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Predicting the Growth of *Salmonella typhimurium* on Beef by Using the Temperature Function Integration Technique

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Lag and generation times for the growth of *Salmonella typhimurium* on sterile lean beef were modeled as functions of cooling time under various carcass-chilling scenarios. Gompertz growth models were fit to the $\log_{10}$ colony counts over time at each of six temperatures in the range of 15 to 40°C. Lag and generation times were defined as the points at which the second and first derivatives, respectively, of each growth curve attained a maximum. Generation time and lag time parameters were modeled as functions of temperature by use of exponential-decay models. The models were applied to typical beef carcass-cooling scenarios to predict the potential growth of *S. typhimurium* during the cooling of beef. Validation studies indicated no significant difference between the observed and predicted bacterial populations on inoculated lean and fatty beef tissues cooled at either 6 or 9°C/h.

Bacterial contamination of fresh meats can occur during normal slaughter and handling procedures, although this contamination can be minimized by adherence to good hygienic practices during slaughter (7, 12, 16, 37). Since the bacteria are confined almost exclusively to the carcass surface (20, 29) rather than to the deep muscle tissue, procedures that can control the survival and growth of bacteria on tissue surfaces are of interest to both the meat industry and regulatory agencies. Chilling, either by forced-air or water spray systems, is used universally to reduce the growth of bacteria on animal carcasses.

Since temperature has a significant effect on bacterial growth rate, the temperature history of food products has been used to estimate the potential bacterial population on a given product or, in practice, to predict relative rates of microbial growth for different cooling scenarios, with this process currently being referred to as temperature function integration (15). The relationship of spoilage rate to temperature has been suggested to be linear (24, 39). Olley and Ratkowsky (32) fitted spoilage data from several flesh foods to the Arrhenius equation and reported a relative spoilage rate equation for these foods on the basis of storage temperature. Daud et al. (10) used this same equation to predict the remaining shelf life of poultry. Ratkowsky et al. (36) refined the relative spoilage rate equation to more accurately reflect bacterial growth; this refinement resulted in an equation relating temperature to the square root of growth rate (the square root equation). This revised equation has been applied to poultry meat products (35), although the authors noted some discrepancies between observed and predicted values.

Although initial research focused on spoilage, this area of research also has applications for pathogenic or indicator bacteria. Smith (38) determined the lag and generation times for *Escherichia coli* and *Salmonella typhimurium* in blended mutton by using the square root equation. Lowry et al. (27) developed linear and quadratic equations describing the growth of *E. coli* on meat products by growing the bacterium in a liquid synthetic meat medium. Gibson et al. (14) used the Gompertz equation to characterize the growth of salmonellae over a range of pH, sodium chloride concentrations, and temperatures in broth media. These data have been incorporated into an integrated computer spreadsheet program for predicting the growth parameters for these organisms under different storage conditions (4). Other food-borne pathogens, including *Listeria* spp. (6) and *Aeromonas* spp. (33), have been integrated into this spreadsheet program, which estimates growth on the basis of pH, a_w, and storage temperature. Although there is some discussion over the terminology (8, 23), the terms "predictive microbiology" and "predictive modeling" will be used in this paper.

Gill et al. (17, 18) used temperature function integration as a technique for assessing beef carcass cooling processes. These researchers used temperature data loggers to record the temperature history of beef carcasses and then used these data to predict the growth of *E. coli* during cooling on the basis of the model of Lowry et al. (27). While the latter model has been demonstrated to provide an accurate estimate of the growth of an indicator bacterium (i.e., *E. coli*) and to be useful in assessing cooling processes, the intent of the research presented here was to construct a predictive model for the growth of salmonellae on beef tissue surfaces, with the specific application of evaluating beef carcass cooling procedures. In addition, the model presented here was developed with intact beef tissue representative of a beef carcass, in contrast to models that were developed with blended mutton (38) or liquid media (14, 27). While these other models are valid, our concern was that the growth characteristics in liquid media might not accurately reflect the growth of bacteria on the surface of a beef carcass.

MATERIALS AND METHODS

Bacterial culture. *S. typhimurium* ATCC 14028 was grown and maintained in tryptic soy broth (BBL). The cultures were transferred to tryptic soy broth and incubated at 37°C for 18 h prior to use. The cultures were harvested in the late logarithmic growth phase by centrifugation (3,000 × *g*, 25°C, 10 min), and the pellets were suspended in Butterfield's phosphate buffer (34).

Tissue preparation. Post-rigor mortis beef tissue was ob-

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TABLE 1. Growth temperature and tissue surface pH combinations used to simulate beef carcass characteristics during chilling

Growth temp (°C)[a]	Estimated pH[b]	Actual surface pH[c]
15	5.70	5.64
20	5.90	5.65
25	6.10	5.93
30	6.30	6.02
35	6.60	6.25
40	6.80	6.19

[a] Temperature used to develop bacterial growth curves.
[b] Estimated pH for beef carcasses during cooling; actual pH of the 0.2 M phosphate buffer used to modify the tissue pH.
[c] Actual pH of the tissue surface after 18 h of immersion in the buffer.

tained as boneless trim from the abattoir at the Roman L. Hruska U.S. Meat Animal Research Center. The tissue was separated into lean and fatty tissues, sliced into 0.5-cm-thick slices, frozen in sealed bags, sterilized with gamma radiation at a minimum dose of 42 kGy, and stored at −20°C until use. Prior to use, the slices were cut into squares of 2.0 by 2.0 cm (sample size, 2.0 × 2.0 × 0.5 cm) and tempered to room temperature. Tissue produced in this manner had previously been determined to be representative of pre-rigor mortis tissue, in terms of numbers of bacteria that would attach and sensitivity of the attached bacteria to organic acids (11). The pH of the lean muscle tissue was altered by immersing the tissue in sterile 0.2 M sodium phosphate buffer (9) at 5°C for 18 h prior to use (approximately 24 tissue samples per 200 ml of buffer). The pH of the buffer was estimated for the respective growth temperature on the basis of beef carcass cooling data (Table 1). The estimated pH of the tissue surface was determined by plotting pH and temperature data from several reports of beef carcass cooling (25, 41, 42) and then constructing a regression line through the data. The actual pH of the tissue surface was determined by use of a flat-surface combination pH probe and a model 140 digital pH meter (Corning Scientific Instruments, Medfield, Mass.). Because there is no difference in the pH of pre- or post-rigor mortis fatty tissue (11), the pH of the fatty tissue was not adjusted (typical pH, 6.2 to 6.3).

Experimental design. The harvested bacteria were diluted 1:1,000 in sterile phosphate buffer. Tissue samples were inoculated by immersion for 5 min, drained briefly, attached to sterile square-jawed alligator clips or hooks, and suspended in sterile containers such that the samples did not contact the sides of the containers. Sterile distilled water was added to the containers to minimize dehydration of the samples, and the containers were covered with plastic film to prevent sample contamination. Samples were incubated at 15, 20, 25, 30, 35, and 40°C and analyzed at 2-h intervals. These temperatures were chosen to reflect the range of growth temperatures that would be encountered during beef carcass cooling. Since Mackey et al. (28) had reported a generation time of approximately 14 h for *Salmonella* spp. on beef at 10°C, 15°C was the lowest temperature evaluated.

Enumeration of bacteria. Tissue samples were homogenized in 99 ml of phosphate buffer for 2 min in a stomacher (model 400; Tekmar Inc., Cincinnati, Ohio). Samples were enumerated on tryptic soy agar (BBL) with a model D spiral plater (Spiral Systems Instruments, Inc., Bethesda, Md.), the plates were incubated at 37°C for 24 h, and the bacterial populations were calculated by use of the methodology appropriate for spiral plates (30).

Model development. The estimates of bacterial populations were converted to $\log_{10}$ CFU per square centimeter, and each temperature-tissue combination was independently tested three times. Data from each growth curve [the bacterial population at time t in CFU per square centimeter, $N(t)$] were fitted to the Gompertz equation:

$$N(t) = A + \{C[e^{-e^{-B(t-M)}}]\} \quad (1)$$

(4, 22) by use of a nonlinear regression procedure (40); A is the initial level of bacteria (CFU per square centimeter), B is the relative growth rate of bacteria at the time, in hours, when the growth rate of the culture is maximal, C is the asymptotic amount of growth that occurs as time increases indefinitely, and M is the time, in hours, when the growth rate is maximal. Recent reviews have concluded that the Gompertz equation is preferred for modeling bacterial growth curves (13, 43). Lag and generation times were defined as the points at which the second and first derivatives, respectively, of each growth curve attained a maximum (5). Generation time was calculated with the following formula:

$$\text{GT (in hours)} = [\log (2)]e/BC \quad (2)$$

where GT is the generation time and B and C are the same parameters as those in the Gompertz equation (4, 14). Although equation 2 describes maximum growth under specific conditions, several researchers have found this equation to provide a good approximation of actual growth under various conditions (6, 13, 14, 33).

Validation of equations. Tissue samples were prepared as previously described and tempered to 40°C in an environmental incubator. Bacterial cultures were prepared as previously described, with the following exceptions: centrifugation was performed at room temperature, and the bacteria were suspended and diluted in 40°C phosphate buffer. Tissue samples were inoculated by immersion (5 min at 40°C), drained briefly, and suspended over sterile distilled water. Samples were cooled in the incubator at a rate of 6 or 9°C/h by a stepwise reduction in the temperature by 2 or 3°C every 20 min, respectively. The incubator typically equilibrated to the lower temperature within 2 min. Surface temperatures of uninoculated samples were monitored every 20 min with a surface temperature dial thermometer (Pacific Transducer Corp., Los Angeles, Calif.). After the sample temperatures reached approximately 10°C, the bacterial populations were enumerated. Lag times were estimated as the numerical average of the calculated lag times on the basis of the maximum and minimum temperatures during the first 2 h of cooling. This process assumes that lag time is a linear function under dynamic temperature conditions. This assumption probably overestimates lag time, resulting in an underestimation of the final bacterial population. Further research is needed to develop a more accurate estimate of lag time under dynamic temperature conditions. At the end of the estimated lag time, the predicted generation time was calculated for each subsequent recorded temperature. Bacterial populations at each time interval were calculated by linear interpolation with the following formula:

$$N_1 = [N(2^G)] \quad (3)$$

where N_1 is the new arithmetic bacterial population, N is the previous arithmetic bacterial population, and G is the number of generations per time interval. In practice, the temperature was recorded every 20 min, so G was expressed as generations per 0.33 h. Each cooling rate experiment was independently repeated twice.

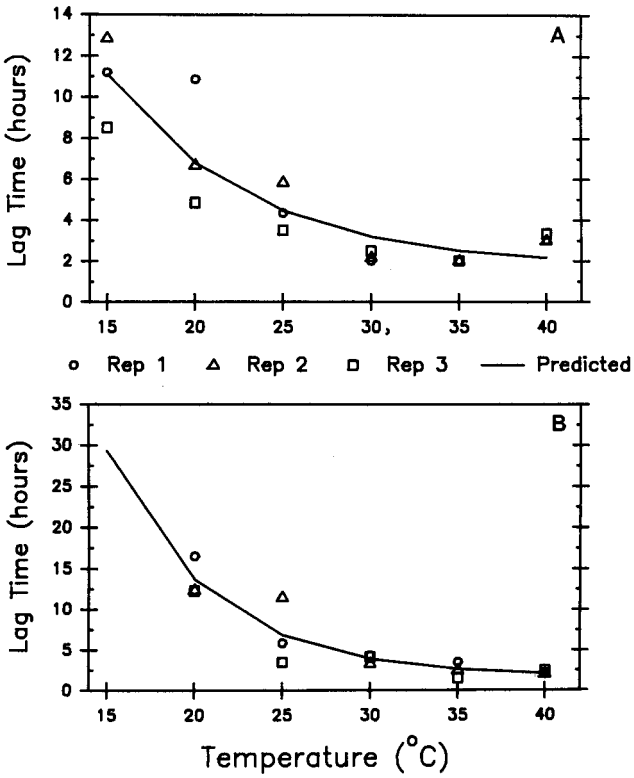

FIG. 1. Observed and predicted lag times for lean (A) and fatty (B) beef tissues. Rep, replicate.

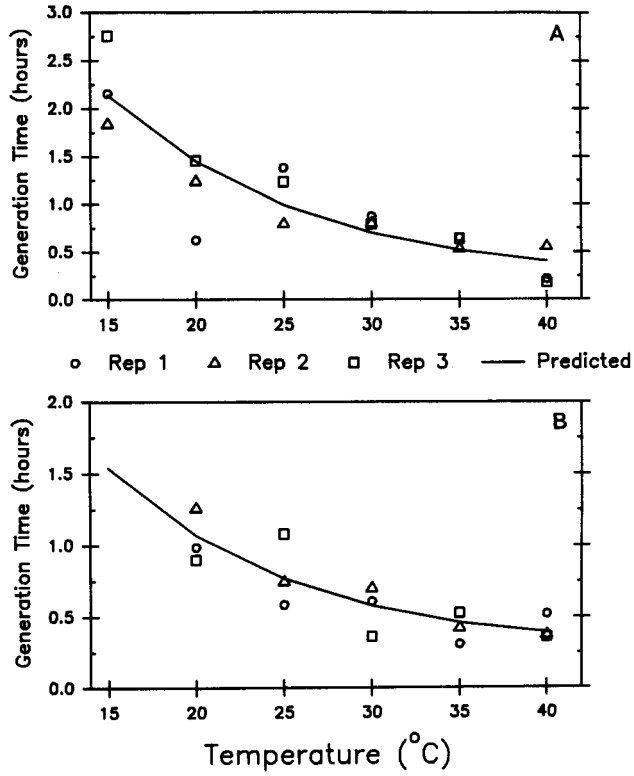

FIG. 2. Observed and predicted generation times for lean (A) and fatty (B) beef tissues. Rep, replicate.

RESULTS AND DISCUSSION

Lag and generation times were plotted for each temperature (Fig. 1 and 2). Lag and generation times were modeled as exponential-decay functions of temperature by use of the following formula:

$$Y = D + \{E[e^{-F(T)}]\} \quad (4)$$

where Y is the lag or generation time in hours, T is the temperature in degrees centigrade, and D, E, and F are derived parameters (Table 2). The choice of this function, although somewhat arbitrary, resulted in predicted values that closely approximated the numerical averages of the laboratory data. Given a function, $T(t)$, describing the temperature T of a carcass at time t, it is evident that the composite functions $(L \circ T)(t)$ and $(G \circ T)(t)$ will describe the lag and generation times, respectively, and will change with the rate of cooling of a carcass.

The estimated lag and generation times for both lean and fatty tissues, based on the models, are shown in Table 3. The lag times were generally longer on fatty tissue than on lean tissue, especially at temperatures at or below 25°C. The cells apparently require a longer period of time to adjust to the environment on the tissue surface. Lean muscle tissue has a moisture content of approximately 75% (26); that of fatty tissue is only 20% (1). It is plausible that the moisture in the lean tissue may have solubilized nutrients in the tissue cells, making them more readily available for bacterial metabolism. Conversely, the reduced moisture in the fatty tissue may have reduced the availability of substrates present on the surface of the fatty tissue. In addition, the lipid material in the fatty tissue increases in viscosity and solidifies as the temperature decreases, possibly further reducing nutrient transfer.

Once the bacterial cells adapted to the environment on the fatty tissue surface, the growth was generally more rapid, again with shorter generation times at temperatures at or below 25°C. Berry et al. (2) suggested that the growth of *Pseudomonas* and *Flavobacterium* spp. on fatty tissue was based on lipid utilization. However, Gill and Newton (21) noted that the high pH and low level of carbohydrates would allow amino acids to be metabolized by nonlipolytic bacteria and compared the bacterial spoilage of moist fatty tissue surfaces to that of dark, firm, dry meat (31). Dark, firm, dry meat will spoil more rapidly than normal muscle tissue (19). These authors also noted that, although low-molecular-weight substrates were present on the surfaces of fatty tissue, possibly from the serum from cut blood vessels, these substrates would be rapidly exhausted by the bacteria (21).

TABLE 2. Derived parameters of the equations for lag and generation times

Tissue	Parameter[a]	Value for the following time:	
		Lag	Generation
Lean	D	1.72	0.188
	E	59.02	7.65
	F	0.12	0.09
Fatty	D	1.68	0.257
	E	338.27	5.104
	F	0.167	0.092

[a] Constant parameter in the equation $Y = D + \{E[e^{-F(T)}]\}$.

TABLE 3. Comparison of derived lag and generation times on beef tissue to previously published data

Time	Growth temp (°C)	Time (h) reported for type of tissue or by previous investigator(s):				
		Lean[a]	Gibson et al. (14)	Smith (38)	Lowry et al. (27)	Fatty[a]
Lag	15	11.48	20.88	5.88	16.51	29.31
	20	7.07	8.00	2.93	7.24	13.67
	25	4.66	4.45	1.75	3.98	6.88
	30	3.33	4.25	1.16	2.69	3.94
	35	2.61	NA[b]	0.83	2.24	2.66
	40	2.21	NA	0.62	2.29	2.10
Generation	15	2.14	4.44	2.57	2.78	1.54
	20	1.45	1.51	1.25	1.37	1.07
	25	0.99	0.60	0.74	0.81	0.77
	30	0.70	0.36	0.49	0.54	0.58
	35	0.52	NA	0.35	0.45	0.46
	40	0.40	NA	0.26	0.38	0.39

[a] Data obtained for intact tissue in this study.
[b] NA, not applicable; data not provided for temperatures above 30°C.

An additional factor that could have affected the observed growth rates, particularly at the lower temperatures, was the lower pH of the lean tissue at these temperatures (Table 1). Again, the pH of lean tissue was adjusted to more closely resemble that of muscle tissue undergoing rigor mortis.

The predicted lag times for *S. typhimurium* on lean and fatty tissue surfaces (Table 3) were substantially higher than those reported by Smith (38), although they were similar to those reported by Lowry et al. (27) and Gibson et al. (14). The differences are a result of both the experimental design and subsequent data analysis. Smith (38) inoculated mutton with either *E. coli* SF or *S. typhimurium* grown to the stationary phase, processed the meat in a commercial blender at 15,000 rpm for 20 s, and then vacuum sealed thin films (ca. 35 μm) of the inoculated meat in polyvinyl chloride pouches. Growth curves were determined by immersing these pouches in a controlled-temperature water bath. The lag and generation times were determined by graphing the data and then were plotted in accordance with the square root model of Ratkowsky et al. (36). Blending the meat tissue ruptured the muscle cells, releasing moisture and nutrients that would be readily available for bacterial metabolism. This abundance of readily available nutrients would be expected to reduce the length of time that the bacteria would require to acclimate to the environment, with a resulting decrease in the lag time.

The predicted lag times reported by Lowry et al. (27) and Gibson et al. (14) were generally intermediate between those derived for lean and fatty tissues. Lowry et al. (27) grew a strain of *E. coli* isolated from sheep liver in a synthetic meat medium consisting of brain heart infusion broth supplemented with hemolyzed whole blood and lactic acid, while Gibson et al. (14) grew several strains of *Salmonella* spp. in tryptone soya broth. The lag times were modeled as a simple quadratic equation (27) or as a Gompertz equation (14). As previously noted, the lag times on fatty tissue at the lower temperatures tended to be longer than those on lean tissue or in broth media.

The predicted generation times for lean and fatty tissues were similar to those previously reported (Table 3). The greatest range occurred at 15°C, at which the generation times ranged from 4.44 h (14) to 1.54 h (fat tissue). Mackey et al. (28) reported that the average generation times for *Salmonella* spp. on chilled beef at 10, 12.5, and 15°C were 13.87, 6.79, and 3.25 h, respectively. However, they also noted that there was considerable variation in the generation times between replications, especially at 10°C, at which the range of generation times was 25.5 to 8.1 h. At temperatures above 15°C, the range of predicted generation times was less than 0.5 h, with smaller ranges seen as the temperature approached 40°C. The implication is that, despite the broad range of media and different bacterial species used in the different experiments, the growth rate of similar bacteria is determined primarily by temperature. The predicted generation times on lean tissue at temperatures at and above 25°C were slightly longer than those in the previously published reports and, as previously noted, those on fatty tissue.

The model uses isothermal growth data to predict growth under dynamic temperature conditions; however, validation studies demonstrated that the growth model closely predicted the observed increase in the population of *S. typhimurium* during cooling of beef tissue (Table 4). Blankenship et al. (3) used isothermal data to predict the growth of *Clostridium perfringens* during the cooling of cooked chili, with a good correlation between observed and predicted populations. Although the predicted populations were slightly smaller than the observed populations, there was generally no significant difference between the predicted and observed populations. It is likely that the frequency of the

TABLE 4. Comparison of observed and predicted populations of *S. typhimurium* on lean and fatty tissues after cooling at rates of 6 or 9°C/h

Tissue	Cooling rate (°C/h)	Increase in bacterial population[a]	
		Observed[b]	Predicted[c]
Lean	6	1.52	1.42
	9	1.03	0.81
Fatty	6	1.90	1.72
	9	1.18	0.91

[a] Determined as $\log_{10}$ final population − $\log_{10}$ initial population. $\log_{10}$ average initial populations before cooling were 7.15 (lean) and 7.02 (fatty) CFU/cm^2. Means within rows are not significantly different; see the text for a discussion. Data are averages for two independent replicates.
[b] Mean of the bacterial population on inoculated tissue after cooling.
[c] Mean of the bacterial population after cooling, based on a temperature history of inoculated tissue samples.

recorded temperatures will affect the relative precision of the estimated populations, with a higher degree of precision being associated with more frequent temperature recording. Although we used a dial thermometer and took readings every 20 min, an electronic data acquisition system could record temperatures much more accurately and at intervals as short as 1 min. The validation studies indicated that equations for lag and generation times are relatively accurate, within experimental error.

In summary, we have achieved the objective of developing a predictive model for the growth of salmonellae on beef tissue surfaces during cooling. The model can be applied to the temperature function integration evaluation of beef carcass-cooling systems, as well as to furthering the understanding of bacterial growth kinetics under changing environmental conditions.

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