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Mathematical Models to Describe the Kinetic Behavior of *Staphylococcus aureus* in Potato and Sweet Potato Salad at Constant and Dynamic Temperatures

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In this study, we developed mathematical models to describe the kinetic behavior of *Staphylococcus aureus*. *S. aureus* was inoculated into potato and sweet potato salads, which were then stored at 10–30°C. The Baranyi model was fitted to the *S. aureus* growth data to calculate the lag phase duration, maximum growth rate, and upper and lower asymptotes. The square root model and polynomial equation were then used to evaluate the effects of storage temperature on *S. aureus* growth parameters. Subsequently, dynamic models were developed under dynamic temperatures. To evaluate the performance of the model, relative error (*RE*) were calculated. Bacterial growth was observed at both constant and dynamic temperatures, and the R^2 of the primary and secondary models were 0.959–0.995 and 0.789–0.976, respectively. In addition, the developed models were determined to be appropriate with -0.0108 – 0.0053 of *RE*. These results suggest that the model is useful for describing the kinetic behavior of *S. aureus* in potato and sweet potato salads under constant and dynamic temperatures.

Key words: *Staphylococcus aureus*, ready-to-eat salad, predictive model, dynamic model.

Various types of ready-to-eat (RTE) foods have been developed in response to consumer preference, and the foodborne outbreaks associated with these foods have increased[9]. Microbial risk assessments for RTE foods have therefore been conducted for *Staphylococcus aureus*, because 20–30% of the global population is infected with *S. aureus*, which can be transferred to foods during handling[9, 23]. In recent years, the consumption of RTE foods, particularly salads, has increased continuously. In 2009, approximately 400 people consumed potato salad contaminated with *S. aureus* in Switzerland, and 30 suffered from acute vomiting and diarrhea due to staphylococcal enterotoxins[22].

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The presence of *S. aureus* in food is an indicator of insufficient hygiene. *S. aureus* is a common cause of foodborne illnesses and acts by producing enterotoxin[7,19]. Staphylococcal enterotoxins A, B, C, D, E, and F are produced in the late exponential and stationary phases of *S. aureus* growth[5,20].

Mathematical models have been used to describe the kinetic behavior of pathogenic bacteria under varying conditions of temperature, pH, and water activity, and can therefore be used to evaluate microbiological food safety and the shelf life of foods[26]. Primary models describe the changes in microbial populations over time under constant environmental conditions and can be used to calculate kinetic parameters such as maximum growth rate and lag phase duration[1,12,25]. Secondary models, including the polynomial and square root model, describe the effects of intrinsic or extrinsic factors on kinetic parameters[24].

Temperature is one of the most critical factors for bacterial growth in foods during processing, distribution, and storage[15]. Although 20% of domestic and commercial refrigerators operate above 10°C in the U.S[8], no studies have been carried out to evaluate the impact of temperature abuse during food distribution and storage on microbial safety.

The objective of this study, therefore, was to develop mathematical models to describe the kinetic behavior of *S. aureus* in potato and sweet potato salads at constant and dynamic temperatures.

MATERIALS AND METHODS

Preparation of inoculum

Five *S. aureus* strains KACC10768, KACC10778, KACC11596, KACC13236, and NCCP10862 were cultured in brain heart infusion (BHI) broth (Difco™, Becton Dickinson and Company, Sparks, MD, USA) at 35°C for 24 h. Each culture (0.1 ml) was then subcultured in 10 ml BHI at 35°C for 24 h. The subcultures of the five strains were mixed and centrifuged at 1,912 × *g* at 4°C for 15 min. The supernatants were discarded, and cell pellets were washed twice with phosphate buffered saline (PBS, pH 7.4; 0.2 g KH_2PO_4, 1.5 g $Na_2HPO_4 \cdot 7H_2O$, 8.0 g NaCl, and 0.2 g KCl in 1 L distilled water) and serially diluted in PBS to prepare the inoculum at 6-7 log CFU/ml.

Sample preparation and inoculation

Commercial potato (55.5% potato, 0.15% sodium) and sweet potato (60% sweet potato, 0.22% sodium) salads were purchased from a local supermarket, and 5 g portions were transferred into Whir-pak® bags (Nasco, Modesto, CA, USA). One hundred microliters of inoculum was added to the samples of potato and sweet potato salad at 5 log CFU/g, and the samples were thoroughly massaged. Samples (n = 4) were then stored at 10 and 15°C for 216 h, 20°C for 120 h, and 25 and 30°C for 48 h.

Microbial analyses

To enumerate *S. aureus* populations, the samples were analyzed at appropriate time intervals. Ten milliliters of 0.1% buffered peptone water (BPW, Difco™) was added to each sample. The samples were then homogenized using a pummeler (BagMixer®, Interscience, St. Nom, France) for 2 min. The homogenate was serially diluted with BPW, and 0.1 ml diluent was spread-plated on to mannitol salt agar (Difco™). Plates were incubated at 35°C for 24 h, and the colonies were manually counted.

Measurements of pH and a_w

The pH of the homogenates were measured using a digital pH meter (Accument®, Denver Instruments, Arvada, CO, USA) after microbial analysis. The a_w values of the potato and sweet potato salad were measured using a water activity meter (Aquaspector®, NAGY Messsystem GmbH, Germany).

Model development

The Baranyi model *(1)* was fitted to the *S. aureus* growth data, using DMFit (In house program at Institute of Food Research, Norwich, UK), which is an MS Excel add-in used to fit sigmoidal curves and calculate the maximum growth rate (μ_{max}; Log CFU/g/h), lag phase duration (*LPD*; h), lower asymptote (N_0; Log CFU/g), and upper asymptote (N_{max}; Log CFU/g). The Baranyi model is:

$$N_t = N_0 + \mu_{max} \times A_t - \ln[1 + \frac{\exp(\mu_{max} \times A_t) - 1}{\exp(N_{max} - N_0)}] \quad ...(1)$$

Where N_t (log CFU/g) is the bacterial cell counts at any time *t*, and A_t is an adjustment function described by Baranyi and Roberts *(1)*.

$$A_t = t + \frac{1}{\mu_{max}} \ln\left(\frac{e^{-\mu_{max} \times t} + q_0}{1 + q_0}\right) \quad ...(2)$$

To evaluate the effects of storage temperature on growth parameters, secondary models were developed as follows:

$$\sqrt{\mu}_{max} = a_\mu (T - T_{min}) \quad ...(3)$$

Where a_μ is the slope of the regression line for μ_{max}, and T_{min} is the theoretical minimum temperature (°C) of growth for *S. aureus*. A polynomial equation was fitted to the *LPD* values as follows:

$$\sqrt{\frac{1}{LPD}} = a_0 + a_1 T + a_2 T^2 \quad ...(4)$$

Where a_1 (where "i" represents any number from 0 to 2) are coefficients, and *T* is the temperature (°C). The secondary models from Eq. 3 and Eq. 4 were solved with MS Excel.

To predict the growth of *S. aureus* in potato and sweet potato salads at changing air temperatures, the mathematical model defined by Baranyi and Roberts *(1)* was used in conjunction

with Eq. 3.

$$\frac{dN(t)}{dt} = \left[\frac{Q(t)}{1+Q(t)}\right] \times \mu_{max} \times \left[1 - \frac{N(t)}{N_{max}}\right] \times N(t) \quad ...(5)$$

$$\frac{dQ(t)}{dt} = \mu_{max} \times Q(t)$$

Where *t* is time, *N* is *S. aureus* cell concentration at time *t*; N_{max} is the maximum cell counts and the parameter *Q* denotes the concentration of a substance critical to growth. The dynamic model includes adjustment function which describes the gradual adaptation of the bacterial population to gain μ_{max} and inhibition function, which causes μ_{max} to decrease asymptotically to 0 (*2*). Changing temperatures were monitored using an electronic temperature recorder (CEM®DT-172, Shenzhen, China).

Validation

The performance of the developed models was evaluated using observed bacterial cell counts (20 and 27°C) for constant storage temperature, obtained from an independent study in our laboratory, because no published data describing the growth of *S. aureus* in potato and sweet potato salads were available. To calculate predicted *S. aureus* cell counts in the salads, parameters were calculated by substituting 20 and 27°C as *T* in the secondary models. The parameters were then used in the primary model to calculate predicted *S. aureus* cell counts at 20 and 27°C. The predicted and observed bacterial cell counts were compared and the relative error (*RE* = predicted bacterial cell count – observed bacterial cell count/predicted cell count) was calculated.

RESULTS AND DISCUSSION

The pH of the potato and sweet potato salads were 5.47–5.51 and 5.81–5.94, and the a_w values were 0.989 and 0.994, respectively, regardless of storage temperature (data not shown). The optimum growth of *S. aureus* growth occurs at a pH 6–7, and a_w of 0.980 *(21)*. The a_w values of both salads were therefore similar to the optimum a_w for *S. aureus* growth, but the pH values were lower. However, the pH values were higher in sweet potato than potato salad, which explains why sweet potato salad had more *S. aureus* growth compared to potato salad (Table 1).

There was no growth of *S. aureus* at 10°C (data not shown). However, the cell counts of *S. aureus* in both salads increased during storage at 15–30°C (Figure 1). Based on this result, the storage of potato and sweet potato salads below 10°C may prevent the growth of *S. aureus*. The effects of storage temperature on the growth of *S. aureus* are an important consideration during the assessment of food safety because 20% of domestic and commercial refrigerators operate above 10°C in the U.S. *(8)*. As storage temperatures increased, the μ_{max} values increased, but *LPD* values decreased (Table 1). The growth curves based on the Baranyi model passed through most data points (Figure 1), and a high degree of goodness of fits (potato salad; R^2 = 0.959–0.995, sweet potato salad; R^2 = 0.973–0.993) was observed (Table 1). These results suggest that the developed primary models were effective.

To evaluate the effects of storage temperature on growth parameters, the polynomial

Table 1. Growth parameters (mean ± standard deviation) of *Staphylococcus aureus* growth in potato and sweet potato salad calculated by the Baranyi model[1]

Salad	Temp (°C)	*LPD* (h)	μ_{max} (Log CFU/g/h)	N_0 (Log CFU/g)	N_{max} (Log CFU/g)	R^2
Potato salad	15	43.99±11.22	0.01±0.00	5.4±0.18	7.0±0.19	0.959-0.972
	20	9.16±2.17	0.08±0.04	5.2±0.00	8.7±0.18	0.984-0.995
	25	4.33±3.14	0.12±0.01	5.3±0.18	8.9±0.00	0.981-0.988
	30	7.26±3.82	0.21±0.01	5.4±0.36	9.1±0.13	0.984-0.986
Sweetpotato salad	15	36.92±12.39	0.03±0.00	5.3±0.08	8.2±0.37	0.973-0.984
	20	4.92±3.69	0.08±0.00	5.3±0.04	9.1±0.16	0.988-0.991
	25	6.05±0.00	0.16±0.01	5.3±0.01	9.0±0.03	0.976-0.993
	30	2.90±0.00	0.26±0.08	5.2±0.11	9.2±0.05	0.975-0.986

Temp: storage temperature *LPD*: lag phage duration μ_{max}: maximum growth rate

equation (*LPD*) and the square root model (μ_{max}) were used as secondary models. Since polynomial models are comparatively easy to describe experimental data, and allow any environmental parameters[18], transformed *LPD* values to $\sqrt{\frac{1}{LPD}}$ were fitted with a quadratic polynomial model. To describe the effect of suboptimal temperature on μ_{max}, the classical Arrhenius equation is inappropriate because the activation energy itself is temperature dependent[18]. Thus, Ratkowsky *et al.*[17] suggested a simple empirical model (Eq. 2) to stabilize their variance and by square-root transformation.

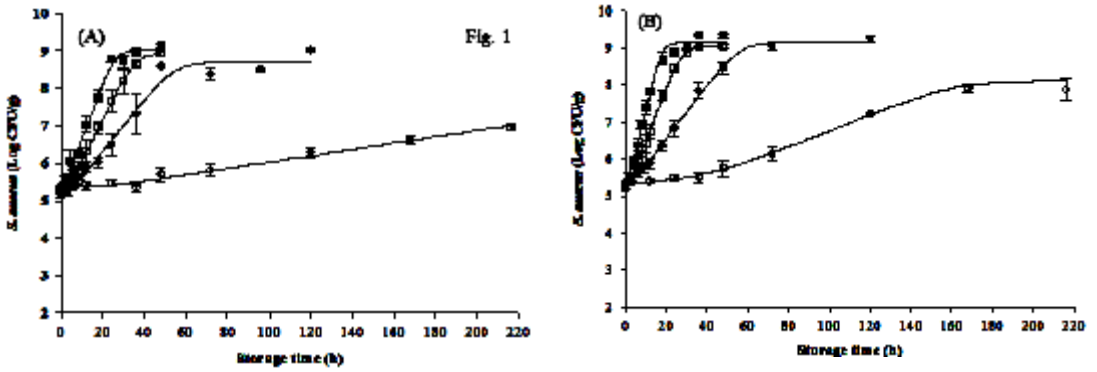

Fig. 1. Bacterial cell counts of *Staphylococcus aureus* in potato salad (A) and sweet potato salad (B) during storage, fitted with the Baranyi model; ○: 15°C, ●: 20°C, □: 25°C, ■30°C

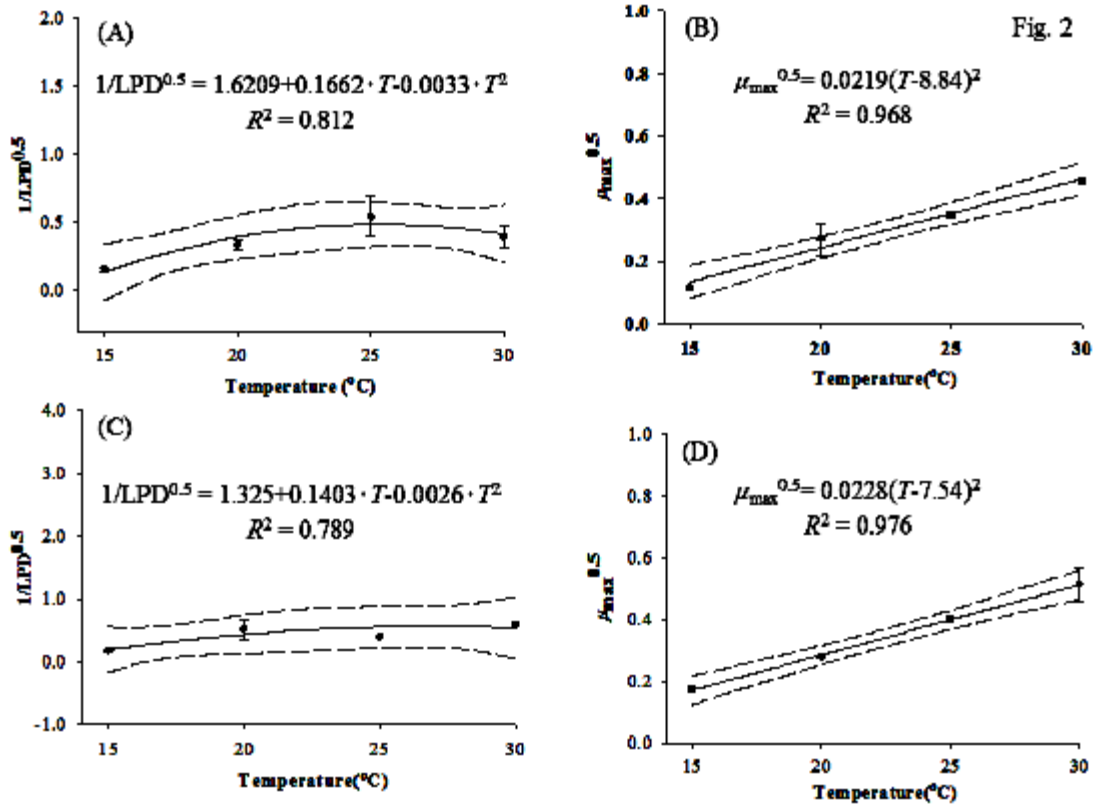

Fig. 2. Secondary modeling the *LPD* and μ_{max} of *Staphylococcus aureus* in potato salad (A and B) and sweet potato salad (C and D); ●: observed value, —: fitted line, - - -: 95% confidence interval

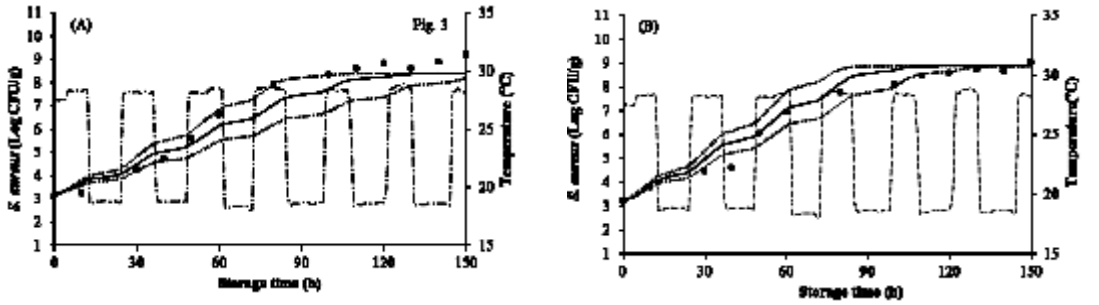

Fig. 3. Predicted *Staphylococcus aureus* growth in potato salad (A) and sweet potato salad (B) under dynamic temperature condition; ●: observed bacterial cell counts, —: predicted bacterial cell counts, - - -: 95% confidence interval, - •-•-: temperature.

The *LPD* and μ_{max} values were proportional to storage temperature (Figure 2). The R^2 values for the *LPD* and μ_{max} secondary models in potato salad were 0.812 and 0.968, respectively. For the sweet potato salad, R^2 values were 0.789 and 0.976 (Figure 2). Although the R^2 values of *LPD* for both potato and sweet potato salads were slightly low, Figure 2 shows that the fitted curves passed through most data points, indicating that the secondary models were effective. The T_{min} values were 8.84°C and 7.54°C for potato and sweet potato salad, respectively (Figures 2B and 2D), which were similar to the T_{min} values of growth media (5.46–8.7°C), but slightly higher than those of milk (5.82°C), and *Carbonara* sauce (5.2°C) (*4, 10, 11*). These data suggest that the food matrix may affect the physiological responses of *S. aureus*[3,16].

Under conditions of dynamic temperature, the growth of *S. aureus* in potato and sweet potato salad was simulated with the h_0 (μ_{max} × *LPD*) of 0.8 (potato salad) and 0.7 (sweet potato salad), indicating the physiological status[6]. This different h_0 values between two salads may be caused by different intrinsic factors such pH and a_w because the factors significantly affect μ_{max} and *LPD*. Observed *S. aureus* cell counts and the line of predicted *S. aureus* were plotted for validation, and most data were close to the predicted line (Figure 3).

In a study by Oscar[14], a limitation of bias (*B*) and accuracy (*A*) factors for model performance evaluation is that criteria for acceptable *B* and *A* factors are not consistent. Hence, the performance of the model at constant storage temperatures was evaluated by calculating *RE*, and *RE* values were 0.0053 (20°C) and -0.0108 (27°C) for potato salad, and -0.0081 (20°C) and -0.0030 (27°C) for sweet potato salad. An *RE* value of less than 0 indicates a 'fail-safe' prediction and greater than 0 indicates a 'fail-dangerous' prediction, and the *RE* range of -0.6 to 0.3 is considered appropriate[13].

In conclusion, the developed mathematical models may be useful in describing the kinetic behavior of *S. aureus* in potato and sweet potato salads under constant and dynamic temperature conditions in the cases of storage temperature abuse and for exposure assessment.

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