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Predictive model for growth of *Clostridium perfringens* in cooked cured pork $\stackrel{\text{$\stackrel{\leftrightarrow}{\sim}$}}{\sim}$

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Abstract

Mathematical models have been developed and used for predicting growth of foodborne pathogens in various food matrices. However, these early models either used microbiological media or other model systems to develop the predictive models. Some of these models have been shown to be inaccurate for applications in meat and specific food matrices, especially under dynamic conditions, such as constantly changing temperatures that are encountered during food processing. The objective of this investigation was to develop a model for predicting growth of *Clostridium perfringens* from spore inocula in cured pork ham. Isothermal growth of *C. perfringens* at various temperatures from 10 to 48.9 °C were evaluated using a methodology that employed a numerical technique to solve a set of differential equations. The estimated theoretical minimum and maximum growth temperatures of *C. perfringens* in cooked cured pork were 13.5 and 50.6 °C, respectively. The kinetic and growth parameters obtained from this study can be used in evaluating growth of *C. perfringens* from spore populations during dynamically changing temperature conditions such as those encountered in meat processing. Further, this model can be successfully used to design microbiologically "safe" cooling regimes for cured pork hams and similar products.

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1. Introduction

Vegetative cells of *Clostridium perfringens* are grampositive, non-motile, rods (Rhodehamel and Harmon, 1998). This organism is one of the most common causes of foodborne illness in the United States. *C. perfringens* causes an estimated 250,000 cases of food poisoning annually, leading to about 41 hospitalizations and seven deaths each year in the U.S. (Mead et al., 1999). As a result, the economic impact associated with this organism was calculated to reach US\$12.5 billion annually (Buzby and Roberts, 1997).

Food poisoning associated with *C. perfringens* is caused by the ingestion of a large number ($\geq 10^6$) of viable vegetative cells

of the organism in temperature-abused foods. Once in the small intestine, the cells sporulate, releasing an enterotoxin that is responsible for the pathological effects in humans as well as the typical symptoms of diarrhea and abdominal pain. The *C. perfringens* outbreaks in North America have been primarily associated with meat and poultry products (Bryan, 1988; Taormina et al., 2003), probably due to the abundance of more than a dozen amino acids and several vitamins necessary for the organism to grow in these products (Brynestad and Granum, 2002; Labbe and Juneja, 2002). The outbreaks usually result from improper handling and preparation of foods at the home, retail, or food service operations, and rarely involve commercial meat processors (Bryan, 1988; Taormina et al., 2003).

C. perfringens spores are widely distributed in soil and dust, the intestinal tract of humans and animals, and raw ingredients, e.g., spices, used in food processing. Because of its ubiquitous distribution, it is difficult if not impossible to exclude spores of this pathogen from various animal or plant food products during processing and its presence must be assumed. Because of the

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high heat resistance of the spores of *C. perfringens*, thermal treatments applied to process meat and poultry products are usually not sufficient to inactivate them. On the contrary, the spores can be heat-activated, outgrow and multiply during subsequent cooling. According to Willardsen et al. (1979, 1978), one strain of *C. perfringens* exhibited a generation time as short as 7.1 min in autoclaved ground beef incubated at 41 °C, with mean generation times for a 8-strain cocktail of 19.5 min at 33 °C and 8.8 min at 45 °C. Optimal *C. perfringens* growth been observed between 30 and 45 °C in meats, the temperature range that cooked meats must pass through during cooling.

Since this organism can grow and multiply rapidly after germination, cooked meat and poultry products must be cooled rapidly to restrict their germination, outgrowth, and the subsequent vegetative growth. To ensure safety, products must be transported, distributed, stored and handled under refrigeration. Juneja et al. (1994) studied germination and growth of C. perfringens spores in beef simulating the cooling conditions that occur in meat processing operations. The authors reported that no appreciable growth (<1.0 \log_{10} CFU/g) occurred if cooling took 15 h or less when cooked ground beef inoculated with heat activated C. perfringens spores was cooled from 54.4 to 7.2 °C at an exponential rate, that being more rapid cooling at the beginning and then slower. However, C. perfringens grew by 4-5 log₁₀ CFU/g if the cooling was greater than 18 h. This implies that C. perfringens is capable of rapid growth in meat systems, making this organism a particular concern to meat processors, as well as to the foodservice industry, if the products are not cooled properly.

Due to its ubiquitous nature and rapid growth in meat products, C. perfringens can be a potential hazard in processed meat and poultry products. The FDA (2001) Food Code dictates that potentially hazardous cooked foods such as meats should be cooled from 60 to 21 °C within 2 h, and from 60 to 5 °C within 6 h. In U.K., it is recommended that uncured cooked meats be cooled from 50 to 12 °C within 6 h and from 12 to 5 °C within 1 h (Gaze et al., 1998). Safe cooling times for cured meats may be up to 25% longer (Gaze et al., 1998). The USDA-FSIS compliance guidelines (USDA, 1999; USDA, 2001) for chilling of thermally processed meat and poultry products state that these products should be chilled following the prescribed chilling rates, or require that process authorities validate the safety of customized chilling rates to control spore-forming bacteria. Specifically, the guidelines state that cooling from 54.4 to 26.7 °C should take no longer than 1.5 h, and cooling from 26.7 to 4.4 °C should take no longer than 5 h (USDA, 2001). Additional guidelines allow for the cooling of certain cured cooked meats from 54.4 to 26.7 °C in 5 h, and from 26.7 to 7.2 °C in 10 h (USDA, 2001). If meat processors are unable to meet the prescribed time-temperature cooling schedule, they must be able to document that the customized or alternative cooling regimen used will result in a less than $1-\log_{10}$ CFU increase in C. perfringens in the finished product. If the cooling guidelines can not be achieved, computer modeling and/or product sampling can be used to evaluate the severity and microbiological risk of the process deviation, and that it may be

necessary to conduct challenge studies to determine if performance standards have been met.

Predictive bacterial growth models that describe C. perfringens spore germination and outgrowth during cooling of food systems have been generated by researchers using constant temperature data. Juneja et al. (1999) presented a model for predicting the relative growth of C. perfringens from spores, through lag, exponential and stationary phases of growth, at temperatures spanning the entire growth temperature range of about 10-50 °C. Huang (2003a,b,c) used different mathematical methods to estimate the growth kinetics of C. perfringens in ground beef during isothermal, square-waved, linear, exponential, and fluctuating cooling temperature profiles. Juneja et al. (2001) developed a predictive cooling model for cooked cured beef based on growth rates of the organism at different temperatures, which estimated that exponential cooling from 51 to 11 °C in 6, 8, or 10 h would result in an increase of 1.43, 3.17, and 11.8 log₁₀ CFU/g, respectively, when assuming the ratio of the mathematical lag time to the generation time for cells in exponential phase of growth is equal to 8.068, the estimated geometric mean. A similar model was later developed for cooked cured chicken (Juneja and Marks, 2002).

The objective of this research was to develop a model that can be used to evaluate microbiological safety of cured pork products, or those supplemented with low levels of preservatives, with respect to *C. perfringens* spore germination and outgrowth.

2. Materials and methods

2.1. 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), obtained from our culture collection, were used in this study. The spores were produced in a modified formulation of Duncan and Strong sporulation medium as previously described (Juneja et al., 1993). After the spore crop of each strain had been washed twice and resuspended in sterile distilled water, the spore suspensions were stored at 4 °C. Spores of individual strains at equal numbers were then mixed to prepare a cocktail. This composite of spore strains was not heat-shocked prior to use.

2.2. Preparation of samples

Ground pork was obtained from a local meat manufacturing company. The proximate analysis of meats performed by the supplier indicated that the pork contained 16% fat, 65% moisture, 2% ash, and 17% protein. Brine (3.5%) was thoroughly mixed in ground pork before the meat was placed into polyethylene bags (100 g/bag) and vacuum sealed. Thereafter, every five of these bags was vacuum-sealed in a barrier pouch (Bell Fibre products Corp., Columbus, GA), frozen at - 40 °C and irradiated (42 kGy) to eliminate indigenous microflora. Irradiation was performed using a self-

contained ¹³⁷Cs Irradiator (Lockheed Georgia Co., Marietta GA) at the Eastern Regional Research Center, ARS, USDA, Wyndmoor, PA. Random samples were tested to verify elimination of the microflora by diluting in 0.1% (wt/vol) peptone water (PW), spiral plating (Spiral Biotech, Bethesda, MD; Model D) on Tryptic soy agar (TSA; BD/Difco, Sparks, MD) and then incubating, both aerobically and anaerobically, at 37 °C for 48 h. After irradiation, samples were maintained in a freezer (-40 °C). Upon experiment, the samples were thawed overnight in a refrigerator (\sim 4 °C).

2.3. Inoculation of samples

Sodium nitrite (120 ppm) and a cocktail of three strains of C. perfringens spores were added (1 ml) to 100 g of the thawed irradiated pork products. Thereafter, the inoculated meat was blended with a stomacher (Seward Stomacher 400, London, UK) for 5 min to ensure even distribution of sodium nitrite and the organisms in the meat samples. Ground meat samples (5 g each) were then weighed aseptically into 30×19 cm sterile filter stomacher bags (Spiral Biotech, Bethesda, MD). Negative controls included bags containing meat samples inoculated with 0.1 ml of 0.1% (w/v) peptone water with no bacterial spores. Thereafter, the bags were compressed into a thin layer (approximately 1-2 mm thick) by pressing against a flat surface, excluding most of the air, and then heat sealed under vacuum (negative pressure of 1000 mbars). One bag, randomly selected, was opened and heatshocked at 75 °C for 20 min. The samples were serial diluted in PW, surface plated with a Spiral plater (Model D, Spiral Biotech, Bethesda, MD) on Shahidi-Ferguson perfringens agar as described earlier (Juneja et al., 1996). The lower limit of detection by this procedure is 21 CFU/g. The total C. perfringens population was determined after 48 h of anaerobic incubation. This was recorded as the initial inoculated numbers of bacterial spores before cooking.

2.4. Cooking temperatures, sampling times and bacterial enumeration

Simulating the conditions that occur in the retail food industry and institutional food service settings, the vacuumpackaged bags containing the meat samples were immersed in a programmable water bath (Techne, ESRB, Cambridge, UK) at 10 °C. The temperature of the water bath was programmed to increase in a linear fashion to achieve 60 °C in a period of 1 h. After cooking, samples were enumerated and the data recorded as spore numbers after cooking. Thereafter, all samples were incubated in a constant temperature water bath stabilized at 10.0, 12.0, 15.6, 19.0, 21.1, 23.9, 26.7, 29.4, 32.2, 35.0, 37.8, 40.6, 43.3, 46.1, or 48.9 °C. At frequent intervals, appropriate for each growth temperature, about 6-7 samples were taken out for C. perfringens count enumeration as described above. Two independent experiments were done at each temperature. For each experiment, an average CFU/g of two platings of each sampling point were recorded and used to determine estimates of the growth kinetics.

2.5. Mathematical modeling of growth of C. perfringens in cooked pork

The methodology developed by Huang (2004) was used to develop mathematical models for describing the growth of *C. perfringens* in cured pork. Huang (2004) used a set of differential equations (Eqs. (1) and (2)), representing different stages of bacterial growth, to describe the dynamics of bacterial growth and multiplication.

$$\frac{\mathrm{d}C_{\mathrm{L}}}{\mathrm{d}t} = -k_{\mathrm{L}}C_{\mathrm{L}} \tag{1}$$

$$\frac{\mathrm{d}C_{\mathrm{D}}}{\mathrm{d}t} = -\frac{\mathrm{d}C_{\mathrm{L}}}{\mathrm{d}t} + k_{\mathrm{D}}C_{\mathrm{D}}\left(1 - \frac{C_{\mathrm{D}}}{C_{\mathrm{max}}}\right). \tag{2}$$

Parameters in Eqs. (1) and (2) are: $C_{\rm L}$: concentration of dormant bacterial cells, CFU/g; $C_{\rm D}$: concentration of actively dividing cells, CFU/g; $C_{\rm max}$: maximum cell concentration that bacteria can grow in the food system, CFU/g; $k_{\rm L}$ and $k_{\rm D}$: rate constants, 1/h.

It is important to note the unit for cell concentration in Eqs. (1) and (2) is CFU per unit mass or volume, not the commonly used the logarithm of bacterial counts or concentration. Eqs. (1) and (2) can be combined to form a new equation

$$\frac{\mathrm{d}C_{\mathrm{D}}}{\mathrm{d}t} = k_{\mathrm{L}}C_{\mathrm{L}} + k_{\mathrm{D}}C_{\mathrm{D}}\left(1 - \frac{C_{\mathrm{D}}}{C_{\mathrm{max}}}\right). \tag{3}$$

Since both dormant and actively dividing cells in a culture are living cells that are capable of growth and have the ability to form new cells; at any given moment, the total cell concentration (C) is the sum of the dormant and actively dividing cell concentrations:

$$C = C_{\rm L} + C_{\rm D} \tag{4}$$

Eqs. (1) through (4) were solved numerically based on the 4th-order Runge-Kutta method (Chandra and Singh, 1995) using the computer algorithm developed by Huang (2004), in which an optimal relationship between $k_{\rm L}$ and $k_{\rm D}$ was $k_{\rm L}$ =0.01 $k_{\rm D}$. This relationship was used in the present study for curving-fitting to search for the best $k_{\rm D}$ value of a growth curve under isothermal conditions.

The determination of kinetic parameter k_D of a growth curve under an isothermal condition requires a set of growth data in the raw form (CFU/g). Both the initial and final concentrations are necessary for the curve-fitting. The initial concentration is needed to initiate the numerical iteration. The final concentration is needed to force the growth curve to converge to the stationary phase. But sometimes a growth curve is not complete. It may only contain the lag and exponential phase data without the stationary phase. This method, however, can also be used to find the kinetic parameter k_D of a growth curve when the stationary phase data are not available. In such a case, the maximum cell concentration can be set to a relative large imaginary stationary phase concentration, which allows a loglinear growth curve in the exponential phase and forces the curve to converge to the imaginary maximum stationary phase concentration. In this study, the imaginary stationary phase concentration was set to 10 log(CFU/g), and was used only when the final stationary phase cell concentration was not available. Use of this number allows the program to choose the best linear curve for the linear phase of the experimental data. The computer algorithm automatically searches for a k_D value that best fits the data from the lag phase to the exponential phase.

2.6. Secondary kinetic model

To evaluate the effect of temperature on *C. perfringens* growth in cooked cured pork, k_D was fitted to temperature using a modified Ratkowsky equation (Zwietering et al., 1991):

$$k_{\rm D}(T) = A(T - T_{\rm min})^2 \{1 - \exp[B(T - T_{\rm max})]\}$$
(5)

In this equation, A and B are coefficients, and T_{min} and T_{max} are the theoretical minimum and maximum growth temperatures for *C. perfringens* in cooked pork. A Windows-based statistical package, NCSS 2000 (Hintze, 1999), was used to obtain the parameters of the equation using nonlinear regression.

3. Results and discussion

3.1. Experimental observations

The present study assessed the growth of C. perfringens from spore inocula in pork supplemented with curing salts. The pork was cooked slowly to 60 °C to simulate cooking of the products in the meat industry, then rapidly cooled to a target temperature and maintained at that specified temperature. Growth of C. perfringens from spores was not observed up to a period of 21 days at both 10 and 12 °C. These observations are in agreement with previous studies. Goepfert and Kim (1975) reported that C. perfringens growth does not initiate in foods stored at 15 °C or below, even after extended storage. However, this may not hold true when hot foods are cooled and the rate of cooling is not sufficiently fast. Solberg and Elkind (1970) reported that C. *perfringens* vegetative cells increased by $3 \log_{10}$ cycles in 3 days at 15 °C and in 5 days at 12 °C, but the growth was restricted at 10 and 5 °C. Further, Amezquita et al. (2004) reported no growth of C. perfringens at 21 and 17 °C after 10 and 21 days, respectively, in cured ham (initial sodium nitrite concentration of 156 ppm). These differences in the minimum growth temperature could be a function of product characteristics, such as the meat species, pH, concentrations of functional ingredients such as NaCl, phosphates and other antimicrobial ingredients such as nitrates/nitrites or salts of organic acids. These ingredients affect growth of C. perfringens from spore inocula in specific meat products (Juneja and Thippareddi, 2004; Thippareddi et al., 2003; Zaika, 2003). Zaika (2003) reported inhibition of C. perfringens germination and growth in cured hams with NaCl concentrations of 3.1% when cooled exponentially from 54.4 to 7.2 °C within 15, 18 or 21 h. While Zaika (2003) evaluated C. perfringens germination and outgrowth in cured hams, the products were obtained from establishments subsequent to processing, and NaCl was

supplemented in ground, processed hams. The residual nitrite concentrations reported for the three hams were 26, 12 and 12 ppm expressed as sodium nitrite. While these nitrite concentrations are representative of the products commercially available, in practice, the *C. perfringens* spores are exposed to greater concentrations of nitrite (ca. 156 ppm) during the initial stages of cooking and cooling and lower residual nitrite concentrations (ca. 30 ppm) at the end of processing. Exposure to these higher concentrations of nitrites during initial meat processing stages can affect *C. perfringens* spore germination and outgrowth (Sanchez, 2004).

Thippareddi et al. (2003) reported complete inhibition of *C. perfringens* spore germination and outgrowth by sodium salts of lactic and citric acids (2.5% and 1.3%, respectively) in roast beef, pork ham and injected turkey products. Thus, the presence of inhibitory agents in the products can affect germination of *C. perfringens* spores, and may also affect the minimum growth temperatures for the germinated spores.

Sodium nitrite has traditionally been used as a preservative in cured meats and can inhibit growth of C. perfringens under certain conditions (Robach et al., 1978). It has been demonstrated that 0.02% and 0.01% sodium nitrite slows germination of both heat resistant and heat sensitive C. perfringens spores (Labbe and Duncan, 1970; Sauter et al., 1977). Gough and Alford (1965) reported that C. perfringens growth was not inhibited at 8000 ppm of sodium nitrite but was inhibited when the concentration was increased to 12,000 ppm. It is worth mentioning that the inhibitory effect of sodium nitrite is enhanced at elevated temperatures (Davidson and Juneja, 1990) since heat injured spores are more sensitive to the effects of nitrite (Chumney and Adams, 1980). Further, the inhibitory effect of nitrite can be enhanced under acidic conditions and by prior heating of the medium containing nitrite (Labbe, 1989; Riha and Solberg, 1975). Most processed meat products contain about 2.75% and 3.25% salt (Maurer, 1983) and sodium nitrite is allowed in cured meats at ingoing (initial) levels of 156 ppm. Gibson and Roberts (1986) reported that the inhibitory concentrations of sodium nitrite can be lowered if combined with other curing salts. In their study, C. perfringens growth at 20 °C was inhibited by 200 µg of nitrite/ml (ppm) and 3% salt or 50 µg of nitrite/ml and 4% salt at pH 6.2 in a laboratory medium. In another study, the levels of sodium nitrite necessary to inhibit the strains tested dropped from 300 ppm to 25 ppm when the concentration of NaCl was increased from 3% to 6% (Roberts and Derrick, 1978).

3.2. Curve-fitting

The differential mathematical models described by Eqs. (1) through (4) can be used to describe a complete growth curve. Fig. 1 shows representative growth curves fitted with the numerical method. As illustrated in these figures, all three phases of growth, the lag, exponential, and stationary phases are clearly represented by the fitted curves. The transition from one phase to another is smooth, and the exponential phase is linear.

In a few instances, the raw data gathered in the study did not contain a stationary phase. The differential equations were used



Fig. 1. Growth of C. perfringens in cooked pork supplemented with sodium nitrite at 26.7, 37.8, 40.6, and 46.1 °C.

to describe these curves using an imaginary stationary phase (Fig. 2). This method, in addition to the method developed by Baranyi and Roberts (1994), allow of fitting incomplete data curves.

3.3. Effect of temperature on growth rate

Inadequate cooling of foods in retail operations is a major food safety problem. In an attempt to determine safe cooling



Fig. 2. Incomplete growth curves without stationary phases fitted with the differential equations.

rates for cooked beef, Juneja et al. (1994) demonstrated the effectiveness and validity of the square-root model under non-isothermal conditions and reported that cooked beef must be cooled to 7.2 °C in 15 h or less to prevent C. perfringens foodborne disease outbreaks. In the present study, we have described the effect of temperature on the kinetic parameter $k_{\rm D}$ by the modified Ratkowski model (Fig. 3). According to the coefficient of the model (Table 1), the minimum and maximum growth temperatures of C. perfringens in cooked pork are 13.5 and 50.6 °C, respectively. Estimations of these temperatures are in agreement with the experimental observations. The estimated maximum growth temperature is consistent with the results reported by Juneia et al. (1993) and Huang (2004). The literature indicated that 50-51 °C is the upper growth limit for this organism (Juneja et al., 1999).



Fig. 3. Effect of temperature on growth rate—the modified Ratkowski model. The broken lines represent upper and lower prediction limits at a 95% confidence level.

Table 1 Estimates and asymptotic standard error of parameters used for estimating growth characteristics

Parameter	Estimated value	Asymptotic standard error
A	2.43×10^{-3}	1.12×10^{-3}
В	0.41	0.44
T_{\min} (°C)	13.5	4.8
T_{\max} (°C)	50.6	1.7

Model: Eq. (5), pseudo- $R^2 = 0.81$.

3.4. Lag phase

According to Huang (2004), $k_{\rm D}$ was the only parameter determined by the numerical method, whereas 3–4 parameters need to be estimated when Gompertz, logistic, or Baranyi growth models are used, along with estimations of the initial and stationary phase cell concentrations. Using the method developed by Huang (2004), the initial and stationary phase concentrations are obtained experimentally and subsequently incorporated into the models to estimate the values of $k_{\rm D}$.

The lag phase duration can be directly calculated from k_D using the following mathematical relationship (Huang, 2004; Juneja and Marks, 2002):

$$\lambda_{\rm F} = \frac{\ln\left(1 + \frac{k_{\rm D}}{k_{\rm L}}\right)}{k_{\rm D}} \tag{6}$$

Since $k_{\rm L}$ is available and is equal to 0.01 $k_{\rm D}$, the lag phase duration of each growth curve can be directly calculated from Eq. (6). Eq. (5) also can be used to estimate the lag phase duration within the limits of the growth temperature.

3.5. Comparison of specific growth rates and lag phase durations

Fig. 4 illustrates the comparison of the specific growth rates and lag phase durations of *C. perfringens* obtained in this study with those from beef (Huang, 2003a) and broth (Juneja et al., 1999). In general, the specific growth rates of *C. perfringens* in pork are similar to those in broth, but both are smaller than those in beef. Faster growth of *C. perfringens* in beef is probably due to the more nutritious components available in beef. While ground pork may also have nutrients for *C. perfringens* to growth, its growth in cured pork may be impeded because of the small amount of sodium nitrite present in these products.

The effect of sodium nitrite is more significant on the lag phase duration (Fig. 4). At temperatures below 35 °C, the lag phases of *C. perfringens* in cured pork are longer than those in beef or broth. This also may be attributed to the inhibitory effect of sodium nitrite on *C. perfringens*. At temperatures between 35 and 50 °C, an optimum range for growth, the length of lag phase duration of *C. perfringens* in cured pork is between uncured beef and broth. Since there was no inhibitory agent in ground beef, *C. perfringens* was able to grow without hindrance due to the ample supply the nutritive components. Therefore, the lag phase of *C. perfringens* is shorter in cooked beef. In broth where

vital growth components are not naturally available, the bacteria need to produce and accumulate sufficient amount of these components before they can germinate, outgrow, and multiply. Therefore, the lag phases of *C. perfringens* in broth are longer than those in cooked beef. Although cured pork also contained a sufficient amount of vital growth components, the germination, outgrowth, and multiplication is relatively inhibited by sodium nitrite. But, because bacteria are grown in the optimum temperature range, the rates at which the bacteria generate vital components are higher than the rates at temperature below the optimum temperatures. Therefore, at optimum growth temperatures, the lag phase duration of *C. perfringens* in cured pork is longer than that in beef, but shorter than that in broth.

In a study by Juneja et al. (2001), when growth of C. perfringens was determined at isothermal temperatures to develop a model to predict the fate of the pathogen at temperatures applicable to the cooling of cooked cured meat products, C. perfringens growth from spores was not observed at 12 °C for up to 3 weeks. Germination, outgrowth, and lag (GOL) time and exponential growth rate (EGR) were determined using a function derived from mechanistic and stochastic considerations and the observed relative growths at specified times (Juneja et al., 2001). A general model to predict the amount of relative growth for an arbitrary temperature was determined by fitting the exponential growth rates to a square root Ratkowsky function, and assuming a constant ratio of GOL and generation times. Juneja et al. (2001) developed a closed form equation that can be used to estimate the relative growth for a general cooling scenario and to determine a standard error of the estimate. Applying multivariate statistical procedures, Juneja et al. (2001)



Fig. 4. Comparison of specific growth rates and lag phase duration in cured pork, uncured beef, and beef broth.

computed a confidence interval on the prediction of the amount of growth for a given temperature. The model by Juneja et al. (2001) predicts, for example, predicts a relative growth of 3.17 with an upper 95% confidence limit of 8.50 when cooling the product from 51 to 11 °C in 8 h, assuming log linear decline in temperature with time. The predictive model presented in this paper and those published earlier (Juneja et al., 2001; Juneja and Marks, 2002) should aid in evaluating the safety of cooked cured products after cooling and, thus, the disposition of products subjected to cooling deviations.

In summary, this paper presents a model for predicting growth of *C. perfringens* at temperature ranges applicable to cooling and subsequent storage of cooked cured pork products. Further research is needed to study the kinetics of growth as a function of the cooling rate to validate assumptions and equations presented in this paper.

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