

Interactive Effects of Temperature, Initial pH, Sodium Chloride, and Sodium Pyrophosphate on the Growth Kinetics of *Clostridium perfringens*[†]

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ABSTRACT

The interactive effects of temperature (12 to 42°C), initial pH (5.5 to 7), sodium chloride (0 to 3%) and sodium pyrophosphate concentrations (0 to 0.3%) on the growth in Trypticase-peptone-glucose-yeast extract broth of a three-strain mixture of *Clostridium perfringens* vegetative cells were determined. The number of viable *C. perfringens* cells was determined at appropriate intervals by plating on tryptose-sulfite-cycloserine agar. Growth data were analyzed by the Gompertz equation; the Gompertz B and M parameters were then used to calculate lag-phase duration, exponential growth rate, generation time, and maximum population-density values. The data indicated that the growth kinetics of *C. perfringens* were dependent on the interaction of the four variables, particularly in regard to exponential growth rates and lag-phase durations. Cubic models based on the natural logarithm transformation of lag-phase duration and generation time were evaluated and appeared to adequately fit the data. The data suggest that sodium pyrophosphate can have significant bacteriostatic activity against *C. perfringens* and may provide processed meats with a degree of protection against this microorganism, particularly if employed in conjunction with a combination of acidic pH, high salt concentrations, and adequate refrigeration.

Key words: *Clostridium perfringens*, predictive equations, modeling, growth kinetics, growth variables

Clostridium perfringens remains a major cause of food-borne illness worldwide and is a continuing concern to the food-service industry (3, 6, 7, 18). It has been implicated in 11.8% of the total bacterial food-borne disease outbreaks in the United States (1); *C. perfringens* is also a leading cause of food poisoning in Canada (19).

Because of the ubiquitous distribution of *C. perfringens*, it is difficult if not impossible to exclude spores of this

bacterium during the processing of various animal or plant products and its presence must be assumed. If the food is to be safe, either the thermal treatment should be adequate to destroy the spores, or their germination and subsequent growth must be prevented by manipulation and control of one or more of several factors such as storage temperature, pH, and preservatives. Thermal processing, if designed to inactivate *C. perfringens* spores, may affect the product quality. The heat treatment given to minimally processed foods could serve as an activation step if it were not lethal to the spores. During cooling, activated spores are likely to germinate, outgrow, and multiply, if the rate and extent of cooling is insufficient. Inadequate cooling practices have been cited as a major cause of food poisoning with *C. perfringens* (1).

With the trend towards minimally processed foods, there has been heightened interest in applying a multiple-barrier approach to controlling the growth of food-borne microorganisms. In a study by Gibson and Roberts (10), when the *C. perfringens* growth-no growth interface was assessed for a combination of factors such as storage temperature, initial pH, sodium chloride content, and sodium nitrite concentration, a combination of 1% salt and 50 µg/ml of nitrite inhibited growth at 15°C. While the study by Gibson and Roberts (10) provided some characterization of the response of *C. perfringens* to the presence of nitrite, concern over the possible health hazards of nitrite and its reaction products had led to an effort to eliminate or reduce nitrite levels in processed meats (13). Additionally, there is a lack of quantitative data on the growth kinetics of *C. perfringens* that could be used to predict the relative role of various factors that influence the vegetative growth of this organism. Accordingly, the objective of the current study was to assess the effects and interactions of sodium chloride (salt), sodium pyrophosphate (SPP), temperature, and pH on the growth kinetics of *C. perfringens*. The data were then used to develop quadratic and cubic polynomial models that could be used to predict the behavior of the bacterium.

MATERIALS AND METHODS

Bacteria

Three strains of *Clostridium perfringens*, NCTC 8238 (Hobbs serotype 2), NCTC 8239 (Hobbs serotype 3), and NCTC 10240 (Hobbs serotype 13), from our in-house culture collection were used in this study. Stock cultures were maintained at 4°C in cooked-meat medium (Difco Laboratories, Detroit, MI).

Experimental design

A fractional factorial design was employed to assess the effects of incubation temperature (12, 19, 28, 37, and 42°C), initial pH (5.5, 6.0, 6.25, 6.5, and 7.0), sodium chloride (Mallinckrodt, Paris, KY) content (0, 1.0, 1.5, 2.0, and 3% [wt/vol]), and sodium pyrophosphate (Fisher Scientific Company, Fair Lawn, NJ) content (0, 0.1, 0.15, 0.2 and 0.3% [wt/vol]). These variables were chosen as representing culture conditions at which, when tested individually, *C. perfringens* would grow. Subsequent iterations of model development and data acquisition employed a central composite design, focusing on those variable combinations needed to generate a cubic model and to enhance model effectiveness. Each variable combination was replicated twice and is given in Table 1.

Culturing techniques

Starter cultures were prepared by transferring 0.1 ml of the stock cultures individually to 9.9 ml of freshly prepared fluid thioglycollate medium (FTM) (Difco). The inoculated medium was then heat shocked at 75°C for 20 min and incubated at 37°C for 6 h. Approximately 1 ml of the 6-h culture was transferred to 9 ml of FTM and incubated for 18 h. One milliliter of each of the three strains was mixed to prepare a cocktail and then 10-fold serial dilutions were made in 0.1% (wt/vol) peptone (Difco) water to a final concentration of 10⁴ CFU/ml. Trypticase-peptone-glucose-yeast extract (TPGY) containing (all wt/vol) 5% Trypticase, 0.5% peptone, 2% yeast extract, 0.1% cysteine hydrochloride (Sigma Chemical Company, Saint Louis, MO), 0.4% dextrose was used as the test-culture basal medium for growth kinetics studies. Except as noted, ingredients were from Difco; Trypticase was from BBL (Cockeysville, MD). The medium was supplemented with the required calculated concentration of 0 to 3% (wt/vol) sodium chloride and 0 to 0.3% sodium pyrophosphate, dispensed in 50-ml portions into 250-ml trypsinizing flasks equipped with a rubber septum inserted in the side-arm sampling port, and sterilized by autoclaving.

Dextrose and cysteine hydrochloride were dissolved in deionized water and filter sterilized (0.22- μ m-pore-size syringe filter, Nalge Company, Rochester, NY). Prior to inoculation, dextrose and cysteine hydrochloride were added aseptically to the basal medium to achieve the required levels and then adjusted to the desired pH (5.5 to 7.0) with HCl or NaOH after autoclaving. Each flask received 0.5 ml of the diluted inoculum to yield a starting level of approximately 3 log CFU/ml. The flasks were then flushed with sterile N₂ for 10 min and sealed with a rubber stopper. All flasks were incubated on a rotary shaker (150 rpm) at 12 to 42°C. At intervals appropriate for the culture conditions, samples were withdrawn through the side-arm septum with a syringe fitted with a hypodermic needle. Serial dilutions made in 0.1% (wt/vol) peptone water were surface plated with a Spiral plater (Model D, Spiral Biotech, Bethesda, MD) onto tryptose-sulfite-cycloserine agar without cycloserine, i.e., SFP agar and egg yolk enrichment. The lower limit of detection by this procedure is 21 CFU/ml. After overlaying with an additional 10 ml of SFP agar, the plates were allowed to solidify before being placed into anaerobic jars. The

total *C. perfringens* population was determined after 48 h of incubation at 37°C in a Gas Pak system (BBL).

Curve fitting and model development

Plate counts were transformed to log values and growth curves were generated from each variable condition by fitting the Gompertz function (8) to the plate count data using ABACUS, a nonlinear regression program that employs a Gauss-Newton integration procedure (5); this Fortran-based program was developed at the Eastern Regional Research Center and is available upon request. The four Gompertz parameters (A, C, B, and M) were subsequently used to calculate kinetic parameters: exponential growth rates (EGR, log (CFU/ml)/h), generation time (GT, h), lag-phase duration (LPD, h), and maximum population densities (MPD, log (CFU/ml)) as described by Gibson et al. (8, 9). Cultures displaying less than a log-cycle increase over the initial inoculum level were designated nongrowing and were not used for curve fitting. In some cultures, population densities declined after attaining a peak. In such instances, the maximum population values were considered during curve fitting and not the data after initiation of the decline. Quadratic and cubic polynomial models on temperature, pH, salt, and phosphate of natural log and square root transformations of the Gompertz B and M values, as well as the maximum population densities (MPD), were generated using the SAS General Linear Model (GLM) procedure (16). In addition, the data were analyzed by an analysis of variance (ANOVA) using an SAS program (16); F-values and significance for individual terms and interactions of the respective equations were calculated.

RESULTS AND DISCUSSION

Data from 90 curves, representing 45 variable combinations, were used to derive the models to predict the growth of *C. perfringens* as a function of temperature, sodium chloride, initial pH and SPP concentrations. Growth curves were generated from the bacterial population data (log transformation of the viable counts) using the Gompertz equation (8), and the exponential growth rates, generation times, lag times, and maximum population densities were calculated. Variable combinations, Gompertz equation parameters and derived growth kinetics values are summarized in Table 1. The *C. perfringens* strain cocktail used in this study did not grow at 12°C under harsh conditions (1.5% salt, 0.15% PO₄ and pH 6.25); at 19°C variable combinations resulting in "no growth" determinations were observed by a continued drop in the microbial population with time to ultimately nondetectable levels (<21 CFU/ml). Following nondetectable counts, sampling was continued for another week to ascertain the organism's nonviability. Analysis of the data from our growth experiments indicated that the four variables studied (temperature, pH, salt, SPP) individually and in combination influenced the growth of *C. perfringens* (Table 2). If the organism grew, it typically achieved an MPD between 10⁶ and 10⁸ CFU/ml. Some suppression of MPDs was observed with nonoptimal conditions, particularly at the lower temperatures and in the presence of various additives. As expected, conditions favoring growth (pH 6.5, temperature >19°C) reduced lag and generation times. The most rapid growth rate in this study occurred at 42°C at pH 6.25, with generation and lag times of 0.20 h and 2.27 h, respectively. Growth was almost as rapid at 37°C regardless

TABLE 1. Culture variables, Gompertz parameters, and calculated kinetic parameters from the growth study of *C. perfringens*; Gompertz and calculated kinetic parameters are the average of duplicate determinations

Obs	Culture variables				Gompertz parameters ^a				Calculated kinetic parameters ^b			
	Temp (°C)	pH	NaCl (% wt/vol)	SPP (% wt/vol)	A	C	B	M	EGR	GT	LPD	MPD
1	12	6.25	1.5	0.15	ng ^c							
2	19	6.00	1.0	0.20	3.80	3.09	1.35	186.72	1.54	4.70	188.98	6.89
3	19	6.00	1.0	0.10	3.52	2.85	0.29	131.52	0.61	23.82	129.80	6.37
4	19	6.00	2.0	0.10	3.52	2.55	0.20	261.60	0.19	39.37	256.60	6.07
5	19	6.00	2.0	0.20	ng							
6	19	6.50	1.0	0.10	3.61	3.14	0.49	286.32	0.59	12.92	284.32	6.76
7	19	6.50	1.0	0.20	3.54	3.22	0.66	98.40	0.78	9.24	96.88	6.81
8	19	6.50	2.0	0.20	ng							
9	19	6.50	2.0	0.10	3.41	3.03	0.33	77.04	0.37	19.93	74.01	6.44
10	19	6.00	1.5	0.10	3.64	2.70	0.24	156.96	0.25	43.37	152.96	6.34
11	19	6.00	2.0	0.15	3.38	2.93	0.27	129.36	0.29	30.93	125.66	6.31
12	19	6.25	1.0	0.15	3.62	2.76	0.33	131.28	0.34	21.73	128.25	6.38
13	19	6.25	2.0	0.20	ng							
14	19	6.25	1.5	0.10	3.53	2.91	0.25	136.08	0.27	19.83	132.08	6.44
15	19	6.50	1.5	0.15	3.61	2.93	1.06	177.12	1.14	6.32	176.18	6.54
16	28	5.50	1.5	0.15	2.42	5.18	0.08	25.38	0.44	1.22	12.19	8.10
17	28	7.00	0	0.15	3.37	4.95	0.60	6.58	1.10	0.28	4.92	8.32
18	28	6.25	3.0	0.15	2.77	5.60	0.08	20.68	0.17	1.74	8.80	8.37
19	28	6.25	1.5	0.15	2.41	5.91	0.11	10.10	0.24	1.30	0.71	8.32
20	28	6.25	1.5	0	2.35	6.06	0.19	5.15	0.43	0.71	0.10	8.37
21	28	6.25	1.5	0.30	2.37	5.91	0.09	11.01	0.20	1.52	0.43	8.27
22	28	6.00	1.0	0.15	2.24	6.37	0.35	9.32	0.80	0.38	6.34	8.61
23	28	6.00	1.5	0.10	2.10	6.38	0.25	9.16	0.58	0.52	5.08	8.47
24	28	6.00	2.0	0.20	2.38	6.05	0.10	12.71	0.21	1.43	2.15	8.42
25	28	6.00	0	0.15	1.86	6.61	0.12	8.63	0.28	1.06	0.10	8.47
26	28	6.25	1.0	0.20	2.26	6.35	0.13	10.59	0.29	1.04	2.57	8.61
27	28	6.25	2.0	0.10	2.14	3.41	0.24	9.11	0.56	0.54	4.88	8.55
28	28	6.50	1.0	0.10	2.50	5.90	0.28	6.77	0.61	0.63	3.10	8.42
29	28	6.50	1.5	0.20	2.81	5.86	0.15	9.41	0.32	0.95	2.63	8.67
30	28	6.50	2.0	0.15	2.54	6.09	0.14	9.01	0.33	0.94	2.03	8.64
31	37	6.00	1.0	0.10	2.70	5.50	0.47	4.45	0.45	0.32	2.34	8.20
32	37	6.00	1.0	0.20	2.74	5.44	0.81	5.60	1.61	0.19	4.36	8.18
33	37	6.00	2.0	0.20	3.03	5.23	0.58	6.13	1.10	0.76	4.65	8.25
34	37	6.00	2.0	0.10	2.79	5.51	0.54	5.70	1.10	0.28	3.85	8.29
35	37	6.50	1.0	0.20	3.23	5.24	0.65	4.41	1.25	0.24	2.86	8.47
36	37	6.50	1.0	0.10	3.03	5.36	0.61	2.87	1.20	0.25	1.23	8.39
37	37	6.50	2.0	0.10	3.18	5.13	0.31	7.35	0.58	0.25	4.11	8.31
38	37	6.50	2.0	0.20	2.91	5.60	0.12	10.38	0.24	1.24	1.88	8.51
39	37	6.00	1.5	0.15	2.83	5.88	0.58	4.73	1.27	0.24	3.04	8.71
40	37	6.25	1.0	0.10	2.70	6.07	0.66	4.48	1.47	0.23	2.96	8.77
41	37	6.25	1.5	0.20	2.53	5.21	0.59	4.69	1.12	0.27	2.98	7.74
42	37	6.25	2.0	0.15	2.65	6.07	0.53	4.67	1.19	0.25	2.78	8.71
43	37	6.50	1.0	0.15	2.78	5.71	0.58	5.04	1.23	0.25	3.32	8.49
44	37	6.50	1.5	0.10	2.80	5.70	0.61	4.11	1.28	0.24	2.78	8.49
45	42	6.25	1.5	0.15	3.57	4.86	0.82	3.49	1.47	0.20	2.27	8.29

^a A, starting count, log CFU/ml; C, growth during the experiment, log CFU/ml; M is the time (h) at which the absolute growth rate is maximum; B, log (CFU/ml)/h, relative growth rate at M.

^b EGR (exponential growth rate), log (CFU/ml)/h; GT (generation time), h; LPD (lag-phase duration), h; MPD (maximum population density), log CFU/ml.

^c ng, no growth.

of the initial pH and the presence of additives. Labbe and Huang (12) dealing with strain NCTC 8798 reported a generation time of 0.14 h at 43°C in fluid thioglycollate medium supplemented with beef. These data support the mesophilic nature of this bacterium and indicate that normal

refrigeration should be more than adequate to restrict the growth of *C. perfringens* vegetative cells in foods.

A total of 10 quadratic and 6 cubic models were developed for the natural log and square root transformations of the Gompertz parameters, the natural log of the

TABLE 2. *F*-values for independent variables and their cross products for the cubic models based on ln transformation of the Gompertz B and M parameters

Culture variable ^a	Gompertz parameter	
	ln (LPD)	ln (GT)
T	314.50 ^b	2371.13 ^b
pH	13.96 ^c	13.66 ^c
S	5.01	76.56 ^b
P	0.27	0.20
T * pH	2.67	28.69 ^b
T * S	14.40 ^c	0.85
T * P	0.09	51.58 ^b
pH * S	9.87 ^c	13.90 ^c
pH * P	0.48	4.27
S * P	2.55	11.95 ^c
T * T	173.75 ^b	214.41 ^b
pH * pH	11.55 ^c	1.60
S * S	6.35	6.70
P * P	25.99 ^b	3.43
T * pH * S	5.76	9.50 ^c
T * pH * P	1.39	1.85
T * S * P	4.18	0.95
pH * S * P	12.13 ^c	4.40
T * T * pH	0.01	0.03
T * T * S	47.34 ^b	12.54 ^c
T * T * P	0.01	22.68 ^b
pH * pH * S	1.90	29.47 ^b
pH * pH * P	1.16	0.39
T * pH * pH	1.81	18.91 ^b
S * S * P	1.39	0.26
pH * S * S	19.67 ^b	8.61 ^c
T * S * S	15.93 ^c	1.38
T * P * P	2.99	1.23
pH * P * P	0.59	2.48
S * P * P	1.47	0.14
T * T * T	1.41	0.00
pH * pH * pH	2.26	1.44
S * S * S	13.06 ^c	1.28
P * P * P	6.59	3.11

^a T, temperature; pH, pH; S, sodium chloride; P, sodium pyrophosphate.

^b $P \leq 0.0001$.

^c $0.01 > P > 0.0001$.

kinetic parameters (GT and LPD) and the Gompertz parameters themselves (Table 3). For each model developed, the goodness of fit or R^2_{adj} was obtained from the SAS/GLM analysis of the growth data. Based on their R^2_{adj} , only models VIII and XI to XVI were selected for further evaluation.

Using the graphing technique described by Buchanan et al. (2), log observed parameters (GT or LPD) were plotted against log predicted parameters along with the $\pm 50\%$ of the parameter values for the seven models indicated above. It was determined that a cubic natural log transformation of GT and LPD yielded the best fit (Figs. 1a and 1b); similar plots for the other models are not shown. The best fit is based on points falling on the line of identity and within the $\pm 50\%$ of the parameter values. Cubic models for the effects and interactions of temperature, pH 5.5 to 6.5, 0 to 3% salt, and 0 to 0.3% SPP, levels on the growth of *C. perfringens*

TABLE 3. Goodness-of-fit parameters for the quadratic and cubic models generated for the anaerobic growth of *C. perfringens*

Model number	Model type	Model designation	Number of observations	R^2_{adj}
I	Quadratic	B	82	0.64
II	Quadratic	M	82	0.82
III	Quadratic	LPD	80	0.65
IV	Quadratic	GT	82	0.88
V	Quadratic	LN(B)	82	0.74
VI	Quadratic	LN(M)	82	0.81
VII	Quadratic	LN(LPD)	80	0.77
VIII	Quadratic	LN(GT)	82	0.95
IX	Quadratic	SQRT(B)	82	0.69
X	Quadratic	SQRT(1/M)	82	0.78
XI	Cubic	LN(B)	82	0.97
XII	Cubic	LN(M)	82	0.96
XIII	Cubic	LN(LPD)	80	0.96
XIV	Cubic	LN(GT)	82	0.99
XV	Cubic	SQRT(B)	82	0.96
XVI	Cubic	SQRT(1/M)	82	0.96

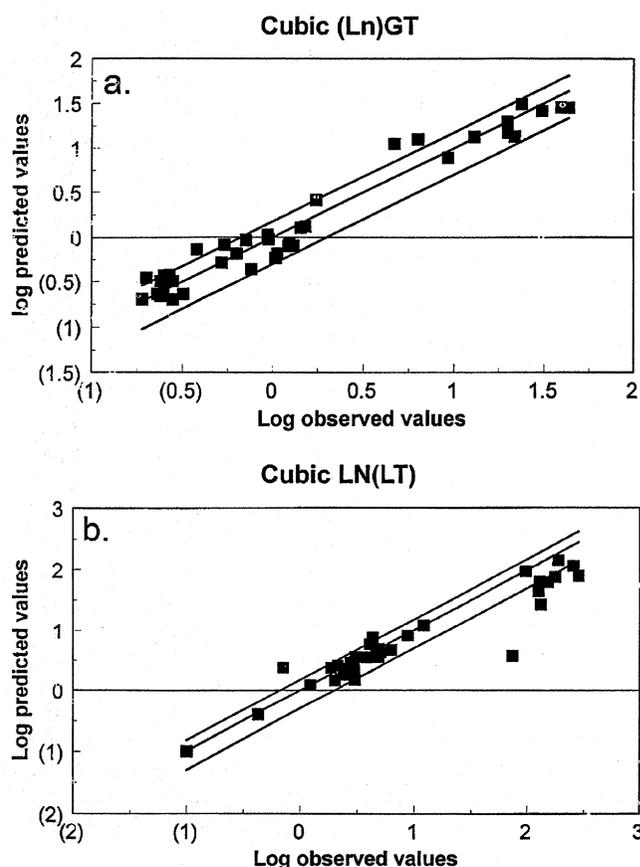


FIGURE 1. Estimated generation and lag times based on experimental data compared with predictions by the ln transformation response surface models for the growth of *C. perfringens* (cubic, ln (GT) and ln (LPD): Models XIII and XIV). The center line is the line of identity; top and bottom lines are $\pm 50\%$ of observed parameters. a., Observed versus predicted generation times; b., observed versus predicted lag times.

nitrite (0 to 400 µg/ml), and at 3 pH values (5.6, 6.2, 6.8) on the growth of *C. perfringens* at various temperatures. In their study, the four variables interacted to affect growth; increasing salt levels decreased the growth rate of the organism, with the effect being greater if the other variables were also nonoptimal.

We studied additional factors that influenced the growth of the microorganism. One of the underlying factors for electing to develop a model based on data generated using sodium pyrophosphate in addition to salt was the health concerns on the use of nitrites. To our knowledge, the efficacy of sodium pyrophosphate in preventing the growth of *C. perfringens* has not been previously assessed. The model should allow food processors involved in new ready-to-eat low-salt food-product development to assess quickly the impact of altering any combination of these variables.

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using ln (GT) and ln (LPD) transformations in conjunction with variable combinations producing no-growth responses being treated as missing values are given in Table 4.

Though the cubic ln (LPD) and ln (GT) models appeared to offer the best fit of the data based on the graphing technique (Fig. 1) and R^2_{adj} (Table 3), the ability of these models to predict the response of the bacterium to food conditions was also evaluated. The food and parameters chosen were roast beef containing 0.5% salt and 0.1% PO₄ (pH 6.0), with storage at temperatures ranging from 12 to 42°C. The predicted GT and LPD along with the 95% confidence limits generated using the SAS GLM procedure are shown in Figure 2. As can be seen, at temperatures above 15°C, the intervals are small and from ca. 20 to 42°C, the ±95% confidence limits are close or identical to those predicted. The wide limits at 12°C may reflect the limited data generated in this region of the model and/or the very slow growth of the bacterium at this temperature.

Craven (4) reviewed growth and sporulation of *C. perfringens* in foods. While the temperature range for growth of *C. perfringens* is reported to be 6 to 52.3°C, (1, 11, 17), relatively few studies have been conducted investigating the growth potential at low temperatures. Inadequate

TABLE 4. Cubic response surface models of the Gompertz B and M values for the growth of *C. perfringens* as a function of incubation temperature (T, °C), initial pH (pH, 5.5 to 7.0), sodium chloride (S, 0 to 3%) and sodium pyrophosphate content (P, 0 to 0.3%)

$$\begin{aligned} \ln(\text{LPD}) = & -1238.672491 - 32.881513 * T + 648.595735 * \\ & \text{pH} - 47.243080 * S + 6727.179420 * P + 10.373588 * \\ & T * \text{pH} - 2.702801 * T * S - 7.563676 * T * P + \\ & 17.849349 * \text{pH} * S - 2385.490259 * \text{pH} * P - 80.008300 * \\ & S * P + 0.073987 * T * T - 109.295418 * \text{pH} * \text{pH} + \\ & 28.703415 * S * S + 6122.446362 * P * P + \\ & 0.416916 * T * \text{pH} * S + 0.171252 * T * \text{pH} * P + \\ & 0.389069 * T * S * P + 9.862304 * \text{pH} * S * P + \\ & 0.000526 * T * T * \text{pH} + 0.001944 * T * T * S + \\ & 0.028592 * T * T * P - 1.404731 * \text{pH} * \text{pH} * S + \\ & 261.362357 * \text{pH} * \text{pH} * P - 0.874443 * T * \text{pH} * \text{pH} - \\ & 11.696847 * S * S * P - 5.483789 * \text{pH} * S * S - \\ & 0.024351 * T * S * S + 15.850520 * T * P * P - \\ & 1149.877675 * \text{pH} * P * P + 85.874407 * S * P * P - \\ & 0.000831 * T * T * T + 6.003902 * \text{pH} * H * \text{pH} + \\ & 1.374338 * S * S * S + 867.514736 * P * P * P. \end{aligned}$$

$$\begin{aligned} \ln(\text{GT}) = & 17.724558 + 15.537606 * T - 111.695685 * \text{pH} + \\ & 390.605063 * S - 2703.617295 * P - 6.430266 * T * \text{pH} - \\ & 1.394666 * T * S + 5.283237 * T * P - 107.378064 * \text{pH} * \\ & S + 887.404347 * \text{pH} * P + 83.189230 * S * P + \\ & 0.150023 * T * T + 36.172833 * \text{pH} * \text{pH} - \\ & 27.310755 * S * S - 1520.898649 * P * P + \\ & 0.182614 * T * \text{pH} * S - 0.088506 * T * \text{pH} * P + \\ & 0.680010 * T * S * P - 22.064429 * \text{pH} * S * P - \\ & 0.018157 * T * T * \text{pH} - 0.001664 * T * T * S - \\ & 0.107028 * T * T * P + 7.466274 * \text{pH} * \text{pH} * S - \\ & 73.223747 * \text{pH} * \text{pH} * P + 0.583340 * T * \text{pH} * \text{pH} + \\ & 3.472668 * S * S * P + 3.887390 * \text{pH} * S * S + \\ & 0.088253 * T * S * S + 3.112956 * T * P * P + \\ & 228.626428 * \text{pH} * P * P + 101.810476 * S * P * P - \\ & 0.000065 * T * T * T - 2.912550 * \text{pH} * \text{pH} * \text{pH} + \\ & 0.060810 * S * S * S - 299.989795 * P * P * P. \end{aligned}$$

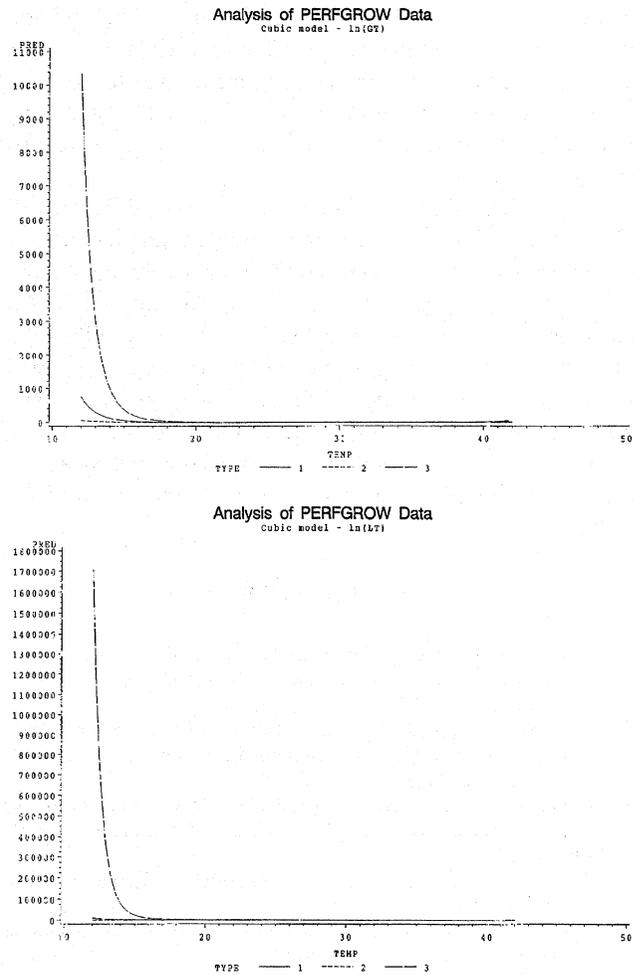


FIGURE 2. Predicted LPD and GT for *C. perfringens* in roast beef (0.5% salt, 0.1% PO₄, pH 6.0) stored at varying temperatures: solid line, predicted; short dashed line lower 95% confidence interval; long dashed line upper 95% confidence interval. a., Generation time; b., lag-phase duration.

cooling and holding foods at warm temperatures have traditionally been associated with *C. perfringens* food poisoning. In a study by Rey et al. (14), when *C. perfringens* growth was studied at low temperatures, 2 of 6 strains grew at 15°C and all failed to grow at 5°C. Our study investigated the combined effects of salt, SPP, and pH on *C. perfringens* growth at low temperatures such as 12 and 19°C in addition to higher temperatures. The data reported in this study cannot be compared with published growth data because published studies have applied different measures of growth, such as time to turbidity or the growth-no growth interface (10, 15). In a study by Rhodehamel and Pierson (15), when the efficacy of sodium hypophosphite (SHP) in inhibiting *C. perfringens* growth at 37°C was assessed in prerduced peptone-yeast extract-glucose broth, the greatest inhibition occurred with 3,000 µg of SHP per ml at pH 6.5, where time-to-growth was delayed 10.5 h when compared to the control (no SHP, pH 6.5, and no added salt). The addition of salt and the decreasing pH of the test medium produced an additive effect on growth inhibition by SHP.

Gibson and Roberts (10) investigated the effects of increasing salt concentrations in combination with sodium