**Growth and Survival of *Salmonella* Paratyphi A in Roasted Marinated
Chicken during Refrigerated Storage: Effect of Temperature Abuse and
Computer Simulation for Cold Chain Management[†]**

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19 Abstract

20 This research was conducted to evaluate the feasibility of using a one-step
21 dynamic numerical analysis and optimization method to directly construct a tertiary
22 model to describe the growth and survival of *Salmonella* Paratyphi A (SPA) in a
23 marinated roasted chicken product. Multiple dynamic growth and survival curves
24 obtained under different fluctuating temperature conditions between 4 and 35°C were
25 used to determine the growth kinetics of SPA. In combination with appropriate
26 secondary models, the study examined both growth and survival of SPA
27 simultaneously by an integrated one-step approach using a set of differential
28 equations.

29 The estimated minimum growth temperature ($T_{\min}$) of SPA was 8.91 °C,
30 matching well with the growth characteristics of this microorganism. The growth at
31 temperatures above $T_{\min}$ and the survival below $T_{\min}$ was accurately simulated by the
32 predictive models. For model development, the root mean square error (RMSE) was
33 0.26 log CFU/g.

34 The predictive models and kinetic parameters were validated using two
35 dynamic growth and survival curves along with one isothermal thermal growth curve.
36 The validation also showed that the models were accurate in predicting the growth
37 and survival of the bacterium, with the RMSE of predictions only 0.52 log CFU/g.
38 The errors of predictions were within normal experimental errors. The results of
39 this work may be used to predict the change in the population of SPA in the marinated

40 roasted chickens in the cold chain and during temperature abuse and to conduct risk
41 assessment of this pathogen.

42 **Keywords:** dynamic modeling, *Salmonella* Paratyphi A, predictive microbiology

43

1. Introduction

Salmonella Paratyphi A is a Gram-negative bacterium widely distributed in nature, and is one of the most common foodborne pathogens in many countries of the world. If inadvertently ingested, it can cause a variety of illness, such as acute gastroenteritis, septicemia, and enteric fever (Li et al., 2015). Currently *Salmonella* has been recognized as one of the most significant foodborne pathogens and public health hazards (Gould et al., 2013; Olsen et al., 2001). In the United States, nontyphoidal *Salmonella* causes about 1.4 million infections, 15,000 hospitalizations, and 580 deaths per year (WHO, 2005). In Southeast Asia, the estimated infection is 22.8 million, causing 37,600 deaths annually (Majowicz et al., 2010, Van et al., 2012). *S. Typhoid* and *S. Paratyphi* caused 51,700 cases of infections and 7 deaths in China between 2011 and 2014 (NBSPRC, 2011-2014). In China, the majority (70-80%) of bacterial foodborne infections is caused by *Salmonella*, and 90% of the foodborne salmonellosis is attributable to contaminated meat products (Yang et al., 2010; EFSA, 2014). In recent years, several outbreaks of foodborne salmonellosis were caused by consumptions of roasted marinated chicken.

Daokou roasted marinated chicken (DRMC) is a popular traditional food in China. This food originated in Daokou of He Nan Province, and has a history of more than 350 years. The traditional technique used in preparation of DRMC requires marination of whole chickens in a sauce that contains a variety of spices and herbs to infuse different flavors. After marination, the chickens are fried and roasted. After cooling, DRMC is packaged for consumption. The final product has a unique

66 flavor and texture, and is well liked by consumers in China.

67 DRMC is a ready-to-eat product and should be stored at 0-4 °C prior to
68 consumption. The cooking process should have inactivated foodborne pathogens
69 such as *S. Paratyphi*. However, most DRMC products are prepared by small shops
70 that lack necessary hygiene measures to prevent cross-contamination, leading to
71 re-contamination of products. In addition, temperature abuse during storage and
72 distribution may allow *S. Paratyphi* to grow and multiply in contaminated products.
73 A survey conducted by our laboratory has determined that *S. Paratyphi* A is the main
74 cause of foodborne salmonellosis in chilled chicken. Therefore, DRMC can be a
75 potential vehicle for transmission of *S. Paratyphi*, causing foodborne salmonellosis
76 (Zhai et al., 2014).

77 Currently, a few mathematical models have been developed to simulate the
78 growth of *Salmonella* in meat and poultry, including chicken and ground beef (Juneja
79 et al. 2007, 2009). These growth models are developed under isothermal conditions.
80 They can be used for prediction of the growth of *Salmonella* under different storage
81 temperatures. Developing these models requires a three-step process. First,
82 isothermal studies must be performed to obtain growth curves under selected
83 temperatures. These growth curves are analyzed and fitted to one or more primary
84 models to derive growth parameters such as lag time and growth rates. After a
85 sufficient number of growth parameters are obtained, a secondary model is identified
86 and used to analyze the primary growth parameters to evaluate the effect of
87 temperature and other factors on these parameters. Once the secondary model is

developed, it can be used in conjunction with the primary model to construct a tertiary model to predict the growth of bacteria under different constant temperature conditions. These models, however, cannot be directly used to predict the growth of *Salmonella* under fluctuating temperature conditions. The models developed using this approach are not able to predict the survival and inactivation of bacteria below the minimum growth temperature.

Based on isothermal models, predictive models applications to fluctuating temperature conditions also have been developed. For example, Velugoti et al. (2011) developed a predictive model for predicting the growth of *Salmonella spp.* in ground pork under different fluctuating temperature conditions. Xing et al. (2013) developed a dynamic microbial growth model of tilapia under fluctuating temperatures to simulate the cold chain temperature fluctuation. The development of these models also requires a three-step process as mentioned previously. The first two steps are the same for determining the kinetic parameters. The difference is in the construction of the tertiary models. After the secondary model is developed, it is used in conjunction with a differential form of the Baranyi model to construct a tertiary model. The differential equation is solved by using a numerical analysis method such as the 4th-order Runge-Kutta method to solve the different equation for prediction the growth of *Salmonella* under fluctuating temperature conditions.

More recently, a new one-step dynamic analysis method was developed to directly construct a tertiary growth model from dynamically changing temperature profiles to predict the growth of *Clostridium perfringens* in cooked beef and turkey

110 meat during cooling (Huang, 2015; Huang and Vinyard, 2016). The models
111 developed using this approach have shown to accurately predict the growth of *C.*
112 *perfringens* in cooked beef under both fluctuating and isothermal conditions (Huang,
113 2015; Huang and Vinyard, 2016). It is also a more efficient method for constructing
114 predictive models as it avoids time-consuming and labor-intensive isothermal
115 experiments. This method avoids the accumulation of errors in the first two steps in
116 the traditional method and therefore, can produce more accurate predictive models
117 (Huang, 2016).

118 In most studies of predictive microbiology, growth and survival (inactivation)
119 of microorganisms are examined in separate experiments. This study attempts to
120 develop a methodology to examine both growth and survival using a dynamic approach
121 that exposes the bacteria to the conditions suitable or unsuitable for growth in storage
122 experiments. Therefore, the objective of this research is to apply the one-step direct
123 dynamic method to construct and validate a tertiary mathematical model to predict the
124 growth and survival of *S. Paratyphi A* in DRMC under fluctuating temperature
125 conditions. The ultimate goal of this study is to apply the mathematical models to
126 predict the growth and survival of *S. Paratyphi A* under fluctuating temperature
127 conditions of a cold chain and use the models for cold chain management and risk
128 assessment.

129

130

131 2. Materials and methods

132 2.1 Preparation of bacterium

133 A *Salmonella* Paratyphi A (SPA) strain, previously isolated from chilled
134 chicken products by the Microbiology Laboratory of the College of Food Science and
135 Technology, He Nan Agricultural University (Zhengzhou, China), was used in this
136 study. This strain was identified by Sangon Biotech (Shanghai) Co., Ltd. as the same
137 as ATCC 9150. The bacterial strain was maintained in 25% glycerol at -40 °C, and
138 thawed in a hot water bath (30 °C) before being propagated for use in the experiments.
139 The thawed culture was first propagated in nutrient broth (NB, Beijing Land Bridge
140 Technology Co., Ltd (BLBTC), China) at 37 °C overnight and then streaked onto a
141 nutrient agar (NA, BLBTC) plate. The cells from a single colony on the NA plate
142 was inoculated into a sterile Erlenmeyer flask containing 50 ml NB and incubated for
143 18 h at 37 °C on an orbital shaker (150 rpm, ZD-A Desktop Rotary Oscillator, Taicang
144 Huamei Biochemical Instrument Factory, China). The stationary phase bacteria
145 were harvested and plated onto XLD agar (XLD, Oxoid, UK) plates.

146

147 2.2 Sample preparation

148 Freshly prepared DRMC samples (700~900 g per chicken), purchased from a
149 company in Zhengzhou, He Nan Province before an experiment, were quickly peeled
150 to remove the skin under sterile conditions. The chicken meat samples were cut into
151 ~ 1 cm³, placed on a layer of cotton gauze, and then steamed with boiling water for 10
152 min to eliminate background microorganisms. The samples (25 ± 0.1g) were

153 aseptically transferred to sterile homogenization bags (BLBTC) under sterile
154 conditions. Each 25 g sample was inoculated with 2.5 ml of the bacterial culture (ca.
155 2~3 log CFU/g). The inoculated samples were pressed in the homogenization bags
156 with a round tube to mix the chicken meat with the inoculum. The samples bags
157 were sealed after evacuating the internal air.

158

159 2.3 Dynamic Storage Study

160 A programmable precision incubator (SANYO Incubator, MIR254, Japan) was
161 used to incubate the inoculated samples in this study. The temperature range of this
162 incubator was -20 to 60 °C. The precision of the incubator was $\pm 0.1\sim 0.2$ °C at a
163 temperature set point. Five dynamic temperature profiles were programmed into the
164 incubator (Figs. 1 and 3). The temperature fluctuation in each temperature profile,
165 ranging from 4 to 35 °C, was programed to simulate the temperature changes in a cold
166 chain with a consideration of temperature abuse. Three dynamic profiles (1-3) in Fig.
167 1 were used to determine the kinetic parameters. Two dynamic profiles (4 and 5) in
168 Fig. 3 were used to validate the accuracy of the models. Each dynamic temperature
169 was unique and independently designed for model development and validation. In
170 addition, one isothermal experiment (15 °C) was conducted to collect the growth data
171 for additional model validation. This temperature was arbitrarily chosen to study the
172 prediction under an isothermal condition.

173 After the incubator was stabilized to the initial temperature of each dynamic
174 temperature profile, the inoculated samples were loaded to the incubator, which was

175 programed to change according to the experimental plans. Samples were
176 periodically removed from the incubator to enumerate SPA. During sampling, five
177 sample bags were randomly selected and retrieved from the incubator. The sample
178 bags were immediately opened to enumerate SPA.

179

180 2.4 Enumeration of SPA

181 To enumerate SPA from the samples, sterile saline (0.85% NaCl, Analytical
182 Pure, Sinopharm Chemical Reagent Co., Ltd, China) was used. Each sample was
183 mixed with 225 ml sterile saline and stomached in a homogenizer (Easy Mix, AES
184 Co., France) at high speed for 2 min. After stomaching, the liquid portion of the
185 sample (0.1 ml) was withdrawn and surface-plated onto to freshly prepared XLD agar
186 plates after serial dilutions. The plates were incubated at 37 °C for 16~20 h. The
187 SPA colonies were counted and converted to logarithms of colony-forming units (log
188 CFU/g). The bacterial counts were used to develop the growth and survival curves
189 of SPA in DRMC under fluctuating and isothermal temperatures and determine the
190 growth kinetics of SPA and validate the growth models.

191

192 2.5 Data analysis and development of growth models

193 2.5.1 *Primary models for bacterial growth and survival*

194 Both growth and inactivation of SPA was observed in dynamic studies. At
195 temperatures (T) above the minimum growth temperature, SPA grew in DRMC. The
196 growth of SPA in DRMC was described using a primary model reported in Huang

(2015). According to this model (Eq. (1)-(3)), the bacterial cells can be divided into two different groups (dormant and actively dividing cells) when they are inoculated to the samples, and the growth of bacteria can be described by a set of two ordinary differential equations (ODEs, Eqs. (1) and (2)). The population of dormant cells is designated as N_L (CFU/g), and the population of dividing cells is represented by N_D (CFU/g). The dormant cells gradually leave the dormant state following the 1st-order kinetics (Eq. (1)). Once the dormant cells leave the dormant state, they become actively dividing cells and begin to divide immediately, following a logistic process (Eq. (2)). To simplify the problem, it is assumed that the dormant cells leave the dormant state at a fraction (α) of the actively dividing cells. In Eq. (2), μ_{max} is the specific growth rate (ln CFU/g h⁻¹). At $t = 0$, all cells are dormant cells. Therefore, $N = N_L$ and $N_D = 0$ when $t = 0$. N_{max} is the maximum cell concentration at the stationary phase (CFU/g). The total population (N , CFU/g) is equal to the sum of the populations of dormant and dividing cells (Eq. (3)).

$$\frac{dN_L}{dt} = -\alpha\mu_{max}N_L \quad (1)$$

$$\frac{dN_D}{dt} = \alpha\mu_{max}N_L + \mu_{max}N_D \left(1 - \frac{N_L + N_D}{N_{max}}\right) \quad (2)$$

$$N = N_L + N_D \quad (3)$$

SPA is a mesophilic bacterium. It gradually dies off when exposed to temperatures below the minimum growth temperature (T_{min}). It is assumed that both dormant and dividing cells die off at temperatures below T_{min} , but at different rates. K is the rate at which the bacteria die off at T below T_{min} . The decline in the dormant cells is described using Eq. (4), and the death of dividing cells is represented

by Eq. (5). The term βKN_L is used to describe that a fraction of the dividing cells are changed to dormant cells at $T < T_{min}$. The total cell concentration is still the sum of both dormant and dividing cells (Eq. (3)).

$$\frac{dN_L}{dt} = -\beta KN_L \quad (4)$$

$$\frac{dN_D}{dt} = \beta KN_L + KN_D \quad (5)$$

224

225 2.5.2 Secondary models

In Eqs. (1) and (2), μ_{max} is a function of temperature, and can be described by the Huang square-root model (Eq. (6)) (Huang et al., 2008; Huang, 2011) at temperatures above the minimum growth temperature. In Eq. (6), a is a regression coefficient, T is the incubation temperature ($^{\circ}\text{C}$), and T_{min} is the minimum growth temperature ($^{\circ}\text{C}$). The advantage of using this model is that it can more accurately estimate the value of T_{min} (Huang, 2011). As for the bacterial survival (inactivation) at temperatures below T_{min} , the rate (K) of decline in the bacterial population was described as a linear function of refrigeration temperature (Eq. 7), with k as the coefficient. Based on the definition of K in Eq. (7), the β and K are negative in Eqs. (4) and (5).

236

$$\sqrt{\mu_{max}} = a(T - T_{min})^{0.75} \quad (6)$$

238

$$K = k(T - T_{min}) \quad (7)$$

240

241 2.5.2. Determination of kinetic parameters

242 To determine the kinetic parameters for both growth and survival of the
 243 bacteria during storage, a more general form of mathematical models for dormant and
 244 dividing cells was used (Eqs. (8)-(9)). In Eqs. (8) and (9), the rate term can be either
 245 $\mu_{\max}$ or K , depending on temperature. If T is above $T_{\min}$, Rate is equal to $\mu_{\max}$. The
 246 Rate is equal to K when T is below $T_{\min}$. Similarly, γ can be either α or β , depending
 247 on temperature. At $T \geq T_{\min}$, $\gamma = \alpha$. At T below $T_{\min}$, γ is β . The parameter m is
 248 also determined by temperature. It is equal to 1 when $T \geq T_{\min}$, and to 0 when T is
 249 below $T_{\min}$. The total population is still expressed as in Eq. (3).

$$250 \quad \frac{dN_L}{dt} = -\gamma \text{Rate} \times N_L \quad (8)$$

$$251 \quad \frac{dN_D}{dt} = \gamma \text{Rate} N_L + \text{Rate} \times N_D \left(1 - m \times \frac{N_L + N_D}{N_{\max}} \right) \quad (9)$$

252 The ODEs in Eqs. (1), (2), (4), and (5) are the theoretical models for
 253 describing the microbial growth and survival, while the ODEs in Eqs. (8) and (9) are
 254 the general equations that are used to describe microbial growth and survival when
 255 developing the computer program. The ODEs in Eqs. (8), (9), and (3), serving as the
 256 primary models, do not have an analytical solution. They must be solved with the
 257 secondary models (Eqs. (6) and (7)). The numerical analysis and optimization
 258 method reported in Huang (2015) was used to solve the ODEs and simultaneously
 259 determine the kinetic parameters for bacterial growth and survival, including a , k , $T_{\min}$,
 260 $Y_{\max}$ (or $\ln(N_{\max})$), α , and β . Three dynamic growth and survival curves
 261 (temperature profiles 1-3) were used and analyzed simultaneously in the

determination of kinetic parameters. The initial conditions for Eqs. (8) and (9) are also $N = N_L$ and $N_D = 0$ when $t = 0$.

The Runge-Kutta method used in Huang (2015) was used to solve the differential equations, and the difference (error, or ϵ) between the observed and model values was calculated for each data point. A least-square method was used to search for the most suitable values of a , k , $T_{\min}$, $Y_{\max}$, α , and β , such that the residual sum of squares (RSS) for the residual error of all three dynamic growth and survival curves was minimized (Eq. (10)), which is the difference (ϵ) between the measured (y , or $\log(N)$) and calculated $\log(N)$, or ($\hat{y}$) of bacterial growth, was minimized for a total number (n) of data points.

$$RSS = \sum_0^n (y_i - \hat{y}_i)^2 \quad (10)$$

Numerical analysis and curve fitting was performed using a computer program written in Python and reported in Huang (2015). In this program, numerical libraries such as Numpy and Scipy were used to solve the ordinary differential equations in Eqs. (8) and (9) using the Runge-Kutta method. After optimization was completed, the mean square error (MSE, Eq. (11)) and root mean square error (RMSE, Eq. (12)) were calculated from RSS. In Eqs. (11) and (12), n is the total number of observations, and p is the number of parameters.

$$MSE = \frac{RSS}{n-p} \quad (11)$$

$$RMSE = \sqrt{\frac{RSS}{n-p}} \quad (12)$$

283 2.6 Model validation

284 Once the kinetic parameters were determined, the models were validated with
285 two dynamic growth and survival curves (temperature profiles 4 and 5) and one
286 isothermal growth curve that were not previously used kinetic analysis for
287 determination of dynamic parameters. Additionally, the growth curve obtained at
288 15°C was also used to validate the models. The residual errors (ϵ), or the difference
289 between y and $\hat{y}$, were analyzed to find the distribution of ϵ using @Risk (Version
290 6.3.1, Professional Edition, Palisade Corp., Ithaca, NY).

291

292

293 3. Results and Discussion

294 3.1 Dynamic analysis and curve fitting

295 Fig. 1 shows the bacterial growth and survival under three temperature profiles
296 (1-3) used in dynamic analysis to determine the kinetic parameters of SPA. These
297 three temperature profiles were designed to expose the bacteria to a range of
298 temperatures and fluctuate at different pace in the course of experiments. Profile 1
299 was designed to fluctuate between 4 and 20 °C, and Profile 2 was designed to
300 fluctuate between 6 and 25 °C, while Profile 3 was designed to fluctuate between 6
301 and 35 °C. These three temperature profiles covered a temperature range from 4 to
302 35 °C, and were designed to expose the bacteria to refrigerated and temperature
303 abuse conditions that a product may experience during storage in a cold chain.

304 Depending on temperature, the population of SPA experienced increases and declines
 305 during storage (Fig. 1).

306 During dynamic analysis, curve fitting was initially performed to search for all
 307 6 kinetic parameters (a , k , $T_{\min}$, $Y_{\max}$, α , and β). The numerical analysis converged
 308 easily. The estimates of a , k , $T_{\min}$, $Y_{\max}$, α , and β were $8.73\text{e-}02$, $3.76\text{e-}03$, 8.89 ,
 309 20.5 , 10.0 and -6.64 . The estimates of a , $T_{\min}$, and $Y_{\max}$, were statistically significant
 310 ($p \ll 0.05$). However, the p values of k , α , and β were equal to $0.7 - 0.95$,
 311 suggesting the numerical analysis was not able to determine these values reliably.
 312 But the numerical analysis and optimization did produce estimates of these values.
 313 They were used as fixed values in the analysis that ensued. In the work reported by
 314 Huang (2015), accurate estimation of minimum temperature was particularly difficult
 315 because the bacteria were only allowed to grow within the growth limits of
 316 temperature. In this work, however, the bacteria were exposed to conditions below
 317 the minimum growth temperature, which caused bacterial death. As a result, the
 318 growth rate will change from positive values to negative values as the temperature
 319 drops below the minimum growth temperature, which makes it possible to accurately
 320 estimate the minimum growth temperature of the bacteria.

321 For inactivation rate, the value of k was fixed at $3.76\text{e-}03$. The absolute
 322 values of estimated α and β are both numerically > 1.0 . The maximum absolute
 323 values for these two parameters should be 1.0 . For α , it was set to 1.0 , assuming that
 324 the dormant cells leave the dormant state at the same rate as the dividing cells. For β ,
 325 it was set as -1.0 , assuming that the dividing cells may convert to dormant cells at the

326 same rate as the dividing cells die off. The data of three dynamic growth curves
327 were reanalyzed to search for the most suitable estimates of a , $T_{\min}$, and $Y_{\max}$. A
328 total of 72 observations from three dynamic curves was used for curve fitting. With
329 3 parameters, the degree of freedom (df) was 69. The data analysis also converged
330 quickly. The smooth curves shown in Fig. 1 are the regression curves of the model.
331 Table 1 lists the estimates of a , $T_{\min}$, and $Y_{\max}$.

332 In Table 1, the estimates of a , $T_{\min}$, and $Y_{\max}$ were statistically different from 0,
333 with their p values $\ll 0.05$, suggesting that the null hypothesis that any of these
334 values being 0 can be rejected. The estimated $Y_{\max}$ is 20.4 ln CFU/g, or 8.87 log
335 CFU/g, with a standard error of 0.50 ln CFU/g, or 0.21 log CFU/g. The estimate of
336 $T_{\min}$ is 8.91 °C, which matches well with the mesophilic nature of the bacterium.
337 Overall, the mean square errors (MSE) of the model is 0.070 (log CFU/g)², while the
338 root mean square error is 0.26 log CFU/g, suggesting that the model can be used to
339 describe the growth of SPA in DRMC under fluctuating temperature conditions and
340 estimate the kinetic parameters. The residual errors (ϵ) of curve fitting were
341 analyzed using @Risk to produce the most suitable function for describing the
342 distribution of ϵ . Fig. 2 shows that the residual errors (ϵ) follow a normal
343 distribution, with a mean at 0.03, and a standard deviation of 0.26 log CFU/g.
344 Overall, the majority (94.5%) of the residual errors are within ± 0.5 log CFU/g of the
345 observations. The distribution of residual errors shows that the model prediction
346 matched very well to the observations.

347

3.2 Validation of predictive models

In this study, a one-step approach was used to directly construct a tertiary model. After the kinetic parameters were determined, the models were validated using both dynamic and isothermal growth curves. Fig. 3 shows that the results of validation using two dynamic curves, and Fig. 4 shows the validation results using samples stored at 15 °C. In Profile 4 of Fig. 3, the temperature ranged from 4 to 35 °C, while the minimum and maximum temperatures in Profile 5 were 6 and 35 °C, respectively. According to Table 1, the minimum temperature of the bacterium is 8.91 °C. Since *Salmonella* are mesophilic, exposure to temperatures below $T_{\min}$ may cause gradual cell death. In both profiles shown in Fig. 3, the temperature frequently fluctuated below and above the minimum growth temperatures. Therefore, both growth and decline in the bacterial population were observed during storage in these two temperature profiles. The decline in the cell population in the samples exposed to the temperatures conditions below $T_{\min}$ was captured by the model, and was accurately predicted (Fig. 3). For the dynamic growth curves shown in Fig. 3 and the isothermal curve shown in Fig. 4, a total of 80 data points was used to validate the model. The RMSE of predictions was 0.52 log CFU/g. Overall, the distribution of residual errors also follows a normal distribution (Fig. 5). The mean and standard deviation of the residual errors was -0.29 log CFU/g and 0.45 log CFU/g, respectively. A majority (64.0%) of the residual errors was within ± 0.5 log CFU/g. The majority of the discrepancy between the predictions and observations is the located in growth curves in Fig. 3 for Profiles 4 and 5 where the bacterial growth was near or reached

the stationary phase. The discrepancy is not so large in Fig. 4. More experiments are needed to examine the cause for the discrepancy found in Profiles 4 and 5. However, since the bacterial growth is near or in the stationary phase, more accurate estimation is not really needed as the food is likely spoiled beyond the point for human consumption.

Any measurement in the laboratory has errors. This was true for all microbiological counts observed in this study. For microbiological testing, a commonly accepted rule that 95% of the observations are within ± 0.5 log units of the mean under normal conditions. That the RMSE of the predictions is 0.52 log CFU/g and 64.0% of the residual errors are with ± 0.5 log CFU/g suggests that the models developed in this study are not perfect, but are reasonably accurate. On average, the model predictions were 0.29 log CFU/g higher than the observations with a standard deviation of 0.45 log CFU/g, but they are well within the range of normal experimental errors.

Conclusion

This study was conducted to evaluate the feasibility of using a one-step dynamic analysis approach to directly construct a tertiary model to determine the growth and survival kinetics of SPA in DRMC from multiple growth curves obtained under different fluctuating temperature conditions. The results of analysis showed that this approach is effective and efficient in the growth kinetics of the bacterium, producing kinetic parameters that can be used to accurately predict the growth and

392 survival of SPA under both dynamic and isothermal conditions. The results of this
393 work may be used to predict the change in the population of SPA in DRMC in the
394 cold chain and during temperature abuse and conduct risk assessment of this
395 pathogen.

396

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403

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471 Table 1. Estimates of kinetic parameters for describing growth and survival of SPA
 472 in DRMC.

	a	T _{min} (°C)	Y _{max} (ln CFU/g)
Estimate	8.86e-2	8.91	20.4
p	1.90e-22	8.22e-23	2.24e-50
t	14.4	14.6	41.3
U95% confidence limit	10.1e-2	10.12	21.4
L95% confidence limit	7.64e-2	7.69	19.4
Standard error	6.16e-3	0.61	0.50
MSE* (log CFU/g) ²		0.070	
RMSE* (log CFU/g)		0.26	
Number of observations		72	
Number of parameters		3	
Degree of freedom		69	

473 * MSE: mean square error; RMSE: root mean square error.

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Figure 1. Modeling and analysis of growth of SPA in DRMC under three fluctuating temperature conditions. Legend: y , raw data; $\hat{y}$: predicted growth; T : temperature.

480 Figure 2. Distribution of residual errors (ϵ) of curve fitting. $\epsilon = y - \hat{y}$.

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Figure 3. Validation of the predictive model under dynamic conditions. Legend: y , raw data; $\hat{y}$ ($\hat{y}$), predicted growth, and T , temperature.

486 Figure 4. Validation of the predictive model under an isothermal condition.

487 Legend: y , raw data; $\hat{y}$ ($\hat{y}$), predicted growth, and T , temperature.

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490 Figure 5. Distribution of residual errors of validation. $\varepsilon = y - \hat{y}$.

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