

Evaluation of a Predictive Model for *Clostridium perfringens* Growth during Cooling

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ABSTRACT

Proper temperature control is essential in minimizing *Clostridium perfringens* germination, growth, and toxin production. The U.S. Department of Agriculture Food Safety and Inspection Service offers two options for the cooling of meat products: follow a standard time-temperature schedule or validate that alternative cooling regimes result in no more than a 1-log CFU/g increase of *C. perfringens* and no growth of *Clostridium botulinum*. The Juneja 1999 model for *C. perfringens* growth during cooling may be helpful in determining whether 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 under a variety of temperature situations. The Juneja 1999 model for *C. perfringens* growth during cooling is fail safe when low (<1 log CFU/ml) or high (>3 log CFU/ml) observed increases occur during exponential cooling. The Juneja 1999 model consistently underpredicted growth at intermediate observed increases (1 to 3 log CFU/ml). The Juneja 1999 model also underpredicted growth whenever exponential cooling took place at two different rates in the first and second portions of the cooling process. This error may be due to faster than predicted growth of *C. perfringens* cells during cooling or to an inaccuracy in the Juneja 1999 model.

Clostridium perfringens continues to be a major concern to the food industry. Fifty-seven confirmed outbreaks of *C. perfringens* infections, 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 (8). *C. perfringens* has been implicated as the cause of foodborne illness in roast beef, turkey, meat-containing Mexican foods, and other meat dishes (7). Angulo et al. (1) reported that the most common cause of *C. perfringens* infection was improper cooling (40.9%) of food products. *C. perfringens* spores may survive cooking, germinate, and produce vegetative cells, which then multiply in cooked foods if the rate and duration of cooling are too slow (5). *C. perfringens* generation time can be as rapid as 7.4 min in autoclaved ground beef, with a reported optimal temperature range of 37 to 45°C and with growth reported at temperatures as low as 6°C (18).

The U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) guidelines for the cooling of meat products suggest that the internal product temperature should not remain between 130 (54.4°C) and 80°F (26.7°C) for more than 1.5 h nor between 80 (26.7°C) and 40°F (4.4°C) for more than 5 h during cooling. If meat processors are unwilling or unable to meet this cooling schedule, they must be able to prove that an alternative regimen will result in less than a 1 log CFU/ml increase in *C. perfringens* and no growth of *C. botulinum*.

Computer models are gaining greater acceptance for helping to solve food microbiology problems (15), includ-

ing situations where microbial growth must be predicted under changing temperatures (4, 6, 20). One such model predicts the growth of *C. perfringens* at changing temperatures associated with the cooling of cooked meat products (12). This model (the Juneja 1999 model) has been incorporated into the USDA's Pathogen Modeling Program (PMP) version 6.1 but has not been validated.

The objective of this research was to validate the Juneja 1999 model for *C. perfringens* under changing temperature and temperature abuse situations. Such validated models may be a useful tool for determining whether the *C. perfringens* performance standard was met if a problem were to 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)—that were previously used to collect the growth data for the Juneja 1999 model were obtained from Dr. V. K. Juneja (USDA Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, Pa.). These strains were maintained and prepared according to procedures described by Juneja et al. (10). The spore cocktail was inoculated into 9 ml of freshly sterilized fluid thioglycollate medium and heat activated at 75°C for 20 min.

Growth medium, inoculation, and sampling. Growth of *C. perfringens* in Trypticase-peptone-glucose-yeast extract (TPGY) containing (wt/vol) 5% Trypticase, 0.5% peptone, 2% yeast extract, 0.1% cysteine hydrochloride, and 0.4% dextrose was monitored during changing temperature cooling schemes. TPGY was sterilized by autoclaving, and 9-ml aliquots were dispensed into

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TABLE 1. Comparison of Juneja et al. (12) results and observed germination, outgrowth, and lag times (GOLs) and exponential growth rates (EGRs) for *C. perfringens* at constant temperatures

Temperature (°C)	GOL (h)		EGR (log CFU/ml/h)	
	Juneja et al.	Observed	Juneja et al.	Observed
19	39.32 ± 1.33	32.35 ± 2.15	0.13 ± 0.01	0.20 ± 0.10
35	3.03 ± 0.29	4.33 ± 1.08	1.31 ± 0.05	1.68 ± 0.35
45	3.39 ± 0.35	3.68 ± 0.48	1.62 ± 0.26	2.29 ± 0.81
50	5.61 ± 0.15	5.22 ± 0.35	1.51 ± 0.04	1.70 ± 0.20

sterile culture tubes. Each tube of broth was inoculated with 0.1 ml of appropriately diluted heat-shocked spores to obtain a final target concentration of 10 or 1,000 CFU/g and subsequently flushed for 30 s with sterile N₂ gas. Culture tubes of broth were closed with screw caps to maintain an anaerobic environment. Thermocouples were inserted into uninoculated tubes and used to record the temperature once every minute during the cooling period. Samples were removed at set intervals (30 or 60 min) throughout each cooling process, diluted, plated, and incubated following the procedures described by Labbe and Norris (13). All experiments were performed in triplicate.

Initial spore concentration influences germination and growth of *C. botulinum* (19) and other spore-forming bacteria (9, 14); therefore, initial concentrations of 10 and 1,000 spores per ml were used for each cooling time to determine the effect of initial spore concentration.

Temperature variation studies. Both static (unchanging) temperature experiments and dynamic (changing) temperature experiments were conducted. Dynamic temperature experiments were of two types: those conducted using a temperature profile arising from a single driving force and those conducted using two driving forces at different times. The rate of temperature change for the single driving force experiments is linear when plotted on a logarithmic temperature scale (single rate experiments). The rate of temperature change for the two driving force experiments shows two linear portions with different slopes when plotted on a logarithmic temperature scale (dual rate experiments). The dual rate experiments followed one cooling rate from 54.4 to 26.7°C and a second rate from 26.7 to 4.4°C.

All experiments were carried out in a programmable water bath (model 1028P, Fisher Scientific, Fair Lawn, N.J.). The temperature was set to maintain a constant temperature (19, 35, 45, or 50°C) during the static experiments. Cooling of food products follows an exponential rate (11), and the water bath was programmed to mimic an exponential cooling rate during the dynamic temperature experiments. The driving force for this exponential rate was assumed to be 0°C.

Single cooling rate experiments were conducted to investigate 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) (11). 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 practice. 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 (17). 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 9, 12, and 15 h were also investigated with proportional variations to the temperature ranges of the FSIS cool-

ing 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 using the PMP. The time-temperature combinations used to program the water bath in these experiments were entered into the cooling profile window for the *C. perfringens* cooling beef broth model in PMP version 6.1, and log CFU/ml increases over time were calculated. Predicted log CFU/ml increases over time were used as outputs for subsequent data analysis.

Data analysis. Estimates of germination, outgrowth, and lag time (GOL) and exponential growth rate (EGR) for static temperature conditions were determined by Gompertz curve-fitting procedures (16) using Sigma Plot version 8.0 (SPSS, Inc., Chicago, Ill.) where

$$\log(L_t) = A + Ce^{-B(t-M)}$$

Log(L_t) is the log count of the number of *C. perfringens* cells at time t , A is the log cell concentration at time $-\infty$, C is the difference between the upper (log maximum population density) and lower (log initial inoculum) asymptote, and e is the base of the natural logarithm. The parameter B is proportional to the relative growth rate at time point M , where M is the time at which the absolute growth rate is at a maximum. GOL is defined as $M - 1/B$, and EGR is defined as $B \times C/e$. Exponential growth phase duration (EGPD) is defined as C/EGR . Primary growth curves were also fit to the logistic function (20) and the Baranyi model (3).

Further analysis was performed on the *C. perfringens* growth data collected during changing temperature conditions. Because all the cooling profiles used involved gradual temperature changes, all the resulting growth curves were essentially sigmoid, which allowed the use of the modified Gompertz equation to model *C. perfringens* growth during cooling. Fitting the modified Gompertz equation allowed the calculation of apparent GOLs, apparent EGRs, and apparent EGPDs for all the observed and predicted growth curves. Comparison of observed and predicted GOLs, EGRs, and EGPDs permitted determination of the exact sources of error in the model predictions. Estimations of bias and accuracy of the Juneja 1999 model predictions versus the data collected here were made using the method proposed by Baranyi et al. (2).

RESULTS AND DISCUSSION

Static temperature. Experiments at selected static (i.e., unchanging) temperatures within the range used by the Juneja 1999 model were conducted to verify that methods in use in our laboratory were able to reproduce those results reported by Juneja et al. (12). The previously published and observed GOLs and EGRs shown in Table 1 are in agreement. Although the observed EGRs are slightly higher than

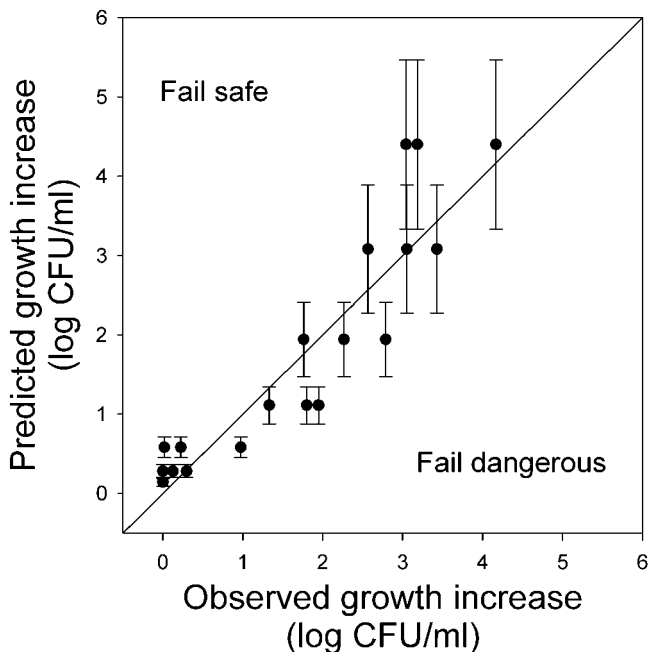


FIGURE 1. Observed increase in *C. perfringens* concentration in broth versus that predicted by the USDA PMP version 6.1 during exponential cooling at a constant rate.

those reported by Juneja et al., the standard deviations of both the GOLs and EGRs generally encompass the Juneja et al. data. The data shown in Table 1 indicate that our methods are able to reproduce the data used to create the Juneja 1999 model for *C. perfringens* growth during cooling.

Single cooling rate. Temperatures recorded using thermocouples inserted into the TPGY broth were compared with profiles programmed into the water bath. No significant differences between the temperature profiles were observed. Extending the duration of exponential cooling curves beyond the 6.5 h currently suggested by FSIS guidelines resulted in *C. perfringens* growth similar to that previously reported (11). Cooling over 6.5, 9.0, and 12.0 h

resulted in <1 log increase of *C. perfringens*. Cooling times of 15, 18, 21, and 24 h resulted in >1 log increase of *C. perfringens*.

When only single-rate exponential cooling patterns are considered, the observed growth and that predicted by the Juneja 1999 model showed reasonably close agreement (Fig. 1). Calculated bias and accuracy values for these data are 1.25 and 2.52, respectively. Figure 1 shows a distinct concave trend, however, where the model overpredicts (is fail safe) for low observed growth increases and high observed growth increases but tends to underpredict (is fail dangerous) at intermediate levels of growth.

Dual cooling rates. Growth of *C. perfringens* was also monitored during cooling at two rates, as outlined in Table 2. Average increases in *C. perfringens* concentration were 0.35 and 0.42 log CFU/ml for 6.5-h cooling experiments, but the response of the *C. perfringens* three-strain cocktail was quite variable (the SDs are similar in magnitude to the concentration increases) for cooling over 6.5 h. The 9-h cooling cycle (divided into 4- and 5-h periods with different cooling rates) resulted in about a 2-log increase. When the 9-h cooling cycle was divided into 3.5- and 5.5-h periods (also with different cooling rates), the increase in *C. perfringens* concentration was slightly <1 log CFU/ml. Cooling over 12 h (in either 5- and 7-h periods or 6- and 6-h periods) or cooling over 15 h (5.5 and 9.5 h) always resulted in >3 log CFU/ml average increase.

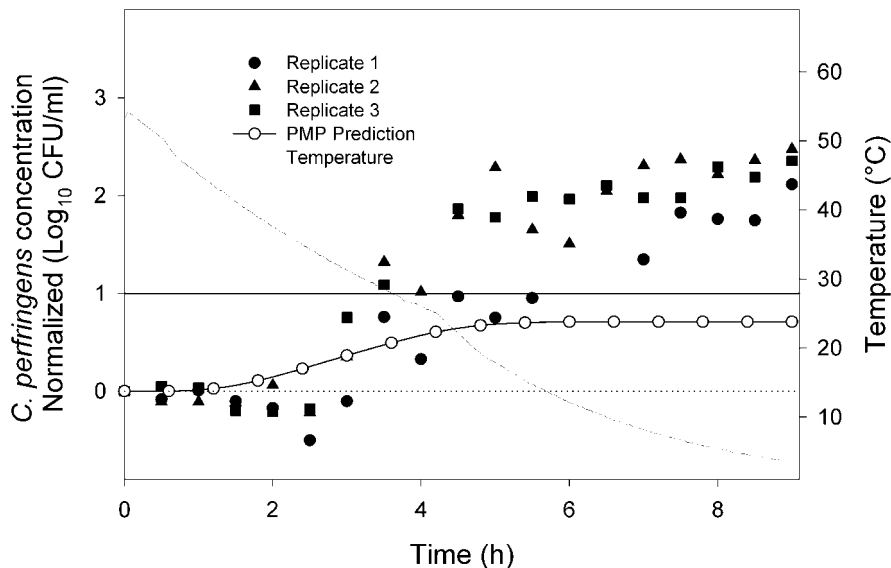
Table 2 also clearly shows that the actual increase in *C. perfringens* concentration always exceeded the increase predicted by the Juneja 1999 model, no matter what time period or cooling rates were considered. This finding may be of special practical importance for the 9-h cooling times, where the predicted increase in *C. perfringens* was <1 log CFU/ml but the actual increase exceeded or was very close to 1 log CFU/ml.

Analysis of model performance. Because of the systematic overprediction seen in Table 2, a more detailed analysis of the observed growth curves and model predic-

TABLE 2. Comparison of observed growth and growth predicted by PMP version 6.1 during dual-rate exponential cooling

Total cooling time (h)	Initial inoculum (log CFU/ml)	Time to cool over parts of range (h)		Mean growth (\pm SD) (log CFU/ml)	
		Upper (54.4–26.7°C)	Lower (26.7–4.4°C)	Actual	Predicted
6.5	3	3.0	3.5	0.35 \pm 0.33	0.04 \pm 0.02
	1	3.0	3.5	0.42 \pm 0.50	0.04 \pm 0.02
9.0	3	4.0	5.0	2.31 \pm 0.18	0.71 \pm 0.13
	1	4.0	5.0	1.99 \pm 0.49	0.71 \pm 0.13
	3	3.5	5.5	0.84 \pm 0.27	0.52 \pm 0.11
	1	3.5	5.5	0.94 \pm 0.20	0.52 \pm 0.11
12.0	3	5.0	7.0	3.07 \pm 0.27	1.40 \pm 0.28
	1	5.0	7.0	3.32 \pm 0.82	1.40 \pm 0.28
	3	6.0	6.0	3.53 \pm 0.31	2.28 \pm 0.49
	1	6.0	6.0	3.51 \pm 0.15	2.28 \pm 0.49
15.0	3	5.5	9.5	3.28 \pm 1.30	2.04 \pm 0.45
	1	5.5	9.5	3.48 \pm 1.12	2.04 \pm 0.45

FIGURE 2. Growth of *C. perfringens* in broth during 9 h of cooling at two rates (54.4 to 26.7°C over 4 h and 26.7 to 4.4°C over 5 h) versus growth predicted by the USDA PMP version 6.1.



tions was undertaken. The objective of this analysis was to determine which aspect of the model predictions contributed most to the prediction error. Were the predictions too low because *C. perfringens* came out of GOL faster than the model predicted or was the error due to a faster than expected EGR? It might also be that the observed EGR was longer than predicted, so the *C. perfringens* cells were able to grow over a longer period than the model predicted and thus were able to reach a greater than expected final concentration.

Figure 2 shows a representative 9-h cooling experiment where the source of the erroneous model prediction is apparent. In this example, the samples are initially cooled at one rate for the first 4 h and then cooled at a different rate for the next 5 h. The observed GOL (for all three replicates) is longer than that predicted by the Juneja 1999 model. The EGR observed is also considerably faster than that predicted by the Juneja 1999 model. Both the observed and model *C. perfringens* populations stop growing after about 5 h, when the temperature of the samples reaches 18°C.

When fitting data to the models, the Gompertz model had slightly better agreement than did the logistic and Baranyi models. Parameters determined from these fits were used in further analysis.

Table 3 presents a detailed analysis of the data collected under dual-rate cooling over 9, 12, and 15 h, including the GOLs, EGRs, and EGPDs for these cooling conditions. There was little influence of inoculum size on GOL, except during the 15-h cooling experiment where the larger inoculum size resulted in a longer lag time. This result is inconsistent with that of other published reports, in which smaller inoculum sizes resulted in longer GOLs for spore-forming bacteria (9, 14, 19). Faster EGRs were observed at smaller inoculum sizes during 9 h of cooling, little difference in EGR was seen during 12 h of cooling, and slower EGRs were seen during 15 h of cooling. Inoculum size had no consistent effect on EGR at any cooling time.

Although the inoculum size results may merit additional study and experimentation before their meaning (if any) is clear, the most striking results seen in Table 3 are

TABLE 3. Comparison of observed and PMP-predicted germination, outgrowth, and lag time (GOL), exponential growth rate (EGR), and exponential growth phase duration (EGPD) for *C. perfringens* during dual-rate exponential cooling

Total cooling time (h)	Time to cool over parts of range (h)		Initial inoculum (log CFU/ml)	GOL (h)		EGR (log CFU/ml/h)		EGPD (h)	
	Upper (54.4–26.7°C)	Lower (26.7–4.4°C)		Observed	Predicted	Observed	Predicted	Observed	Predicted
9	4.0	5.0	3	2.51	1.55	0.68	0.26	1.20	3.80
	4.0	5.0	1	2.53	1.55	1.11	0.26	1.34	3.80
	3.5	5.5	3	3.21	1.29	0.76	0.21	3.20	5.16
	3.5	5.5	1	3.45	1.29	1.56	0.21	3.03	5.16
12	6.0	6.0	3	3.52	2.55	1.03	0.61	0.82	1.18
	6.0	6.0	1	3.23	2.55	1.32	0.61	0.76	1.18
	5.0	7.0	3	3.93	2.03	0.68	0.41	0.74	1.92
	5.0	7.0	1	2.96	2.03	0.71	0.41	0.78	1.92
15	5.5	9.5	3	6.17	2.21	1.61	0.51	0.82	1.32
	5.5	9.5	1	3.78	2.21	0.78	0.51	0.78	1.32

the marked differences between the observed and predicted GOLs, EGRs, and EGPDs. In every case, the observed GOLs were longer than those predicted by the Juneja 1999 model. If this were the only difference (i.e., EGRs and EGPDs were as predicted), the model predictions shown in Table 2 would exceed the observations, which is clearly not the case. Table 3 also shows that the EGR observed exceeds that predicted by the Juneja 1999 model in every case, whereas the EGPD is not as long as would be expected from the predictions. The difference in EGPD (as was the case for GOL) would also result in model predictions that exceed the observations. Because the observations consistently exceed the model predictions (Table 2), the difference in the EGRs more than compensates for the longer observed GOLs and observed EGPDs.

Data from static growth curves can be used to predict growth of organisms during changing temperature situations (4, 6, 12, 20). Trends seen in this study when comparing experimental data to predicted values from single cooling rate studies suggest that the Juneja 1999 model used to describe growth of *C. perfringens* during exponential cooling could be improved. Our findings may also have important implications relative to the *C. perfringens* performance standard when the Juneja 1999 model is used to estimate the expected increase in *C. perfringens* concentration, especially in those instances where the observed increase is very close to or exceeds 1 log CFU/ml and the predicted increase is less.

The results presented here indicate that the Juneja 1999 model for *C. perfringens* growth overpredicts at low (<1 log CFU/ml) and high (>3 log CFU/ml) observed increases during single rate exponential cooling. The Juneja 1999 model for *C. perfringens* growth also consistently underpredicts concentration increases when exponential cooling takes place at two different rates in the first and second portions of the cooling process. This underprediction is apparently due to a much faster than predicted EGR. A possible explanation for this result is that the Juneja 1999 model incorrectly models static data under dynamic conditions.

This work was not intended to create a new model to describe growth during dynamic cooling but to validate the accuracy and applicability of the current model. Because the Juneja 1999 model is inadequate, work is currently underway to use the large quantity of growth data collected under dynamically changing conditions to develop a new model more suitable for use under changing conditions.

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