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Response Surface Models for Effects of Temperature and Previous Temperature on Lag Time and Specific Growth Rate of *Salmonella* Typhimurium on Cooked Ground Chicken Breast†

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ABSTRACT

Response surface models were developed for effects of temperature (16 to 34°C) and previous temperature (pretemperature; 16 to 34°C) on lag time (λ) and specific growth rate (μ) of *Salmonella* Typhimurium on cooked ground chicken breast. The primary objective was to determine whether pretemperature is a major factor affecting growth of *Salmonella* Typhimurium. Growth curves for model development (n = 32) and model testing (n = 18) were fit to a two-phase linear equation that directly estimated λ and μ. Response surface models for ln λ and ln μ as a function of temperature and pretemperature were obtained by regression analysis. Lag time and μ of *Salmonella* Typhimurium were affected by temperature but not pretemperature. Models were tested against data not used in their development. Prediction error (model accuracy) was 13.4% for λ and 11.3% for μ, whereas the median relative error of predictions (model bias) was −3.0% for λ and 6.8% for μ. Results indicated that the models provide reliable predictions of λ and μ of *Salmonella* Typhimurium on cooked ground chicken breast within the matrix of conditions modeled. In addition, pretemperature (16 to 34°C) is not a major factor affecting growth of *Salmonella* Typhimurium.

Predictive models for growth of human pathogens are a valuable tool for assessing the microbiological safety of temperature-abused food *(3, 12)*. Current models for *Salmonella* spp. predict growth as a function of temperature, pH, and water activity *(1, 2, 7, 11)*. However, other factors, such as previous growth conditions, may also be important for predicting growth of *Salmonella* spp. during temperature abuse. In fact, previous growth temperature has been shown to alter lag time (λ) but not growth of *Aeromonas hydrophila (8)* and *Listeria monocytogenes (4)*. In the present study, response surface models were developed for effects of temperature (16 to 34°C) and previous temperature (pretemperature; 16 to 34°C) on λ and specific growth rate (μ) of *Salmonella* Typhimurium on cooked ground chicken breast. The primary objective was to determine whether previous growth temperature is an important factor affecting growth of *Salmonella* Typhimurium.

MATERIALS AND METHODS

Stock and starter cultures. Stock cultures of a single strain (ATCC 14028) of *Salmonella* Typhimurium (American Type Culture Collection, Rockville, Md.) were maintained at −20°C in brain heart infusion broth that contained 15% glycerol. Starter cultures were grown to the stationary phase in 5 ml of brain heart infusion broth, pH 6.4, in 25-ml Erlenmeyer flasks sealed with foam plugs and shaken at 150 rpm. The initial level of *Salmonella* Typhimurium was 1.6×10^3 per ml. Starter cultures were incubated for 71 h at 16 and 19°C, 47 h at 22 and 25°C, and 23 h at 28, 31, and 34°C.

Experimental designs. The experiment for model development was a full 4-by-4 factorial arrangement of temperature (16, 22, 28, and 34°C) and pretemperature (16, 22, 28, and 34°C) and was conducted in duplicate, giving a total of 32 growth curves. The experiment for model testing was a full 3-by-3 factorial arrangement of temperature (19, 25, and 31°C) and pretemperature (19, 25, and 31°C) and was conducted in duplicate, giving a total of 18 growth curves.

Preparation and inoculation of cooked ground chicken breast. Chicken breast was obtained from a local supermarket and was ground twice through a $^3/_{16}$-in. plate of a hand-powered meat grinder. Ten grams of ground chicken was formed into a circular patty by finger-kneading and then flattened with a 100-ml beaker. An indentation (1.2 cm^2) was made in the center of the chicken patty with a dilution tube cap to serve as an inoculation well. Eight 10-g patties were made for each growth curve. Background microflora were removed by autoclaving (15 min at 121°C). After cooling, cooked ground chicken breasts were transferred under sterile conditions to petri dishes and stored at 4°C in plastic bags until used (usually within 4 days).

Cooked ground chicken breasts were incubated for 16 h at the proper temperature (16 to 34°C) before inoculation. A sterile repeater pipette was used to inoculate the surface of the cooked ground chicken breast with 100 μl of sterile, distilled water that contained 1.6×10^5 *Salmonella* Typhimurium from the appropriate starter culture. Incubations were conducted in plastic bags to prevent drying of the chicken breast.

Determination of viable counts. At selected times after inoculation, cooked ground chicken breasts (6 g after autoclaving)

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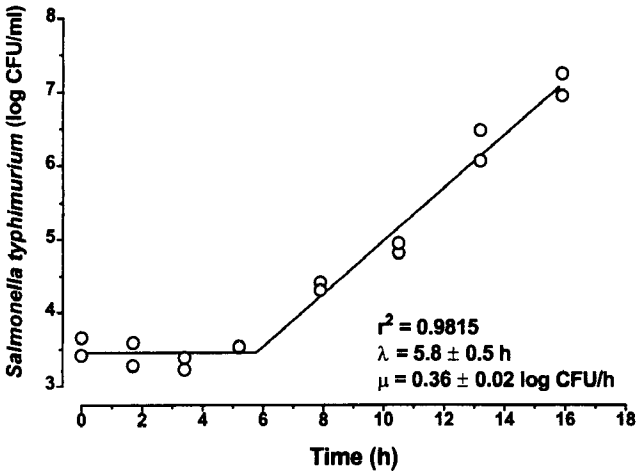

FIGURE 1. *Typical growth curve for* Salmonella *Typhimurium on cooked ground chicken breast at 22°C and a pretemperature of 34°C.*

were homogenized (model 400 Stomacher, Seward, London, UK) for 2 min in 94 ml of sterile, distilled water. Undiluted and diluted samples of homogenate were spiral plated (Whitley automatic spiral plater, Don Whitley Scientific Ltd., West Yorkshire, UK) onto brain heart infusion agar and then incubated at 30°C for 24 h before automated colony counting (Protos colony counter, Synoptics, Cambridge, UK).

Curve-fitting. Growth curves were iteratively fit (PRIZM, GraphPad Software, San Diego, Calif.) to a two-phase linear equation *(5)*:

$$Y = Y_0 + \mathrm{IF}[t \leq \lambda, 0, \mu \times (t - \lambda)]$$

where Y = viable cell count at sampling time t, Y_0 = initial viable cell count, λ = lag time (h), and μ = specific growth rate (log CFU/h).

Response surface modeling. A data set containing model variables and ln λ and ln μ from 32 curve fits was created and subjected to regression analysis (Statistical Analysis System, Cary, N.C.) using the following response surface model:

$$\ln \lambda \text{ or } \ln \mu = b_0 + b_1A + b_2B + b_3AB + b_4A^2 + b_5B^2 + \epsilon$$

where A = temperature, B = pretemperature, b_0 to b_5 = regression coefficients, and ϵ = random error.

Model testing. Models were tested against data not used in their development. The relative error (RE) of each prediction case was determined using the following equation *(6)*:

$$\mathrm{RE} = \frac{(X_p - X_o)}{X_o}$$

where X_p = predicted λ or μ and X_o = observed λ or μ. The prediction error (PE) of each model and data set combination was calculated using the following equation *(6)*:

$$\mathrm{PE} = \frac{1}{n} \times \sum_{i=1}^{n} |\mathrm{RE}|_i$$

RESULTS AND DISCUSSION

Viable count data for growth of *Salmonella* Typhimurium on cooked ground chicken breast over time at different temperatures and pretemperatures were fit to a two-phase linear equation that directly estimated λ and μ (Fig. 1). The mean $r^2 \pm$ SEM for growth curve fits was 0.981 ± 0.002 for data used in model development (n = 32) and 0.986 ± 0.003 for data used in model testing (n = 18). A mean r^2 ± SEM of 0.992 ± 0.001 for growth curve fits using the two-phase linear equation was obtained for the same strain of *Salmonella* Typhimurium in brain heart infusion broth *(9)*. Thus, the two-phase linear equation provides a high degree of goodness-of-fit to *Salmonella* Typhimurium growth data on cooked ground chicken breast and in brain heart infusion broth.

A data set containing model variables, ln λ, and ln μ from 32 curve fits was created and subjected to regression analysis to yield response surface models (Table 1). Lag time and μ were log transformed to stabilize model variance *(7)*. Both models had high r^2 values, indicating a high degree of goodness-of-fit to the data. Regression analysis indicated that temperature had a large effect on λ and μ of *Salmonella* Typhimurium, whereas pretemperature did not alter λ and μ of *Salmonella* Typhimurium. As expected, λ decreased and μ increased as temperature increased from 16 to 34°C.

Pretemperature affects λ but not maximum population density, μ, or generation time (τ) of *L. monocytogenes (4)*. Lag time of *L. monocytogenes* at 5°C increases from 37 h with a pretemperature of 5 to 28°C to 50 h with a pretemperature of 37 to 42°C. Likewise, pretemperature alters λ but not τ of *A. hydrophila (8)*. Lag time of *A. hydrophila* at 5°C increased from 12 to 354 h (28-fold) for an ATCC strain and from 4.5 to 18 h for a food strain as pretemperature increased from 5 to 35°C. These studies indicate that

TABLE 1. *Response surface models for effects of previous temperature (pT) and temperature (T) on lag time (λ) and specific growth rate (μ) of* Salmonella *Typhimurium on cooked ground chicken breast*

	ln λ (h)			ln μ (log CFU/h)		
Parameter	Estimate	F	P	Estimate	F	P
Intercept	4.0615			−6.5016		
pT	0.0092	0.03	0.8662	0.0119	0.17	0.6862
T	−0.1640	9.12	0.0056	0.3308	128.91	0.0001
pT × T	−0.0009	1.38	0.2508	−0.0009	0.04	0.8406
pT × pT	0.0004	0.18	0.6765	−0.0001	0.04	0.8481
T × T	0.0018	3.39	0.0772	−0.0046	73.62	0.0001
r^2	0.9247			0.9791		

TABLE 2. *Relative error and prediction error for the lag time (λ) and specific growth rate (μ) models and data used in model development and data used in model testing for growth of* Salmonella *Typhimurium on cooked ground chicken breast*

Model	Data	Cases	Prediction error ± SEM (%)	Relative error (%)		
				Median	Minimum	Maximum
λ	Model development	32	14.7 ± 2.1	0.9	−34.9	44.0
	Model testing	18	13.4 ± 1.8	−3.0	−30.2	19.4
μ	Model development	32	7.3 ± 1.3	0.3	−17.7	24.8
	Model testing	18	11.3 ± 2.4	6.8	−5.8	33.8

shifting human pathogens from high to low temperatures increases λ.

In agreement with findings of previous studies *(4, 8)*, the results of this study confirm that pretemperature does not alter μ of human food pathogens. In contrast to findings of previous studies *(4, 8)*, results of this study indicate that pretemperature does not alter λ of *Salmonella* Typhimurium. Differences in the type and strain of pathogen, temperature range studied, and other experimental protocols likely contribute to these divergent findings.

Testing the ability of response surface models to interpolate is a critical step in their development but one that is rarely done in modeling experiments *(9)*. Proper testing of response surface model predictions should be against new data obtained using the same strain(s) and protocol as used during model development so that test results are not confounded. Furthermore, an analysis method that quantifies the accuracy and bias of model predictions should be used *(6, 10)* so that the reliability of model predictions can be objectively assessed and compared between studies.

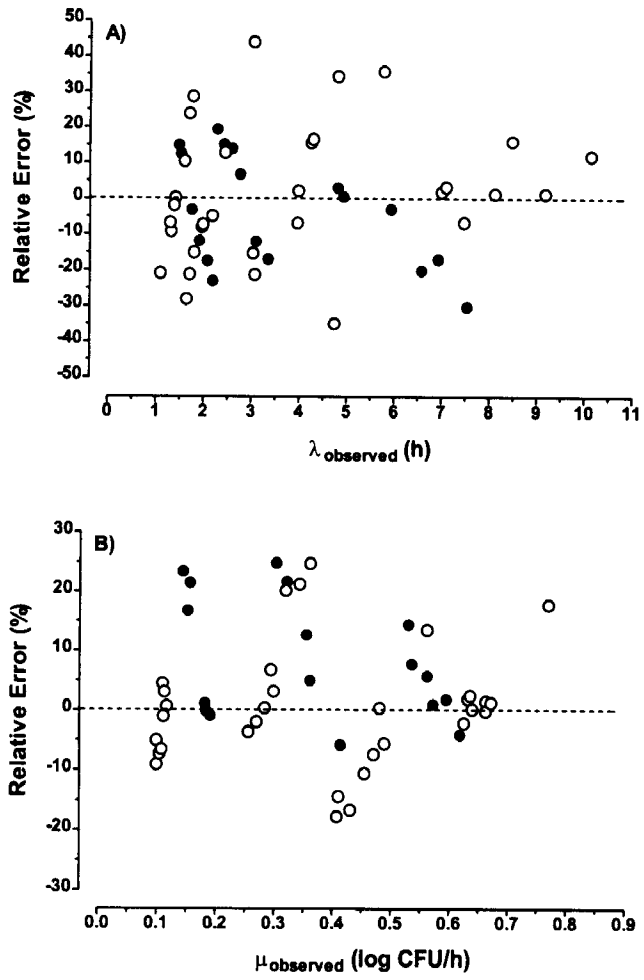

FIGURE 2. *Scatter plot of relative errors of (A) lag time (λ) and (B) specific growth rate (μ) prediction cases for data used in model development (○) and data used in model testing (●).*

In the current study, the ability of our models to predict growth of *Salmonella* Typhimurium was tested using the aforementioned criteria. Lag time and μ from growth curves (n = 18) measured at intermediate levels of temperature and pretemperature were obtained and used to calculate prediction error (model accuracy) and median relative error of predictions (model bias) for each response surface model. For comparison, prediction accuracy and bias were also calculated for data used in model development. Prediction errors of λ and μ models were low and were similar for data used in model development and new data (Table 2). In general, the models did not demonstrate prediction bias, which was indicated by the random distribution of relative errors around 0% (Fig. 2) and closeness of median relative error to 0% (Table 2). The one exception involved the μ model and new data. Here, the μ model predicted slightly faster μ than were observed; thus, its predictions were fail-safe.

Delignette-Muller et al. *(6)* pooled 468 prediction cases from seven response surface modeling papers and calculated prediction errors of 40.3% for λ and 36.2% for τ. Oscar *(9)* calculated prediction errors for 16 models representing 823 prediction cases in nine response surface modeling papers. Prediction error ranged from 18.5 to 74.8%. Thus, the prediction errors of models in this study were lower than those of published models. The one exception is models for λ and μ of *Salmonella* Typhimurium in brain heart infusion broth as a function of temperature, pH, and pre-pH, for which prediction error was 9.2% for λ and 9.1% for μ *(9)*. Nonetheless, the low prediction error of models in this study and the absence of significant prediction bias indicate that the models provide reliable predictions of λ and μ of *Salmonella* Typhimurium on cooked ground chicken breast within the matrix of variables modeled.

Development of response surface models requires extensive kinetic data on microbial growth in response to multiple combinations of environmental and food formulation factors. Collection of such data in food is labor intensive, making it difficult to obtain sufficient data for development of an accurate predictive model *(7)*. Consequently, predic-

tive models are routinely developed using kinetic data collected in broth culture. In the present study, we developed response surface models for growth of *Salmonella* Typhimurium as a function of temperature and pretemperature using cooked ground chicken breast as the growth medium. We demonstrated that it is possible to develop accurate predictive models using food. A key to our success was the use of a two-phase linear equation to fit microbial growth data. Use of this equation reduced by 50% the amount of kinetic data needed to obtain accurate estimates of λ and μ when compared with traditional predictive modeling studies that use the Gompertz equation. Thus, adoption of the two-phase linear equation for microbial modeling studies will facilitate use of food as the growth medium.

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