

Proteolytic *Clostridium botulinum* growth at 12–48°C simulating the cooling of cooked meat: development of a predictive model

V. K. Juneja^{1*} and H. M. Marks²

The objective of this study was to develop a model to predict the germination, outgrowth and lag (GOL), and exponential growth rates of Clostridium botulinum from spores at temperatures (12–48°C) applicable to the cooling of cooked meat products. The growth medium, Reinforced Clostridial medium (RCM) supplemented with oxyrase enzyme to create suitable anaerobic conditions, was inoculated with approximately $4 \log_{10}$ spores ml^{-1} . Clostridium botulinum populations were determined at appropriate intervals by plating onto RCM. Clostridium botulinum growth from spores was not observed at temperatures $< 12^\circ C$ or $> 48^\circ C$ for up to 3 weeks. Growth curves were determined by fitting Gompertz functions to the data. From the parameters of the Gompertz function the growth characteristics, GOL times and exponential growth rates were calculated. These growth characteristics were subsequently described by Ratkowsky functions using temperature as the independent variable. Closed form equations were developed that allow for predicting relative growth for a general cooling scenario. By applying multivariate statistical procedures, the standard errors and confidence intervals were computed on the predictions of the amount of relative growth for a cooling scenario. The predictive model is capable of predicting spore outgrowth and multiplication for general cooling scenarios, for suitable but unverified mathematical assumptions, and should aid in evaluating the safety of cooked products after cooling.

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Received:
8 October 1998

Introduction

Clostridium botulinum is a Gram-positive, spore-forming, anaerobic, rod-shaped, catalase-negative, soil organism that produces a potent neurotoxic protein known as botulinum neurotoxin. This toxin causes a neuroparalytic

disease called botulism. The organism is the most hazardous spore-forming foodborne pathogen and is ubiquitous world-wide. *Clostridium botulinum* spores may find their way into processed food through raw materials or by post-processing contamination of food. It is not possible to be certain that any food will not contain spores of *C. botulinum* other than the foods that have been aseptically packed or have received sporocidal heat treatment. It is worth emphasizing that the presence of even less than 1 spore g^{-1} is enough to justify strict

¹US Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Lane, 19038, USA Pennsylvania, Wyndmoor,
²US Department of Agriculture, Food Safety Inspection Service, 14th and Independence S.W., Washington D.C. 20250, USA

*Corresponding author.

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prophylactic measures to be taken because consumption of even small amounts of the foods in which growth has occurred and toxins have formed can be sufficient to produce symptoms of human botulism.

The demand by consumers for fresh tasting, high-quality, low salt, preservative free, convenience meals has resulted in increased production of minimally processed, ready-to-eat, extended shelf-life refrigerated foods. The mild heat treatment given to such foods is aimed at the destruction of vegetative cells of spoilage and pathogenic bacteria; heat-resistant spore-forming foodborne pathogens, including *C. botulinum* spores can survive the thermal process. In such minimally processed foods, if the rate and extent of cooling after cooking is not sufficient, heat-activated surviving spores pose a potential public health hazard because they are likely to germinate, outgrow, and multiply into toxin producing vegetative cells. Bryan (1978) identified inadequate cooling and lapse of a day or more between food preparation and service as primary factors contributing to outbreaks of foodborne disease. Improper storage or holding temperature was the factor reported to contribute for 34% of *C. botulinum* outbreaks (Bean and Griffin 1990). Such outbreaks clearly stress the importance of cooling foods quickly after cooking. The time/temperature guidelines for cooling heated products recommend that the maximum internal temperature should not remain between 54.4 (130°F) and 26.7°C (80°F) for more than 1.5 h nor between 26.7 and 4.4°C (40°F) for more than 5 h (USDA 1989). The US Food and Drug Administration (FDA) Division of Retail Food Protection recognized that inadequate cooling was a major food safety problem and established a recommendation that all food should be cooled from 60 to 21°C (140–70°F) in 2 h and from 21 to 5°C (70–41°F) in 4 h (FDA Division of Retail Food Protection 1997).

In a study by Juneja et al. (1994), when ground beef samples inoculated with spores of *C. botulinum* were cooled from 54.4 to 7.2°C using cooling times varying from 6 to 21 h, spores germinated and grew, and the population densities increased by 1 log unit in 21 h. While the study by Juneja et al. (1994) provided some characterization regarding growth of

C. botulinum during cooling of cooked meat, there appears to be no work available on growth from spores of *C. botulinum* over the entire temperature range which foods must pass through during cooling after cooking. Thus, a gap in the scientific literature for determining the safety of cooked foods subjected to longer cooling times than those recommended prompted us to perform this investigation of relative growth at different temperatures. Accordingly, the present study was undertaken with the intention of developing a model that can be used to predict the relative growth of *C. botulinum* from spores during a cooling period of cooked meat.

Materials and Methods

Strains and spore suspension

Proteolytic *C. botulinum* type A and B strains and their sources included in this study are listed in Table 1. Proteolytic *C. botulinum* type A and B spores were prepared by anaerobically growing each strain in BAM broth (Huhtanen 1975) at 35°C for 3 weeks. Spores were harvested by centrifugation at 4000 g for 10 min and then washed three times in sterile distilled water by repeated suspension and centrifugation at 4000 g for 5 min. Each spore preparation was stored at 4°C in sterile distilled water. Spore population was enumerated by spiral plating (Spiral Biotech, Bethesda, Maryland, USA; Model D) appropriate dilutions (in 0.1% peptone water), in duplicate, on Reinforced clostridial medium (RCM; Difco) followed by incubation of plates anaerobically for 48 h at 35°C. A spore cocktail containing all

Table 1. Strains used in the study and their sources

Species and strains	Source
<i>Clostridium botulinum</i>	
Proteolytic type A	
Strains: 62A	FDA
33	US Army Lab. Natick
Proteolytic type B	
Strains: 999	FDA
C11 (7949)	ATCC

three strains of *C. botulinum* was prepared immediately prior to experimentation by mixing equal numbers of spores from each suspension. Spore suspensions were heated for 10 min at 80°C immediately before use to stimulate spore germination.

Growth medium, inoculation and sampling

Sterile, oxygen reducing, membrane fragments produced from *E. coli* b/r strain, commercially available as Oxyrase™ and designed for anaerobic cultivation of bacteria were purchased from Oxyrase™ Inc., Ashland, Ohio, USA. Reinforced clostridial medium (RCM; Difco) was used for determination of growth rates. The medium (50 ml) was dispensed in tubes and sterilized by autoclaving. The medium was supplemented with oxyrase (0.1 ml 5 ml⁻¹ broth medium) and then incubated at 35°C for 30 min prior to the experiments. Each tube received 0.5 ml of the heat-shocked spores to obtain an initial count of about 4 log₁₀ spores ml⁻¹ of the growth medium. All tubes were incubated at 10–50°C. At intervals appropriate for each growth temperature, samples were withdrawn and serial dilutions were made in 0.1% peptone-water (w/v) supplemented with oxyrase (0.1 ml 5 ml⁻¹). The dilution tubes were transferred to an Bactron anaerobic chamber (Sheldon Manufact. Inc., Cornelius, Oregon, USA) and spread-plated onto dishes containing RCM agar. The total *C. botulinum* population was determined after 48 h of incubation at 37°C in the anaerobic chamber.

Three replicate experiments were conducted at each temperature, with the exception of 44°C, for which there were three sets of three replicates. One of these sets was not included because of rapid growth suggesting incubation problems. At the temperatures of 11°C and 50°C no growth was measured in a 3-week period.

Results

Fitting growth curves

The procedures for determining a model follow those given in a previous paper (Juneja et al.

1999) on modeling growth for *C. perfringens*. A brief account of the modelling will be given here. The growth of an organism as a function of time, can be described by

$$L(t) = A + (P - A)f(t|M, B) \quad (1)$$

where $L(t)$ is the common logarithm of $N(t)$, the number of organisms at time t , $f(t|M, B)$ is a non-decreasing function of time between 0 and 1, M and B are non-negative parameters that describe the slope and location of the curve along the t -axis and are functions of the relative growth rate, EGR (log₁₀(cfu ml⁻¹) h⁻¹) and the time for germination, outgrowth and lag (GOL) in hours, A is an asymptotic minimum value, and P is an asymptotic maximum value and represents the maximum population density. In generating growth curves using data from controlled experiments, it is often assumed (Gibson et al. 1988, Buchanan 1990) that P is a constant quantity and $A = \log_{10}(N_0)$, where N_0 is the initial number of organisms. To estimate the parameters for equation 1, for a given temperature/replicate growth experiment, the value of A was computed to be the average of the log₁₀ transformed measured population densities that occurred at a time equal to or less than the time of the minimum measured population density. Specifically, let $D(t)$ be the measured density at time t , and t_0 be the maximum time of occurrence of the minimal density, that is, $D(t_0) \leq D(t)$ for all t . Then A is

$$A = \frac{\sum_{t \leq t_0} \log_{10}(D(t))}{N(t \leq t_0)} \quad (2)$$

where $N(t \leq t_0)$ are the number of measured densities in the closed interval $[0, t_0]$.

Data analysis on the maximum measured population densities obtained from the different experiments suggested that the maximum population density did not depend on temperature. The experiments lasted a specified length, thus a maximum possible density might not be reached. There was not an outlier and the highest few log density results were: 10.11, 10.1, 10.05, 10.0. Using an average would create a negative bias, however, fortunately, various possible choices are close in magnitude and the choice of one over the another would not

affect the predicted values by much. Thus, a value 10.2 was chosen to represent P , the maximum possible density.

Comparisons of growth curves using the Gompertz, $g(t|M, B) = \exp(-\exp(-B(t - M)))$ and logistic, $h(t|M, B) = 1/(1 + \exp(-B(t - M)))$, functions (Gibson et al. 1988) were made. Estimates of B and M were derived using SAS/STAT®, PC, edition 6.12, PROC NLIN procedure. Both curves appear for practical and statistical purposes to perform equally well. Since the primary concern is predicting relative growth for small expected relative growth, the primary criterion for deciding upon a curve was the behavior of predictions in the low relative growth region (defined here to be where relative growth is less than $0.5 \log_{10}$). In this region the root mean square error of the residuals (observed minus predicted \log_{10} relative growths) was equal to 0.208 using the logistic function, and equal to 0.204 using the Gompertz function. The slightly higher value associated with the logistic function was caused by a higher, in absolute value, average of the residuals. The averages of the residuals were -0.065 , using the logistic function, and 0.025 , using the Gompertz function. Thus, the Gompertz function was used for modeling the relative growth.

From estimated values of M and B , estimates of the EGR and GOL time were computed. The exponential growth rate, EGR, is defined to be the maximal relative growth rate, $d(L(t))/dt$, (Gibson et al. 1988, McMeekin et al. 1993). For the paper, it is important to note that EGR is expressed in common logarithm rather than natural logarithm units. The GOL time is defined as the value of time of the point of intersection of the line containing the point $(M, L(M))$ with slope equal to the exponential growth rate and the horizontal line at $L(0)$ (McMeekin et al. 1993). The growth characteristics, EGR and GOL, for the Gompertz function can be expressed as:

$$\begin{aligned} \text{EGR} &= B(P - A)/e \\ \text{GOL} &= M + (eg(0|M, B) - 1)/B \end{aligned} \quad (3)$$

where $e = \exp(1)$. Usually $g(0|M, B)$ is small so that GOL is approximated as $M - 1/B$. The

estimated values of EGR and GOL using the approximation, and assuming $A = \log_{10}(N_0)$ (Gibson et al. 1988) are presented in Table 2.

Modelling growth characteristics

The above equations apply for a constant temperature. However, for a cooling scenario, the temperature would be changing, so that, for predicting the amount of growth or relative growth, $N(t)/N_0$, it is necessary to express the growth characteristics, EGR and GOL time, as functions of temperature. For generic bacteria, it has been found by researchers (Ratkowsky et al. 1983) that the square root of the exponential growth rate, or the inverse of the GOL, k , as the dependent variable, and the most general form of the Ratkowsky model:

$$k^{1/2}(T) = a(T - T_{\min})(1 - \exp(b(T - T_{\max})))^\alpha \quad (4)$$

where a, b, T_{\min} and T_{\max} are unknown positive parameters, α is usually either 1 or 1/2, providing a good statistical fit. The Ratkowsky equation describes a curve for which, starting from zero at temperature T_{\min} , there is a near linear increase of the dependent variable, $k^{1/2}$, with increasing temperature, until reaching a maximum value, followed then by a rapid decline to zero at temperature T_{\max} . The choice of α depends upon the curvature at the maximum level and the rapidity of the decline for high temperatures. The root mean square errors of the residuals from the regressions when $\alpha = 1/2$ were smaller than that for when $\alpha = 1$. Without many data points near the point of curvature, at temperatures less than and greater than the temperature for which the curve is at its maximum it is difficult to estimate well the curvature. The consequence, statistically, is that the estimate of α from a regression analysis is highly correlated with the estimates of the other parameters resulting in very unstable estimates. Thus α was not used as an unknown parameter and was set equal to 1/2 for the regression analysis.

Table 2. Estimated GOL times (h) and exponential growth rate ($\log_{10}(\text{cfu ml}^{-1})\text{h}^{-1}$) of *Clostridium botulinum* in RCM broth with Gompertz curves

Experiment	Temperature	Approximate GOL ^a time Gompertz	Exponential growth rate Gompertz
1	12	66.597	0.0610
2	12	69.285	0.0613
3	12	71.981	0.0629
4	15	34.652	0.0681
5	15	38.946	0.0732
6	15	43.129	0.0857
7	19	23.317	0.3919
8	19	22.218	0.3282
9	19	13.926	0.2144
10	21	12.648	0.1036
11	21	23.158	0.3172
12	21	17.100	0.1331
13	25	19.223	0.6557
14	25	20.130	0.7527
15	25	18.222	0.6152
16	30	7.199	0.5785
17	30	7.077	0.5633
18	30	7.835	0.6462
19	32	5.337	0.7805
20	32	3.693	0.4469
21	32	4.838	0.5401
22	37	5.969	0.3347
23	37	6.573	0.6457
24	37	5.826	0.5326
25	40	6.306	1.2112
26	40	6.447	1.3002
27	40	6.588	1.5547
28	44	5.585	1.2282
29	44	4.539	0.8778
30	44	4.346	0.8828
31	44	5.249	1.1597
32	44	5.272	1.0630
33	44	5.501	1.1738
34	46	2.943	0.4723
35	46	3.844	0.5176
36	46	3.759	0.4982
37	48	2.425	0.5840
38	48	3.523	0.6865
39	48	3.559	0.6923

^aGermination, outgrowth and lag.

For modelling GOL and EGR, there are two equations, similar to eqn (4), with a possibility of eight parameters,

$$1/\text{GOL}^{1/2} = a_l(T - T_{\min})(\exp(b_l(T - T_{\max})))^{1/2}$$

$$\text{EGR}^{1/2} = a_g(T - T_{\min})(\exp(b_g(T - T_{\max})))^{1/2}$$

(5)

with parameters a_l, b_l, a_g, b_g and parameters T_{\min} and T_{\max} describing minimum and

maximum temperatures for the ranges in which the dependent variables are defined and are greater than zero. These latter temperature parameters could be different for the GOL and EGR variables. Assuming different T_{\min} and T_{\max} values for GOL and EGR and using the same estimation procedures as described below, the estimate of $T_{\min}(\text{EGR}) = 7.22^\circ\text{C} > T_{\min}(\text{GOL}) = 5.29^\circ\text{C}$, while both $T_{\max}(\text{GOL})$ and $T_{\max}(\text{EGR})$ were approximately equal to

50°C. The two estimates, $T_{\min}(\text{GOL})$ and $T_{\min}(\text{EGR})$, are not, statistically, significantly different. For the final model, it will be assumed that $T_{\min}(\text{GOL}) = T_{\min}(\text{EGR})$ and $T_{\max}(\text{GOL}) = T_{\max}(\text{EGR})$. These assumptions decrease the number of parameters from eight to six and thus increases the stability of the estimates and the degrees of freedom associated with the estimates. They are also considered reasonable because cells are assumed to begin in stationary phase and thus it is necessary for them to go through the germination, outgrowth and lag phase before exponential growth could begin. By definition, once they leave the lag phase they are in the exponential phase of growth. Thus, for modelling the growth characteristics, there are two equations, given in eqn (5) with six unknown parameters, $a_l, b_l, a_g, b_g, T_{\min}$ and T_{\max} .

For each temperature, the means of the estimated EGRs and GOL times were calculated (Table 3) and used to determine the unknown parameters. The regression analyses were performed on the means, which could reasonably be assumed to be independent and closer to having a normal distribution than the individual replicate measurements. Using the mean values rather than the individual replicate values helps simplify calculations of standard errors and confidence intervals. At 11°C and 50°C no growth was observed for 3 weeks. For these temperatures, imputed values of GOL and EGR were made by assuming that the GOL time was 5 weeks or 840 h, and a small amount of relative growth equal to 20% at 3 weeks or 540 h. In order to decrease the 'influence' of the imputed value in the regression, the weight for these values were set equal to 0.5. The estimates of the six parameters were not very sensitive to the imputed values, so that if imputed values were slightly more or less, the estimates would not be greatly affected. Estimates of the six parameters were made (Table 4), simultaneously, using the seemingly unrelated regression (SUR) procedure of the PROC MODEL routine of the SAS/ETS module on PC-SAS®, edition 6.12. Convergence was not obtained by the SUR-SAS® procedure when the parameters were estimated directly using eqn (5) however, convergence was obtained when the mean EGR and GOL values and the model

predicted functions were transformed by the natural logarithm.

The observed and estimated or predicted EGRs and GOL times together with standard errors of the estimates and the correlations between them are presented in Table 3. The covariance matrix was computed by approximating the EGR and GOL time as functions of the parameters, using the linear (first partial derivatives) terms of a Taylor series (Rao 1973). Confidence intervals can be approximated by using the *t*-distribution with 11 degrees of freedom, which is the degrees of freedom assigned to the estimates by the SAS® program. Specifically, the program assigns degrees of freedom for equations using a simple formula, specifically, in our case, $df = N - p_1 - p_2/2$, where p_1 is the number of parameters that are not in common to both equations, p_2 are the number of parameters that are in common and N is the number of observations. For us, $N = 14$, $p_1 = 2$ and $p_2 = 2$, so that 11 degrees of freedom are associated with the estimate of the standard error of the residuals for each equation. Thus, 95% confidence intervals are obtained by adding and subtracting 2.201 times the standard error (the 97.5th percentile of the *t*-distribution with 11 degrees of freedom is 2.201). Figs 1 and 2 are plots of the experimentally determined natural log transformed mean values, together with predicted and 97.5% upper and lower confidence limits. While the figures appear to show a 'reasonable' agreement between the predicted and observed transformed mean values, there are large standard errors of prediction of EGR and GOL time. To help assure estimates of growth that would be protective of public safety, upper confidence limits of estimated relative growth should be used.

Discussion

The model developed in the previous section can be used to predict growth or relative growth for specified temperatures. For general cooling scenarios, the temperature is changing constantly. Discontinuous cooling followed by a rise in temperature (often due to equipment malfunction or electrical

Table 3. Mean of estimated GOL^a times (h) and exponential growth rate (EGR), ($\log_{10}(\text{cfu ml}^{-1})\text{h}^{-1}$), of *Clostridium botulinum* in RCM broth and corresponding predictions from regressions

Temperature	Observed mean		Predicted		Standard error of		Observed mean		Predicted		Standard error of		Correlation of	
	GOL	EGR	GOL	EGR	GOL prediction	EGR prediction	GOL	EGR	GOL	EGR	GOL prediction	EGR prediction	EGR and GOL	
11.0	672.0 ^b	0.01 ^b	196.31	0.02	74.60	0.01	196.31	0.02	74.60	0.01	0.02	0.01	-0.86	
12.0	69.29	0.06	133.48	0.04	39.24	0.01	133.48	0.04	39.24	0.01	0.04	0.01	-0.78	
15.0	38.91	0.08	57.30	0.08	10.34	0.02	57.30	0.08	10.34	0.02	0.08	0.02	-0.49	
19.0	19.82	0.31	26.89	0.18	4.16	0.03	26.89	0.18	4.16	0.03	0.18	0.03	-0.40	
21.0	17.64	0.18	20.07	0.24	3.16	0.05	20.07	0.24	3.16	0.05	0.24	0.05	-0.43	
25.0	19.19	0.67	12.41	0.38	2.09	0.07	12.41	0.38	2.09	0.07	0.38	0.07	-0.50	
30.0	7.37	0.60	7.73	0.60	1.40	0.11	7.73	0.60	1.40	0.11	0.60	0.11	-0.56	
32.0	4.62	0.59	6.58	0.69	1.21	0.13	6.58	0.69	1.21	0.13	0.69	0.13	-0.57	
37.0	6.12	0.50	4.65	0.92	0.84	0.15	4.65	0.92	0.84	0.15	0.92	0.15	-0.54	
40.0	6.45	1.36	3.93	1.01	0.66	0.17	3.93	1.01	0.66	0.17	1.01	0.17	-0.47	
44.0	5.08	1.06	3.45	0.99	0.58	0.21	3.45	0.99	0.58	0.21	0.99	0.21	-0.47	
46.0	3.52	0.50	3.60	0.85	0.80	0.22	3.60	0.85	0.80	0.22	0.85	0.22	-0.51	
48.0	3.17	0.65	4.84	0.55	1.66	0.18	4.84	0.55	1.66	0.18	0.55	0.18	-0.54	
50.0	672.0 ^b	0.01 ^b	536.43	0.00	330.89	0.00	536.43	0.00	330.89	0.00	0.00	0.00	-0.74	

^aGermination, outgrowth and lag.

^bImputed values.

Table 4. Estimates, standard errors and 95% confidence intervals of parameters used for estimating growth characteristics

Parameter ^a	Standard		Lower limit ^b	Upper limit ^b
	Estimate	Error		
a_1	0.015	0.002	0.011	0.019
b_1^c	0.361	0.231	0.088	1.478
a_g	0.033	0.005	0.022	0.044
b_g^c	0.167	0.096	0.047	0.592
T_{min}^c	6.299	1.301	3.997	9.926
T_{max}	50.012	0.009	49.993	50.031

^a $1/GOL^{1/2} = a_1(t - T_{min})(1 - \exp(b_1(t - T_{max})))^{1/2}$, where GOL is the germination, outgrowth and lag time (h), $EGR^{1/2} = a_g(t - T_{min})(1 - \exp(b_g(t - T_{max})))^{1/2}$, where EGR is exponential growth rate ($\log_{10}(\text{cfu ml}^{-1})\text{h}^{-1}$).

^bConfidence limits computed with 11 degrees of freedom.

^cBased on estimate of natural log transformation, to assure positive confidence limits.

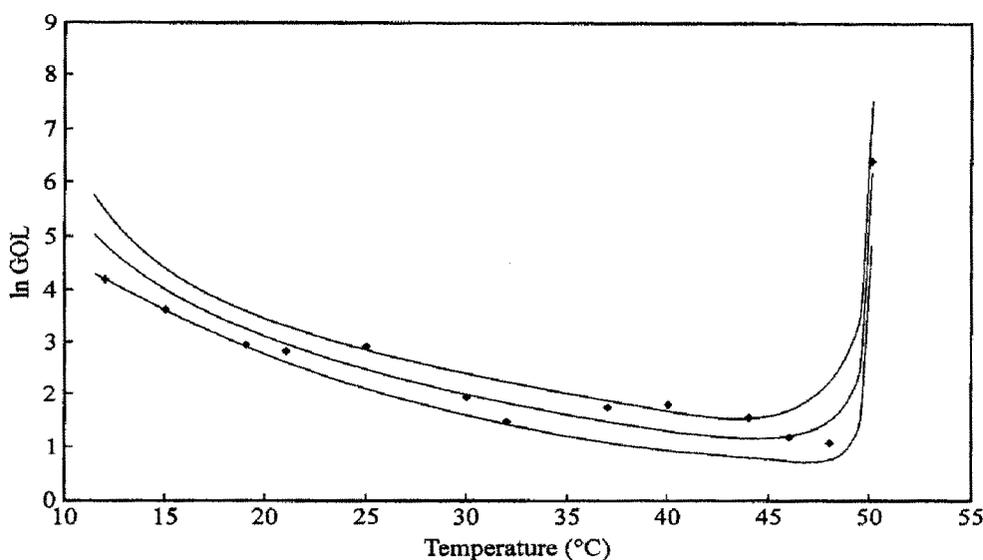


Figure 1. The natural logarithm of the GOL time (h) of *C. botulinum* in RCM broth, predictions and 95% confidence limits vs temperature (°C).

outage) and subsequent continuation of cooling may occur in the food industry and retail food service establishments. The regulatory agencies and the personnel involved in the food preparation need to determine if the product remains safe after such cooling deviations. Thus a closed form expression for predicting relative growth for cooling scenarios would be useful. A closed form expression of the predicted relative growth expressed as a function of the parameters estimated from the Ratkowsky equations would enable the direct calculation of the standard errors of the predictions.

In the usual scenario, temperatures of the warmest section of the product, where organisms might be, would be monitored. Between times for which temperatures are known, assuming that the ambient air temperature remains the same, it is assumed that temperatures change exponentially (Juneja et al. 1994). Thus, it is assumed that the temperature, T , changes with time by some known continuous function of time, $q(t)$. However, because Eqns (1) and (5) apply for constant temperature one cannot just apply the calculus for calculating relative growth without some assumptions or actual knowledge regarding the impact of

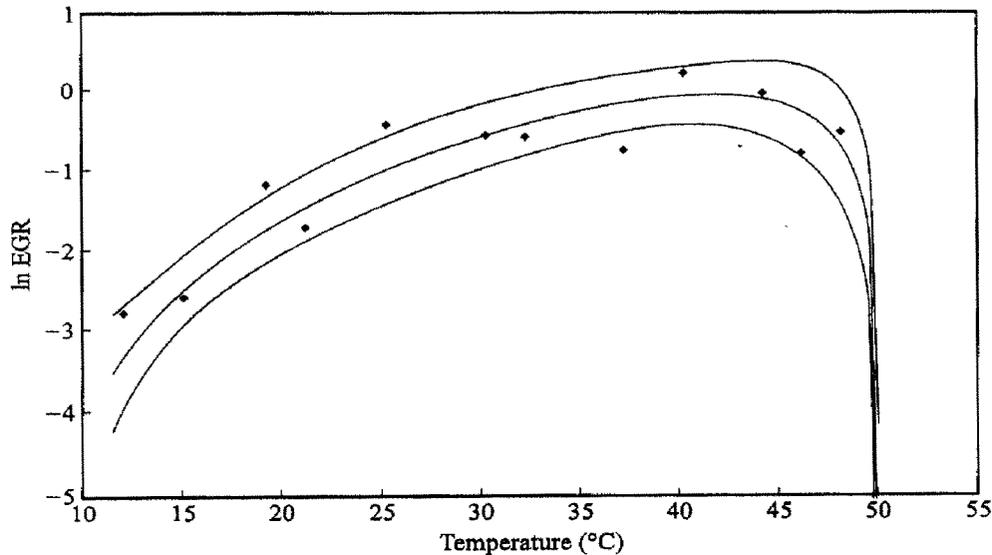


Figure 2. The natural logarithm of the exponential growth rate, EGR ($\log_{10} (\text{cfu ml}^{-1})\text{h}^{-1}$) of *C. botulinum* in RCM broth, predictions and 95% confidence limits vs temperature ($^{\circ}\text{C}$).

prior environmental history on the growth parameters. It is generally assumed that the exponential growth rate is not dependent upon previous environments, but that the GOL time does depend upon previous environments (Baranyi and Roberts 1994). Thus, as the temperature, T , changes, the growth curve changes.

The problem is where (for what value of time) do we begin to measure the change on different growth curves. A complete discussion of this issue is given in our previous paper (Juneja et al. 1999). In that paper, an expression for the expected value of the common logarithm of the relative growth is given. Assume that $L(t|T)$ can be written as a function $H(k \cdot (t - z(T)), \eta)$ where k , z and η are functions of temperature. Let τ be the time such that $q(\tau) = T$. Define $f(\tau)$ to be a function defining the translation from the pivot point $z(q(\tau))$ along the time axis reflecting the accumulated ratios of length of times to pivot points 'spent' on previous growth curves up to time τ , that is, $f(\tau) = z(q(\tau))(1 - \int_0^{\tau} z^{-1}(q(s))ds)$. Note, this function will be negative once the time exceeds 100% of the GOL times. Then the expected value of $\log_{10}(N(t)/N_0) = E(L(t))$, can be expressed as:

$$E(L(t)) = \int_0^t H'(k(q(\tau)) \cdot f(z(\tau)), \eta(q(\tau)))d\tau \quad (6)$$

where H' is the derivative of H with respect to time, holding temperature constant (Juneja et al. 1999). The variance of $E(L(t))$, reflecting the uncertainty of the estimated parameters derived from the Ratkowsky equations, can be determined by computing partial derivatives of the expression in eqn (6) and using the Taylor series linear approximation, as described above.

Two suggested pivot points are: M , the time for which the rate of relative growth is equal to the maximal rate of relative growth; or the GOL time. Our calculations indicate that the choice of a pivot point is not critical. For continuous or slow temperature change, it would be expected that the GOL times changes proportionally, as described above by the function f . For our calculations, eqn (6) is used, assuming that parameter, A , in eqn (1) is equal to 3 (reflecting experimental conditions), with the function, f , defined above, that uses the GOL time as the pivot point. In order to reflect the uncertainty of the estimates, a 97.5% upper confidence limit is calculated by adding to the predicted value 2.201 times the estimated standard error of prediction obtained from the Taylor series approximation. The calculations were made using Mathcad[®] version 7.0. For example, for cooling from 50 $^{\circ}\text{C}$ to 10 $^{\circ}\text{C}$ in 8 h, assuming an ambient temperature of 0 $^{\circ}\text{C}$, the predicted relative growth is 1.33 (0.124 \log_{10})

and the upper 97.5% confidence limit of the relative growth is 2.41 ($0.382 \log_{10}$).

Conclusion

In summary, this paper presents Ratkowsky-type equations (models) for predicting the effect of temperature on GOL and EGR of *C. botulinum* during cooling of certain cooked meat products. From these equations and assumptions, the expected growth that would occur with the changing temperatures during the cooling of meat products can be calculated. Research is being planned to validate these assumptions and equations presented in this paper.

References

- Baranyi, J. and Roberts, T. A. (1994) A dynamic approach to predicting bacterial growth in food. *Int. J. of Food Microbiol.* **23**, 277–294.
- Bean, N. H. and Griffin, P. M. (1990) Foodborne disease outbreaks in the United States, 1973–1987: pathogens, vehicles, and trends. *J. Food Prot.* **53**, 804–817.
- Bryan, F. L. (1978) Factors that contribute to outbreaks of foodborne disease. *J. Food Prot.* **41**:816.
- Buchanan, R. L. (1990) Using spreadsheet software for predictive microbiology applications, *J. Food Safety* **11**, 123–133.
- FDA Division of Retail Food Protection (1997) *Food Code. US Department of Health and Human Services, Public Health Service. Food and Drug Administration, Pub. No. PB97-141204.* Washington, DC.
- Gibson, A. M., Bratchell, N. and Roberts, T. A. (1988) Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride, and storage temperature. *Intl. J. Food Microbiol.* **6**, 155–178.
- Huhtanen, C. N. (1975). Some observations on a perigo-type inhibition of *Clostridium botulinum* in a simplified medium. *J. Milk Food Technol.* **38**, 762–763.
- Juneja, V. K., Snyder, O. P. and Cygnarowicz-Provost, M. (1994) Influence of cooling rate on outgrowth of *Clostridium perfringens* spores in cooked ground beef. *J. Food Prot.* **57**, 1063–1067.
- Juneja, V. K., Whiting, R. C., Marks, H. M. and Snyder, O. P. (1999) Predictive model for growth of *Clostridium perfringens* at temperatures applicable to cooling of cooked meats. *Food Microbiol.* **16**, 335–350.
- McMeekin, T. A., Olley, J. N., Ross, T. and Ratkowsky, D. A. (1993) *Predictive Microbiology: Theory and Application.* New York, J. Wiley & Sons, Inc.
- Rao, C. R. (1973) *Linear Statistical Inference and its Applications.* New York, J. Wiley & Sons, Inc.
- Ratkowsky, D. A., Lowry, R. K., McMeekin, T. A., Stokes, A. N., Chandler, R. E. (1983) Model for bacterial culture growth rate throughout the entire biokinetic temperature range. *J. Bacteriol.* **154**, 1222–1226.
- US Department of Agriculture, Food Safety and Inspection Service (1989). *Time/temperature Guidelines for Cooling Heated Products.* FSIS Directive 7110.3 Rev. 1.