

Evaluation of a *Clostridium perfringens* Predictive Model, Developed under Isothermal Conditions in Broth, To Predict Growth in Ground Beef during Cooling

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Proper temperature control is essential in minimizing *Clostridium perfringens* germination, growth, and toxin production. The U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) offers two options for the cooling of meat products: follow a standard time-temperature schedule or validate that alternative cooling regimens result in no more than a 1-log₁₀ CFU/g increase of *C. perfringens* and no growth of *Clostridium botulinum*. A mathematical model developed by Juneja et al. (Food Microbiol. 16:335-349, 1999) may be helpful in determining if the *C. perfringens* performance standard has been achieved, but this model has not been extensively validated. The objective of this study was to validate the Juneja 1999 model in ground beef under a variety of changing temperature and temperature abuse situations. The Juneja 1999 model consistently underpredicted growth of *C. perfringens* during exponential cooling of ground beef. The model also underpredicted growth of *C. perfringens* in ground beef cooled at two different rates. The results presented here show generally good agreement with published data on the growth of *C. perfringens* in similar products. The model error may be due to faster-than-expected exponential growth rates in ground beef during cooling or an error in the mathematical formulation of the model.

Clostridium perfringens is a contaminant in meat and poultry products and continues to be a major concern to the food industry. In one recent study, *C. perfringens* vegetative cells or spores were isolated from 38.9% of uncured ground or emulsified meat samples with maximum populations of cells and spores of 2.92 and 2.11 log₁₀ CFU/g, respectively (17). *C. perfringens* has been implicated as the cause of food-borne illness in roast beef, turkey, meat-containing Mexican foods, and other meat dishes (6). Fifty-seven confirmed *C. perfringens* outbreaks, involving 2,772 cases, occurred between 1993 and 1997, and more than one-third (35%) of these cases were attributed to meat products or mixed dishes containing meat (7). Food-borne illness has been shown to be a result of spores surviving the cooking process and germinating into vegetative cells, which are able to multiply in cooked foods if the rate and duration of cooling are too slow (4). Angulo et al. (1) reported that the most common cause of *C. perfringens* outbreaks was improper cooling (40.9%) of food products. *C. perfringens* generation time has been reported to be as rapid as 7.4 min in autoclaved ground beef under isothermal conditions, with an optimal growth range of 37 to 45°C, and growth has been reported at temperatures as low as 6°C (20). After food is consumed, the vegetative cells sporulate and have the ability to release enterotoxin within the intestinal tract.

Food Safety Inspection Service (FSIS) guidelines for the cooling of meat products suggest that uncured meat and poultry products be cooled from 130 (54.4°C) to 80°F (26.7°C) in no more than 1.5 h and from 80 (26.7°C) to 40°F (4.4°C) within 5 h. If meat processors are unwilling or unable to meet this

cooling schedule, they must be able to prove that an alternate cooling regimen will result in less than a 1-log₁₀-CFU increase in *C. perfringens* and no growth of *Clostridium botulinum*.

Computer models are gaining greater visibility and utility for helping to solve food microbiology problems (16). There is a class of models that can describe microbial behavior under changing temperature conditions (3, 5, 22). One such model predicts the growth of *C. perfringens* under changing temperatures associated with cooling of cooked meat products (13). This model (hereafter referred to as the Juneja 1999 model) has been incorporated into the U.S. Department of Agriculture's (USDA's) Pathogen Modeling Program (PMP) version 6.1, but it has not been extensively validated. The Juneja 1999 model was created with growth curves collected at static temperatures in a representative beef broth.

The objective of this research was to evaluate the Juneja 1999 model for *C. perfringens* under changing temperature and temperature abuse situations in a ground beef system. Such validated models may be useful tools in determining if the *C. perfringens* performance standard has been met should a problem occur during the cooling of processed meat products.

MATERIALS AND METHODS

Test organisms and spore production. Three strains of *C. perfringens*, NCTC 8238 (Hobbs serotype 2), NCTC 8239 (Hobbs serotype 3), and NCTC 10240 (Hobbs serotype 13), were obtained from V. K. Juneja at the USDA Agricultural Research Service, Eastern Regional Research Center. They were maintained and prepared according to procedures described by Juneja et al. (11). Appropriate volumes of a spore cocktail (approximately 10⁸CFU/ml) were inoculated into 908 g of ground beef (25% fat), obtained at a retail store, to result in initial inoculum levels of between 10¹ or 10³ concentrations of spores/g. Initial spore concentration has been shown to influence germination and growth of *C. botulinum* (21) and other spore-forming bacteria (8, 15), so initial concentrations of 10¹ and 10³ spores/g were used for each cooling time to determine the effect of initial spore concentration.

The ground beef and culture were blended for 10 min on high speed (level 6)

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in the sterilized bowl of a Kitchen Aid mixer (model no. K45SS; Kitchen Aid, Inc., Greenville, Ohio). This mixing process ensured even dispersion of spores. The mixing was halted once every minute to break apart large meat pieces and remove residual meat from the sides of the bowl and mixing paddle. Three grams of inoculated ground beef was aseptically weighed into sterile stomacher filter bags (SFB-510; Spiral Biotech, Boston, Mass.). Bags were vacuum-sealed using a retail vacuum packaging system (Food Saver Vac 300; Tilia, Inc., San Francisco, Calif.) and frozen until used (no longer than 4 weeks). Initial spore concentrations were evaluated over the 4 weeks of frozen storage to ensure that levels did not decrease during storage. Pouches were thawed at 4.4°C overnight before heat-activation of spores at 75°C for 20 min in a circulating water bath.

Temperature variation studies. Dynamic temperature experiments were of two types: those conducted at a single cooling rate and those conducted at two cooling rates. The dual-rate experiments followed one cooling rate from 54.4°C to 26.7°C and a second rate from 26.7°C to 4.4°C. All experiments were carried out in a programmable water bath (model no. 1028P; Fisher Scientific). Cooling of food products has previously been shown to follow an exponential rate (12), so the water bath was programmable to mimic an exponential cooling rate during the dynamic temperature studies. The driving force for this cooling was assumed to be 0°C. Samples were removed at appropriate intervals throughout each cooling process. Samples were diluted 1:10 in 0.1% sterile peptone water and pumped in a Stomacher (Lab Blender 400, model BA6021; Cooke Laboratory Products, Alexandria, Va.) for 2 min. Aliquots of 1 ml were pour plated with 12 to 15 ml of tryptose-sulfite-cycloserine agar with egg yolk emulsion and incubated at 37°C in GasPak containers (Baltimore Biological Laboratory, Cockeysville, Md.) to ensure an anaerobic environment.

Single-cooling-rate experiments investigated cooling from 54.4 to 4.4°C, over 6.5, 9, 12, 15, 18, 21, or 24 h. This range of cooling times includes the USDA recommended maximum time (6.5 h) and times that resulted in significant growth of *C. perfringens* in previous studies (18 h) (12). Longer cooling times (21 and 24 h) were also investigated to ensure thorough model validation.

Dual-cooling-rate experiments were performed to simulate cooling deviations that might occur in the processed meat industry. FSIS guidelines state that a meat product should not remain between 54.4 and 26.7°C for longer than 1.5 h or between 26.7 and 4.4°C for longer than 5 h (18). The time periods associated with the proper 6.5-h cooling schedules were altered from 1.5 and 5 h to 3 and 3.5 h. Cooling times of 7 and 9 h were also investigated with proportional variations to the temperature ranges of the USDA-FSIS cooling guidelines. Predictions from the Juneja 1999 model were used to guide the selection of experimental conditions to ensure that a range of growth responses would be observed.

Predicted growth curves. Predicted growth curves were obtained by using the PMP version 6.1. The time-temperature combinations used in the experiments described above were entered into the cooling profile window for the *C. perfringens* beef broth model in PMP version 6.1, and increases in log₁₀ CFU per milliliter over time were calculated. Predicted increases in log₁₀ CFU per milliliter over time were used as outputs for subsequent data analysis.

Analysis of growth curves to determine model failure. To aid in the analysis of model failure, we needed a method to compare different aspects of model predictions to observed data. Because the temperature change in these studies was relatively gradual (relative to the rate of growth of the organism), typical sigmoidal microbial growth curves were obtained for all experiments. Comparison of the "apparent" germination outgrowth and lag (GOL) time and "apparent" exponential growth rates (EGR) given by the Juneja 1999 model and our data allowed this analysis of model failure, even though these data were collected under changing temperature conditions.

Estimates of GOL time and EGR for dynamic temperature conditions were determined from the Baranyi and Roberts model (2) using the DMFit 1.0 Microsoft Excel add-in program (Institute of Food Research, Norwich Research Park, Norwich, United Kingdom; <http://www.ifr.bbsrc.ac.uk>). DMFit 1.0 calculates a lag time and EGR for each fitted curve. The exponential growth phase duration (EGPD) was calculated by using parameter values given by DMFit 1.0 and the equation $EGPD = (y_{End} - y_0)/EGR$, where y_{End} is the upper asymptote of the sigmoid curve and y_0 is the initial point on the sigmoid curve.

RESULTS

Initial inoculum effect. There was no significant influence of inoculum size on the net growth during single- or dual-cooling-rate experiments. All six replicates (three experiments with an initial inoculum level of 10¹ log₁₀ CFU/g and three experi-

TABLE 1. Comparison of growth of *C. perfringens* predicted by the Juneja 1999 model and observed growth during one-rate exponential cooling from 54.4°C to 4.4°C in ground beef

Length of expt (h)	Growth (log ₁₀ CFU/g) ^a	
	Predicted	Observed
6.5	0.14 ± 0.05	0.56 ± 0.30
9	0.28 ± 0.08	0.77 ± 0.37
12	0.58 ± 0.13	1.99 ± 0.53
15	1.11 ± 0.24	3.73 ± 0.51
18	1.94 ± 0.47	5.10 ± 0.39
21	3.08 ± 0.80	5.64 ± 0.40
24	4.40 ± 1.07	5.71 ± 0.71

^a Predicted values are the mean growth ± 90% confidence interval. Observed values are the averages of six replicates ± standard deviation.

ments with an initial inoculum level of 10³ log₁₀ CFU/g) were pooled together for subsequent analysis.

Single cooling rate. Extending the duration of one-rate exponential cooling curves, as compared to the 6.5-h regulations currently suggested by FSIS, resulted in *C. perfringens* growth greater than in previously reported studies (Table 1) (12). Cooling over 6.5 h resulted in less than a 1-log₁₀ increase of *C. perfringens*. Cooling from 54.4 to 4.4°C in 9 h resulted in less than a 1-log₁₀ increase in five of the six replicates tested, while one replicate exhibited net growth greater than 1 log₁₀. Cooling times of 12, 15, 18, 21, and 24 h resulted in a more than a 1-log₁₀ increase of *C. perfringens*. The result observed differs greatly from Juneja 1999 model predictions (Table 1). More growth was observed during every cooling condition tested compared to the growth predicted by the current model. These underpredictions by the Juneja 1999 model could have serious implications if used to evaluate cooling deviations. For instance, when cooling from 54.4°C to 4.4°C over 12 h the amount of *C. perfringens* growth predicted was <1 log₁₀ (0.58 log₁₀ CFU/g), but the observed value was approximately 2 log₁₀ CFU/g.

Dual cooling rate. Growth of *C. perfringens* was also monitored during cooling at two rates as outlined in Table 2. As in the single-cooling-rate experiments, the observed growth during exponential cooling conditions surpassed that predicted by the Juneja 1999 model no matter what time period or cooling rates were tested. As noted, during the single-cooling-rate experiments these results could have practical importance for many of the cooling regimens tested as the current model predicted a <1-log₁₀-CFU/g increase in *C. perfringens* population, while >1-log₁₀-CFU/g increases were observed.

Experiments in which the total cooling time was 6.5 h but the ground beef was cooled from 54.4°C to 26.7°C in 2 or 3 h resulted in >1 log₁₀ CFU/g, whereas the ground beef that passed through the upper temperatures in 1.5 or 1 h resulted in a <1-log₁₀-CFU/g increase. It was interesting to note that a slightly smaller (but not significantly different) increase in *C. perfringens* population was observed during 6.5 h of cooling, when 1.5 h was spent between 54.4 and 26.7°C versus 1 h of cooling through the same temperatures. This may be a result of vegetative cell inactivation at temperatures greater than 50°C as such cells would have spent a slightly longer time at these higher temperatures when cooling from 54.4°C to 26.7°C in 1.5 h.

TABLE 2. Comparison of growth of *C. perfringens* predicted by the Juneja 1999 model and observed growth during dual-rate exponential cooling in ground beef

Total cooling time (h)	Time to cool (h)		Net growth (\log_{10} CFU/g) ^a	
	54.4°C–26.7°C	26.7°C–4.4°C	Predicted	Observed
6.5	3.0	3.5	0.32 ± 0.08	1.89 ± 0.69
	2.0	4.5	0.16 ± 0.05	1.29 ± 0.35
	1.5	5.0	0.10 ± 0.04	0.40 ± 0.20
	1.0	5.5	0.06 ± 0.03	0.57 ± 0.20
7.0	2.0	5.0	0.16 ± 0.06	1.78 ± 0.23
	1.5	5.5	0.11 ± 0.05	1.53 ± 0.14
	1.25	5.75	0.09 ± 0.04	0.56 ± 0.17
	1.0	6.0	0.07 ± 0.03	0.68 ± 0.33
9.0	4.0	5.0	0.71 ± 0.12	3.53 ± 0.23
	3.5	5.5	0.52 ± 0.10	3.19 ± 0.67
	3.0	6.0	0.39 ± 0.09	2.84 ± 0.52
	2.0	7.0	0.19 ± 0.06	0.82 ± 0.10

^a Predicted values are the mean growth ± 90% confidence interval. Observed values are the averages of six replicates ± standard deviation.

It was possible to extend cooling times from 6.5 h to 7 or 9 h and still meet the performance standard of less than a 1- \log_{10} -CFU/g increase in *C. perfringens*. Ground beef cooled from 54.4°C to 26.7°C in 1 h and then from 26.7°C to 4.4°C in 6 h showed less than a 1- \log_{10} -CFU/g increase in *C. perfringens*. Ground beef cooled from 54.4°C to 26.7°C in 2 h and then from 26.7°C to 4.4°C in 7 h also showed less than a 1- \log_{10} -CFU/g increase in *C. perfringens* (Table 2).

Optimal growth temperatures for *C. perfringens* have been reported to be between 37 and 45°C (20), so the first cooling rate within the dual-rate experiments might be expected to have the largest impact on outgrowth, germination, and multiplication, but our data show that the rate of cooling from 26.7°C to 4.4°C is also important. Overall cooling times of 6.5 and 9 h where the ground beef was cooled from 54.4°C to 26.7°C in 3 h resulted in net increases of *C. perfringens* of 1.89 and 2.84 \log_{10} CFU/g, respectively. The slower cooling from 26.7°C to 4.4°C over 6 h resulted in an additional 1- \log_{10} -CFU/g increase in *C. perfringens* in the final concentration compared to cooling from 26.7°C to 4.4°C in 3.5 h. Similarly, ground beef samples cooled over 6.5 and 7 h (54.4°C to 26.7°C in 1 or 1.5 h) also showed less growth with faster cooling from 26.7°C to 4.4°C. The trend is less clear when considering 6.5-, 7-, and 9-h total cooling times where the product was cooled from 54.4°C to 26.7°C in 2 h. The smallest increase in cell population (0.82 \log_{10} CFU/g) was seen after 9 h of total cooling when compared to 6.5-h (1.29 \log_{10} CFU/g) and 7-h (1.78 \log_{10} CFU/g) cooling times.

Analysis of model failure. Because of the systematic underprediction seen in Tables 1 and 2, a more detailed analysis of the observed growth curves and model predictions was undertaken. The objective of this analysis was to determine which aspect of the model predictions contributed most to the prediction error: e.g., were the predictions too low because *C. perfringens* had shorter GOL times in the beef system than expected, or was the error due to an unexpectedly fast growth rate?

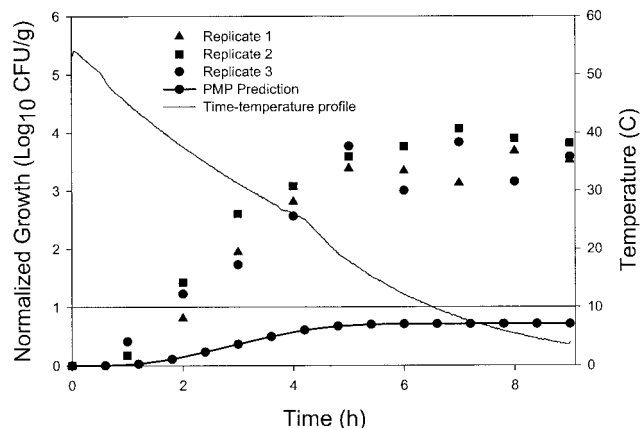


FIG. 1. Growth of *C. perfringens* in ground beef during 9 h of cooling at two rates (54.4 to 26.7°C in 4 h and 26.7 to 4.4°C in 5 h) versus growth predicted by the Juneja 1999 model.

Figure 1 shows a representative 9-h cooling experiment where the source of erroneous model predictions is apparent. In this example, the samples were cooled at one rate for the first 4 h and then at a different rate for the next 5 h. It is clear from Fig. 1 that while the observed GOL times are similar to those predicted by the Juneja 1999 model (~1 h), the observed EGR is considerably faster than predicted. Both observed and model *C. perfringens* populations reach the stationary phase after about 5 h, when the temperature of the samples has reached approximately 18°C.

Figures 2 to 4 show a detailed analysis of the data collected under single-rate cooling over 6.5, 9, 12, 15, 18, 21, and 24 h. Figure 2 shows that a majority of the GOL times calculated for growth curves resulting from one-rate cooling fall into the “fail safe” region of the graph, so the observed GOL times are longer than those predicted by the Juneja 1999 model. There are some (~6) GOL times that fall in the “fail dangerous” region, because the observed GOL time was shorter than the

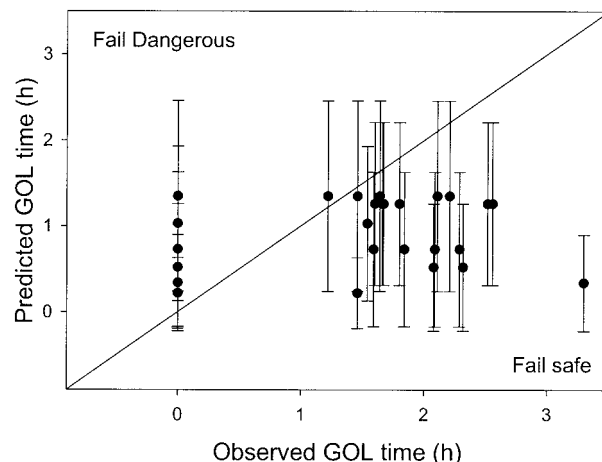


FIG. 2. GOL times predicted for one-rate cooling by the Juneja 1999 model versus observed GOL times of *C. perfringens* in ground beef. Error bars represent 90% confidence intervals given by USDA PMP version 6.1. All parameters were calculated by using the DMFit 1.0 Microsoft Excel add-in program.

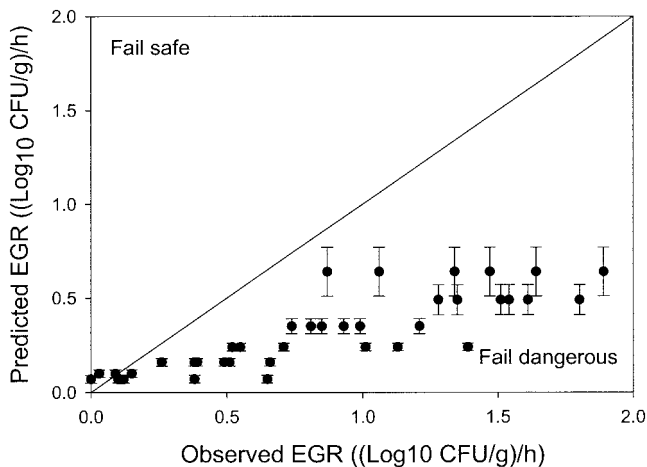


FIG. 3. Exponential growth rates (\log_{10} CFU per gram per hour) predicted for one-rate cooling by the Juneja 1999 model versus observed exponential growth rates of *C. perfringens* in ground beef. Error bars represent 90% confidence intervals given by the USDA PMP version 6.1. All parameters were calculated by using DMFit 1.0 Microsoft Excel add-in program.

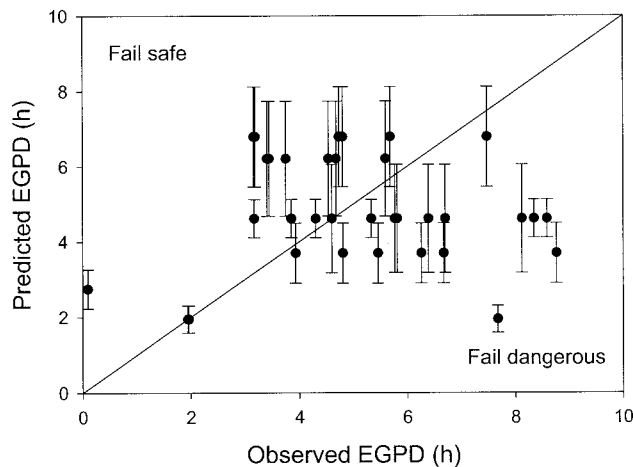


FIG. 4. Exponential-growth-phase durations predicted for one-rate cooling by the Juneja 1999 model versus observed exponential-growth-phase durations of *C. perfringens* in ground beef. Error bars represent 90% confidence intervals given by the USDA PMP version 6.1. All parameters were calculated by using the DMFit 1.0 Microsoft Excel add-in program.

time predicted by the Juneja 1999 model for the same growth curve. It should be noted that all of the points that fell in the fail dangerous region had a DMFit-calculated GOL time of zero. In certain situations, DMFit assumes the lag time to be zero for the sake of parsimony. Typically these are situations where the lag times are very close to zero or the data points in the growth portion of the curve are sufficiently variable such that the lag time cannot be determined reliably. These few fail dangerous predictions are not sufficient to suggest that the GOL time predictions from the Juneja 1999 model are the main cause of model failure.

Figure 3 shows that almost all of the observed EGR values were larger than the predicted values from the Juneja 1999 model (i.e., fail dangerous). Virtually all of the situations where the predicted EGRs were greater than $0.1 \log_{10}$ CFU/g/h resulted in observed EGRs that were fail dangerous. These data further support the limited number of observations shown previously (Fig. 1).

Figure 4 shows that EGPD observations spanned both sides of the line of equality (i.e., were both fail safe and fail dangerous). It appears that EGPD does not play a systematic role in explaining the model discrepancies.

Similar trends were seen in Fig. 5, 6, and 7 for the dual-cooling-rate growth curves. A majority of the observed GOL times were greater than the predicted times: i.e., fail safe (Fig. 5). A few values that resulted in GOL times of zero were classified as fail dangerous, again due to DMFit's assumption of zero lag time for the sake of parsimony.

The vast majority of the observed EGR values for dual-rate cooling were greater than the predicted EGR values (Fig. 6). In some cases, the predicted EGRs were many fold greater than that predicted by the Juneja 1999 model.

As with the single-rate cooling, predicted values for EGPD were at times shorter and other times longer than the observed EGPD. While no overall trend could be seen in the correlation between predicted and observed EGPD, it should be noted

that the range of observed EGPDs was much greater (0.5 to 5.5 h) than the range of predicted EGPDs (1 to 3.5 h). It appears that a majority of the discrepancies seen between the observed and predicted values during dual-rate cooling can be attributed to the EGR.

DISCUSSION

Initial inoculum size (either 10^1 or 10^3 CFU/g) did not influence the GOL time or EGRs measured in these experiments, despite the fact that previous studies with other spore-forming bacteria (*C. botulinum* and *Bacillus megaterium*) (8, 19, 21) did show significant inoculum size effects. While no inoculum size effect was shown here, future studies investigat-

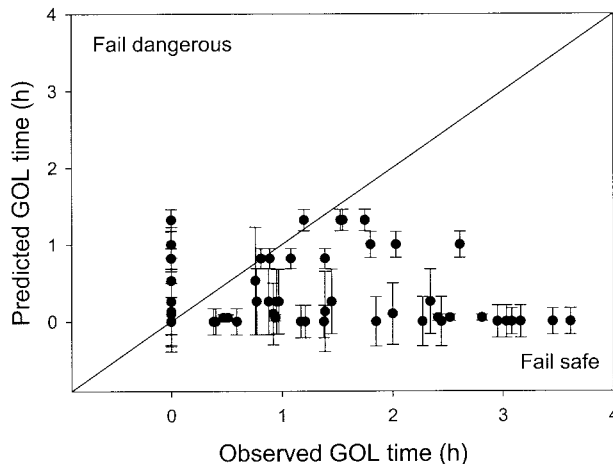


FIG. 5. GOL times predicted for dual-rate cooling by the Juneja 1999 model versus observed GOL times of *C. perfringens* in ground beef. Error bars represent 90% confidence intervals given by the USDA PMP version 6.1. All parameters were calculated by using the DMFit 1.0 Microsoft Excel add-in program.

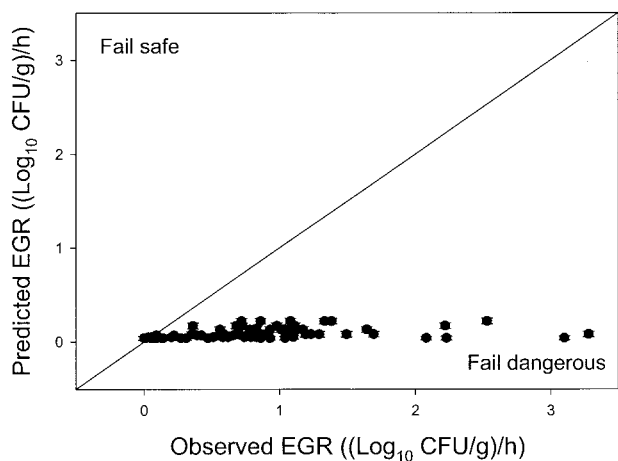


FIG. 6. Exponential growth rates (\log_{10} CFU per gram per hour) predicted for dual-rate cooling by the Juneja 1999 model versus observed exponential growth rates of *C. perfringens* in ground beef. Error bars represent 90% confidence intervals given by the USDA PMP version 6.1. All parameters were calculated by using the DMFit 1.0 Microsoft Excel add-in program.

ing higher and lower inoculum sizes might reveal such an effect.

Microbial models are often developed in broth systems with an assumption that such systems represent a worst-case scenario and that growth will generally be slower in real food systems. Data presented here and in the published literature show that this assumption is not valid for the Juneja 1999 model. *C. perfringens* showed faster growth rates and higher overall net growth in ground beef as compared to the predictions using data collected in beef broth.

Our results are in agreement with Willardsen et al. (20), who reported generation and GOL times of 8.9 min and 1.2 h in autoclaved ground beef versus 12.2 min and 1.9 h in fluid

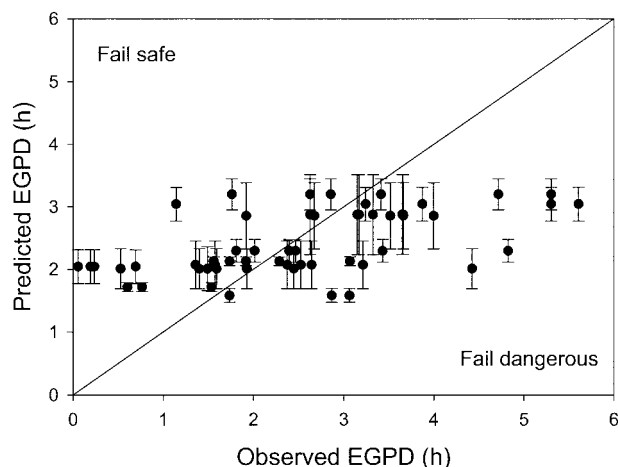


FIG. 7. Exponential-growth-phase durations predicted for dual-rate cooling by the Juneja 1999 model versus observed exponential-growth-phase durations of *C. perfringens* in ground beef. Error bars represent 90% confidence intervals given by the USDA PMP version 6.1. All parameters were calculated by using the DMFit 1.0 Microsoft Excel add-in program.

thioglycolate medium during storage at 45°C. They also observed the same trend for *C. perfringens* growth at temperatures increasing linearly with time from 25 to 60°C. The most rapid generation time observed during rising temperatures of 7.5°C/h in autoclaved ground beef was 8.1 min as compared with 11.0 min in fluid thioglycolate medium.

Our results also show good agreement with those of Kalinowski et al. (14), who demonstrated a net growth of more than 2 \log_{10} CFU/g was observed during cooling uncured turkey samples from 48.9 to 12.8°C over 6 h, which is a rate approximately equivalent to the 12-h cooling process shown in Table 1. Again, the Juneja 1999 model underpredicts the growth observed by Kalinowski et al.

The net growth observed during cooling in this study was more than that seen by Blankenship et al. (4), who observed about 3- and 2.5- \log_{10} -CFU/g increases in *C. perfringens* in a cooked chili product when cooling from 50°C to 25°C in 6 and 4 h, respectively. Differences in *C. perfringens* growth rates may be attributable to inhibitory spices in chili versus ground beef or other aspects of the experimental design. It should be noted, however, that although our data shown more growth than those of Blankenship et al., the Juneja 1999 model underpredicts both data sets.

Danler et al. (9) reported a 0.52- \log_{10} -CFU/g increase in *C. perfringens* after cooling ground beef samples from 54.4°C to 26.7°C in 2 h and from 26.7°C to 4.4°C in 5 h (a total of 7 h). These results are in good agreement with our results for cooling over 6.5 or 9 h (Table 1), and again, the Juneja 1999 model underpredicts both data sets.

When data from the literature were available, growth curves were fit to the Baranyi model by using DMFit 1.0 for comparison of model parameters. A growth curve resulting from an 18-h one-rate cooling time (54.4 to 7.2°C) exhibited GOL-phase and EGR values of 2.25 h and 0.61 \log_{10} CFU/g/h, respectively (12). A representative growth curve from a one-rate 18-h cooling in this study exhibited a shorter GOL phase (1.54 h) and a faster EGR (1.21 \log_{10} CFU/g/h). Differences here could be due to study methodologies, as we have found the type of bag or pouch used in cooling studies can have a large effect on the GOL phase and EGR of *C. perfringens* (S. Smith, V. K. Juneja, and D.W. Schaffner, submitted for publication).

The predictive microbiology literature contains several examples in which data from static growth curves were successfully used to predict growth of organisms during changing temperature situations (3, 5, 22). Despite repeated attempts to demonstrate a temperature history effect, few examples have been reported (10). Trends seen in this study when comparing nonisothermal experimental data collected in ground beef to predicted values from both single- and dual-rate-cooling experiments suggest that the Juneja 1999 model could be improved and that a temperature history effect may be operating.

Our findings may also have important implications relative to the current *C. perfringens* performance standard if the Juneja 1999 model is used to estimate the expected increase in *C. perfringens* concentration. This is especially important in those instances where the observed increase was very close to or exceeded 1 \log_{10} CFU/g while the predicted increase was less, as the model will indicate that the cooling process met the

performance standard, although our data indicate that this may not always be the case.

The main objective of this study was to assess the accuracy and applicability of the Juneja 1999 model to nonisothermal conditions in a ground beef system. Having found possible weaknesses in the Juneja 1999 model, work is currently under way to use the large quantity of growth data collected under dynamically changing conditions to develop a new model more suitable for use under cooling conditions.

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REFERENCES

1. Angulo, F. J., A. C. Voetsch, D. Vugia, J. L. Hadler, M. Farley, C. Hedberg, P. Cieslak, D. Morse, D. Dwyer, and D. L. Swerdlow. 1998. Determining the burden of human illness from food borne diseases—CDC's emerging infectious disease program food borne diseases active surveillance network (FoodNet). *Vet. Clin. N. Am. Food Anim. Pract.* **14**:165–172.
2. Baranyi, J., and T. A. Roberts. 1994. A dynamic approach to predicting bacterial growth in food. *Int. J. Food Microbiol.* **23**:277–294.
3. Baranyi, J., T. P. Robinson, A. Kaloti, and B. M. Mackey. 1995. Predicting growth of *Brochothrix thermosphacta* at changing temperature. *Int. J. Food Microbiol.* **27**:61–75.
4. Blankenship, L. C., S. C. Craven, R. G. Leffler, and C. Custer. 1988. Growth of *Clostridium perfringens* in cooked chili during cooling. *Appl. Environ. Microbiol.* **54**:1104–1108.
5. Bovill, R., J. Bew, N. Cook, M. DAgostino, N. Wilkinson, and J. Baranyi. 2000. Predictions of growth for *Listeria monocytogenes* and *Salmonella* during fluctuating temperature. *Int. J. Food Microbiol.* **59**:157–165.
6. Bryan, F. L. 1988. Risks of practices, procedures and processes that lead to outbreaks of foodborne diseases. *J. Food Prot.* **51**:663–673.
7. Bryan, F. L., and C. A. Bartleson. 1985. Mexican style foodservice operations: hazard analyses, critical control points and monitoring. *J. Food Prot.* **48**:509–524.
8. Caipo, M. L., S. Duffy, L. Zhao, and D. W. Schaffner. 2002. *Bacillus megaterium* spore germination is influenced by inoculum size. *J. Appl. Microbiol.* **92**:879–884.
9. Danler, R. J., E. A. E. Boyle, C. L. Kastner, H. Thippareddi, D. Y. C. Fung, and R. K. Phebus. 2003. Effects of chilling rate on outgrowth of *Clostridium perfringens* spores in vacuum-packaged cooked beef and pork. *J. Food Prot.* **66**:501–503.
10. Fu, B., P. S. Taoukis, and T. P. Labuza. 1991. Predictive microbiology for monitoring spoilage of dairy products with time-temperature integrators. *J. Food Sci.* **56**:1209–1215.
11. Juneja, V. K., B. S. Marmar, and A. J. Miller. 1994. Growth and sporulation potential of *Clostridium perfringens* in aerobic and vacuum-packaged cooked beef. *J. Food Prot.* **57**:393–398.
12. Juneja, V. K., O. P. Snyder, and M. Cygnarowicz-Provost. 1994. Influence of cooling rate on outgrowth of *Clostridium perfringens* spores in cooked ground beef. *J. Food Prot.* **57**:1063–1067.
13. Juneja, V. K., R. C. Whiting, H. M. Marks, and O. P. Snyder. 1999. Predictive model for growth of *Clostridium perfringens* at temperatures applicable to cooling of cooked meat. *Food Microbiol.* **16**:335–349.
14. Kalinowski, R. M., R. B. Tompkin, P. W. Bodnaruk, and W. P. Pruett. 2003. Impact of cooking, cooling, and subsequent refrigeration on the growth or survival of *Clostridium perfringens* in cooked meat and poultry products. *J. Food Prot.* **66**:1227–1232.
15. Llaudes, M., L. Zhao, S. Duffy, and D. W. Schaffner. 2001. Simulation and modeling of the effect of small inoculum size on the time to spoilage by *Bacillus stearothermophilus*. *Food Microbiol.* **18**:395–405.
16. McMeekin, T. A., J. Olley, D. A. Ratkowsky, and T. Ross. 2002. Predictive microbiology: towards the interface and beyond. *Int. J. Food Microbiol.* **73**:395–407.
17. Taormina, P. J., G. W. Bartholomew, and W. J. Dorsa. 2003. Incidence of *Clostridium perfringens* in commercially produced cured raw meat product mixtures and behavior in cooked products during chilling and refrigerated storage. *J. Food Prot.* **66**:72–81.
18. U.S. Department of Agriculture-Food Safety Inspection Service. June 1999. Appendix B—compliance guidelines for cooling heat-treated meat and poultry products (stabilization). [Online.] <http://www.fsis.usda.gov/OA/fr/95033Fb.htm>.
19. Whiting, R. C., and T. P. Strobaugh. 1998. Expansion of the time-to-turbidity model for proteolytic *Clostridium botulinum* to include spore numbers. *Food Microbiol.* **15**:449–453.
20. Willardsen, R. R., F. F. Busta, and C. E. Allen. 1979. Growth of *Clostridium perfringens* in three different beef media and fluid thioglycollate medium at static and constantly rising temperatures. *J. Food Prot.* **42**:144–148.
21. Zhao, L., T. J. Montville, and D. W. Schaffner. 2000. Inoculum size of *Clostridium botulinum* 56A spores influence time-to-detection and percent growth-positive samples. *J. Food Sci.* **65**:1369–1375.
22. Zwietering, M. H., J. C. de Wit, H. G. A. M. Cuppers, and K. van't Riet. 1994. Modeling of bacterial growth with shifts in temperature. *Appl. Environ. Microbiol.* **60**:204–213.