath temperature of 60°C (come-up time), providing close to ideal isothermal conditions. The survival curves of *E. coli* in the capillary tubes were log linear at the three tested temperatures, i.e., the thermal inactivation kinetics of *E. coli* K-12 followed first-order kinetics under isothermal conditions.

The come-up times (the time to reach within 0.5°C of the set temperature) for the large test tubes (13 and 20 mm) at 60°C were about 3 and 7 min, respectively, thus resulting in nonisothermal conditions during those periods. A broad shoulder was observed in the survival curves, and the shoulder for the 20-mm tubes was larger than that for the 13-mm tubes (Fig. 2). After an initial period, the survival curve became log linear. D-values were obtained from the log-linear portions of the curves after the come-up time by linear regression ($P < 0.05$).

Average D-values for *E. coli* at 57, 60, and 63°C obtained from the tests with capillary tubes and the larger tubes (13 and 20 mm) are summarized in Table 3. The D-values for *E. coli* in all three tubes decreased sharply as the temperature increased from 57 to 63°C. At each temperature, the D-values in the capillary tubes were 1.6 to 4.5 times smaller than those in the large test tubes ($P < 0.05$). Similar observations have been reported by others *(3, 10, 32)*. The D-values obtained from the capillary tubes should reflect the intrinsic heat resistance of the original bacterial culture for short heat treatments because of the uniform heating.

Figure 3 shows relationships between D-values and

TABLE 3. D- *and* z*-values for* Escherichia coli *K-12 at different temperatures in tubes of different sizes*[a]

Tube type	*D*-values (min)			*z*-value (°C)
	57°C	60°C	63°C	
Capillary (3 mm)	0.96 ± 0.03 A	0.32 ± 0.00 A	0.11 ± 0.01 A	6.48 ± 0.37 A
13 mm	1.50 ± 0.17 B	0.62 ± 0.02 B	0.34 ± 0.01 B	9.41 ± 0.85 B
20 mm	1.62 ± 0.15 B	0.58 ± 0.01 B	0.51 ± 0.00 C	11.98 ± 1.00 B

[a] Values are mean ± standard deviation of two replicate experiments. Within each column, means followed by different letters are significantly different ($P < 0.05$).

temperatures for *E. coli* in the three sizes of test tubes. The *z*-values were obtained by linear regression of the log *D*-values and temperatures ($R^2 \geq 0.830$) and are listed in Table 3. The *z*-values obtained for the 13- and 20-mm test tubes were not significantly different. However, *z*-values obtained for the capillary tubes were 45 to 85% smaller than those for the larger tubes ($P < 0.05$).

Heat transfer model validation. Before using the computation model to explain the large difference in *D*-values observed between samples tested in capillary tubes versus larger tubes at the same temperature, the finite difference model was validated by comparing predicted and measured sample temperatures. Figure 4 shows an example of predicted and measured sample core temperatures in three different tubes heated in hot water at 60°C and then cooled in ice water. The differences (means ± standard deviation) between the measured and simulated sample center temperatures in the 13- and 20-mm tubes were 0.65 ± 0.81°C and 0.30 ± 0.26°C, respectively, when heated at 60°C, during the come-up, holding, and cooling periods. The main difference was observed at the beginning of the come-up period, but the effect of those deviations at low temperatures on the reduction of microbial population was negligible. The mean differences were comparable to the accuracy of temperature measurements with the calibrated thermocouples (±0.5°C).

Figure 5 shows an example of the temperature distribution obtained with the commercial FEMLAB finite element software over the cross section of the sample in a 20-mm tube. The temperatures of the glass tube wall and head space increased rapidly to the set-point temperature, but the sample temperature increased gradually from the outer layer to the center because of slow conduction. The temperature profile confirmed the general shapes of isothermal layers used in the development of the finite difference simulation model. The sample temperature at the measurement point predicted by the commercial FEBLAB software was close to that obtained with the finite difference model (<0.3°C difference).

Microbial reduction predicted with the finite difference model. Typical simulated survival curves of *E. coli* at 60°C are shown in Figure 6. The computer simulation model confirmed that the shouldering in the survival curves obtained with the large tubes were indeed the result of the thermal lag caused by slow heat conduction in the samples. However, the model that considered the influence of heat transfer alone did not fully follow the experimental curves for the large tubes. The computer-simulated curves gradually departed from the experimental curves with increasing treatment time for samples in the 13- and 20-mm test tubes (Fig. 6). In particular, the slopes of the linear portion of the simulated curves were significantly steeper than experimental counterparts. The observed departures of the simulated curves from the experimental curves suggest the influence of factors other than heat transfer. Overall, the *E. coli* samples in the large test tubes were more heat resistant

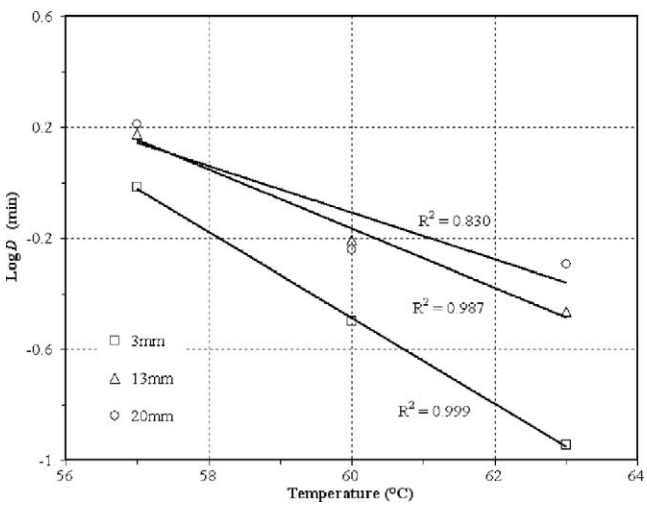

FIGURE 3. *Thermal death time curves for* Escherichia coli *K-12 in 3-, 13-, and 20-mm tubes.*

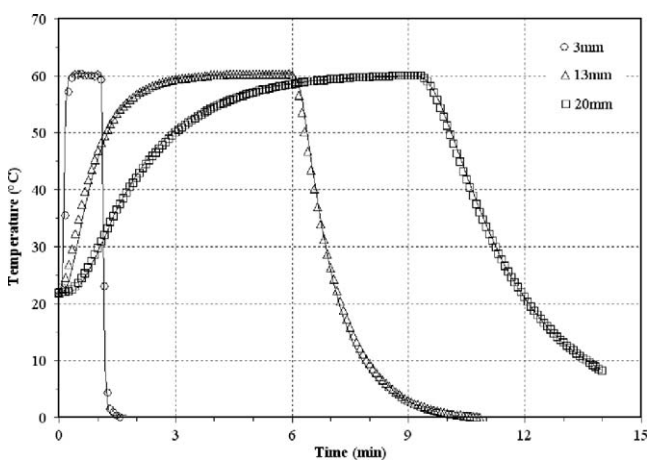

FIGURE 4. *Temperatures in the center of three types of tubes as obtained by experiment (symbols) and simulation (solid line) when subjected to heating in hot water at 60°C and cooling in ice water.*

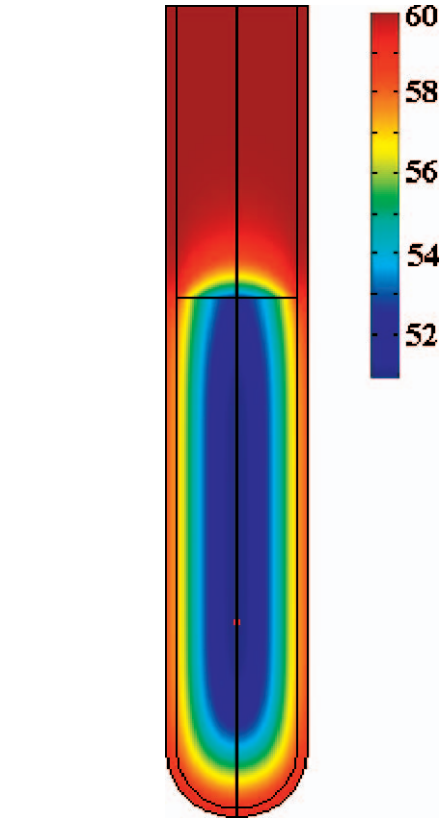

FIGURE 5. *Temperature distribution in the 20-mm tube after 3 min of heating in a 60°C water bath as determined with commercial software. Temperatures are given in degrees Celsius.*

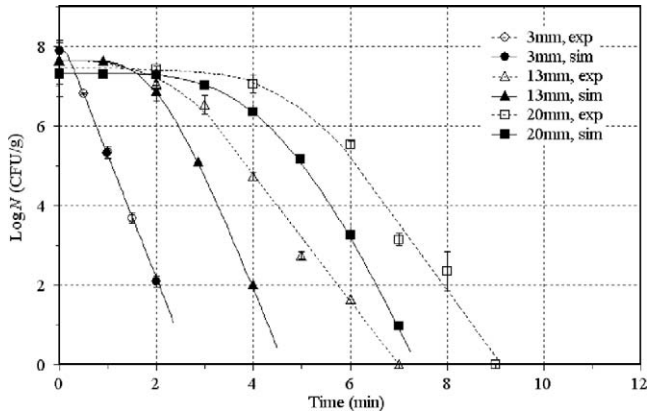

FIGURE 6. *Semilogarithmic survival curves for* Escherichia coli *K-12 in tubes of three different sizes at 60°C as obtained from experiment (exp) and simulation (sim) without preconditioning.*

than those in the capillary tubes considered in the computer simulation. This finding might have been the result of enhanced thermal resistance of *E. coli* in the internal layers of the large tubes where the bacteria could be preconditioned during the nonisothermal heating-up period, as illustrated by the simulated temperature profile (Fig. 7).

We hypothesized that the difference between the experimental and computer simulation results were due to induction of heat shock proteins in *E. coli* during the relatively long come-up times for the 13- and 20-mm tubes. Stephens et al. *(33)* reported significant deviations of experimental kill from the predicted kill due to significant changes in the population's thermotolerance at slow heating rates of $\leq$7.0°C min^{-1}. Mackey and Derrick *(24)* observed that the slower the temperature increases in meat, the larger the increase in the heat resistance of *Salmonella enterica* serovar Typhimurium. In other studies, increased thermal resistance of bacteria after heat shocking has been reported. Juneja et al. *(17)* reported that heat shocking *E. coli* O157:H7 at 46°C for 15 to 30 min in ground beef resulted in induction of heat shock proteins and a consequent increase in the thermotolerance of *E. coli* O157:H7 at 60°C. Bunning et al. *(7)* studied the thermotolerance of *Listeria monocytogenes* and *Salmonella* Typhimurium at 57.8°C after heat shocking at 35, 42, 48, and 52°C for 5 to 60 min.

Effect of preconditioning on the heat resistance of *E. coli*. Table 4 lists the *D*-values obtained from isothermal heat treatments using capillary tubes at 57, 60, and 63°C for *E. coli* cells preconditioned at 45°C for 5 min and compared with controls. Consistently, the survival curves of the control and preconditioned *E. coli* K-12 were all log linear at the tested temperatures ($R^2 \geq 0.939$) (Fig. 8). *D*-values for the control were not significantly different from those in the earlier tests (Table 3). However, preconditioned *E. coli* had much higher heat resistance at all three treatment temperatures ($P < 0.05$). *D*-values of preconditioned *E. coli* increased from 0.99 to 1.72 min at 57°C, from 0.32 to 0.43 min at 60°C, and from 0.12 to 0.15 min at 63°C (Table 4).

After incorporating into the computer simulation the *D*- and *z*-values obtained from samples that had been conditioned at 45°C for 5 min in capillary tubes, the predicted survival curves were much closer to the experimental curves in the larger tubes (Fig. 9). Thus, the observed increase in the heat resistance of *E. coli* in the larger tubes might have been caused by preconditioning during the warm-up period.

Comparison of the survival curves for the *E. coli* in fully exposed capillary tubes with those for the culture in the center of 20-mm tubes at 60°C provide further support for the above hypothesis (Fig. 10). The control in the capillary tubes fully exposed to the water bath at 60°C demonstrated log-linear inactivation kinetics after a very short lag period. The *D*-value for the linear portion of the curve after the come-up time was 0.29 $\pm$ 0.01 min, consistent with the previous data for the control at 60°C (0.32 $\pm$ 0.04 min, Table 4). The survival curve for *E. coli* heated in the center of the 20-mm tubes had a significant lag due to the slow heat conduction through the mashed potato in the large test tubes (Fig. 10). More importantly, the *D*-value taken from the linear portion of the curve after the come-up time was 0.68 $\pm$ 0.01 min, more than twice the time of 0.29 $\pm$ 0.01 min for the culture in capillary tubes directly exposed to the water bath at 60°C. This finding indicates that the thermal lag in the 20-mm tube significantly increased the heat resistance of the culture in the tube center. This increased *D*-value was even higher than that of *E. coli* in the capillary tube at 60°C after preconditioning at 45°C for 5 min (0.43 $\pm$ 0.05 min, Table 4). The above results supported our hypothesis that the difference between the

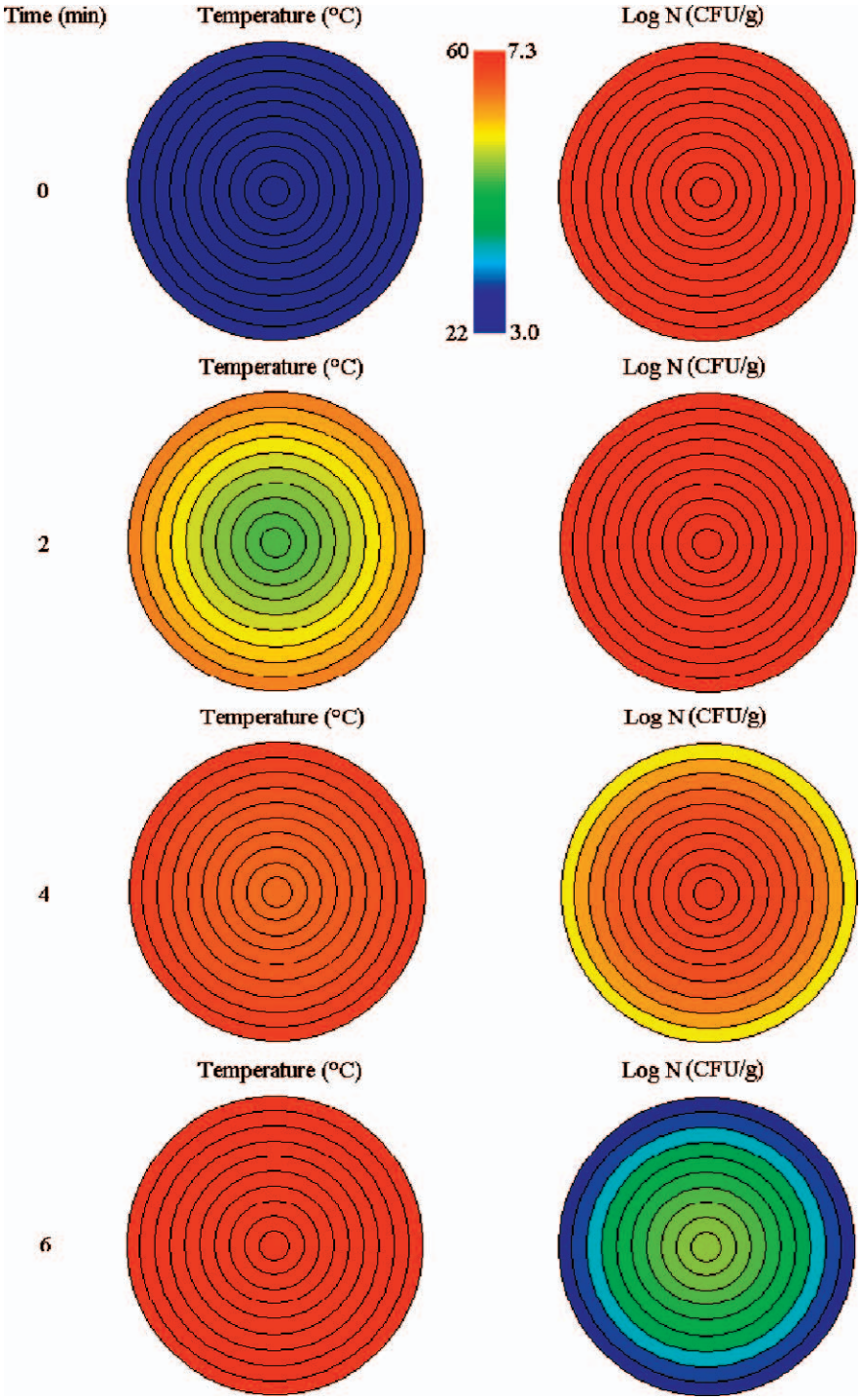

FIGURE 7. *Predicted temperature and microbial reductions in 10 layers within 20-mm tubes after heating for 0, 2, 4, and 6 min at 60°C.*

TABLE 4. D-*values of* Escherichia coli *K-12 at different temperatures before and after preconditioning at 45°C for 5 min with the capillary tube method*

	D-values (min)[a]	
Treatment temp (°C)	Control	Preconditioned
57	0.99 ± 0.09 A	1.72 ± 0.06 B
60	0.32 ± 0.04 A	0.43 ± 0.05 B
63	0.12 ± 0.01 A	0.15 ± 0.01 B

[a] Values are mean ± standard deviation of two replicate experiments. Within each row, means followed by different letters are significantly different ($P < 0.05$).

experimental microbial reduction and the computer simulation that accounted for only heat transfer in the larger tubes was due to increased heat resistance in the bacteria during the long come-up period.

Farber and Brown *(11)* stated that from a food safety standpoint heat-shocked cells may need to be heated twice as long as non–heat-shocked cells to achieve the same lethality. Murano and Pierson *(28)* reported that heat shock at 42°C for 5 min resulted in a 110% increase in the D_{55} for *E. coli* O157:H7. Gadzella and Ingham *(13)* reported that application of a 46°C 1-h heat shock to early stationary phase *E. coli* ATCC 35922 cells resulted in significantly larger *D*-values at 52, 54, and 56°C. Heat shocking *E. coli*

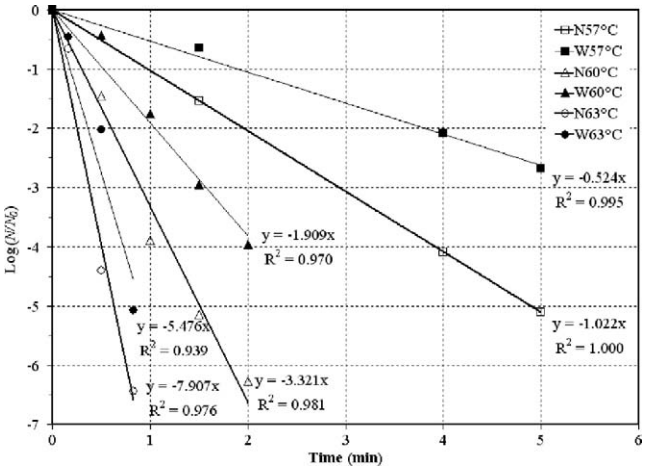

FIGURE 8. *Survival curves for* Escherichia coli *K-12 in capillary tubes at different temperatures with (W) and without (N) preconditioning at 45°C for 5 min.*

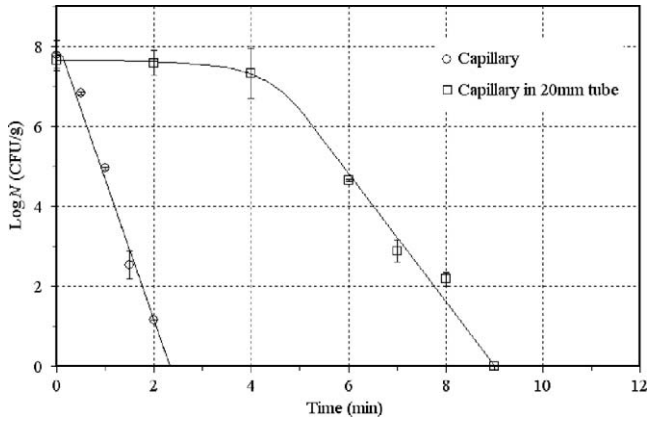

FIGURE 10. *Survival curves for* Escherichia coli *K-12 in samples heated at 60°C in capillary tubes alone and in capillary tubes inserted into the axial center of a 20-mm tube.*

O157:H7 at 45 to 46°C for 15 to 30 min produced appreciable thermal adaptation *(17, 22).* Recently, Yuk and Marshall *(40)* reported that heat shocking at 42 and 45°C resulted in a significant increase in *D*-values in both pathogenic and nonpathogenic *E. coli.* These findings are in agreement with the results in our study. Similar effects were observed when *Salmonella* Thompson was preheated at 48°C and then heated to 54 to 60°C in tryptone soya broth, liquid whole egg, 10% (wt/vol) or 40% (wt/vol) reconstituted dried milk, or minced beef *(23).*

Mackey and Derrick *(23)* reported that the extent of induced thermotolerance was inversely related to the rate of heating, i.e., the slower the temperature rise, the greater the increase in resistance. Quintavalla and Campanini *(30)* evaluated the thermotolerance of *L. monocytogenes* 5S heated at 60, 63, and 66°C in a meat emulsion at a rate of 5°C min^{-1} compared with instantaneous heating. These authors obtained twofold higher *D*-values for the cells heated slowly than for the cells heated instantaneously at all heating temperatures.

The phenomenon of a heat shock response and induced thermotolerance is of great practical importance to food processors for products normally heated at temperatures below 65°C. The slow heating rate used in preparing these foods allows pathogens to be exposed to conditions similar to heat shock, thus making them more heat resistant. Thermotolerance also becomes a concern in meat products kept on warming trays before a final heating or reheating step or when there is an interrupted cooking cycle due to equipment failure during processing. Thus, increased heat resistance due to prior heat shock must be considered when designing thermal processes to assure the microbiological safety of thermally processed foods.

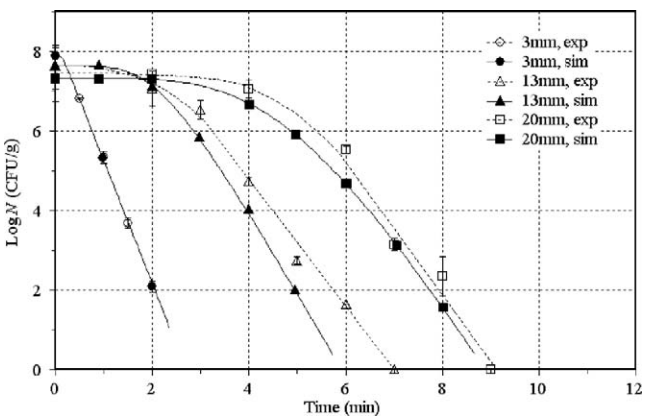

FIGURE 9. *Semilogarithmic survival curves for* Escherichia coli *K-12 in tubes of three different sizes at 60°C as obtained from experiment (exp) and simulation (sim) with preconditioning.*

In our study, thermotolerance parameters obtained with tube methods were influenced by tube sizes. Bacteria with intrinsically log-linear inactivation kinetics under isothermal heat treatment conditions (with or without preconditioning) exhibited nonlinear logarithmic behavior due to nonisothermal test conditions when using a larger test tube. Slow heat transfer in large tubes resulted in shouldering in survival curves. The preconditioning of microbial populations in the inner layers of large samples during the slow come-up time enhanced microbial heat resistance and increased *D*-values. The validated finite difference simulation model provided useful insights into this phenomenon. These results also highlight the importance of considering thermal treatment and pretreatment conditions when designing experiments to characterize the heat resistance of pathogens.

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