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Analysis of Temperature Effects on Microbial Growth Parameters and Estimation of Food Shelf Life with Confidence Band

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Abstract

As a way to account for the variability of the primary model parameters in the secondary modeling of microbial growth, three different regression approaches were compared in determining the confidence interval of the temperature-dependent primary model parameters and the estimated microbial growth during storage: bootstrapped regression with all the individual primary model parameter values; bootstrapped regression with average values at each temperature; and simple regression with regression lines of 2.5% and 97.5% percentile values. Temperature dependences of converted parameters (log q_o, $\mu_{max}^{1/2}$, log N_{max}) of hypothetical initial physiological state, maximum specific growth rate, and maximum cell density in Baranyi's model were subjected to the regression by quadratic, linear, and linear function, respectively. With an advantage of extracting the primary model parameters instantaneously at any temperature by using mathematical functions, regression lines of 2.5% and 97.5% percentile values were capable of accounting for variation in experimental data of microbial growth under constant and fluctuating temperature conditions.

Key words: microbial spoilage model, confidence band, secondary model, regression, temperature dependence

INTRODUCTION

Controlling the microbial load of refrigerated perishable foods is of prime importance for assuring safety and quality. Shelf life monitoring and control based on microbial criteria are essential for the assured quality and safety of the products. However, determination and control of shelf life based on the direct measurement of microbial count is very difficult because it is time consuming and requires expertise not readily available, and thus has limited practical applications. Even though there have been attempts to measure the microbial quality by indirect rapid detection and analysis of physical and chemical indexes, they are still in infancy, not allowing their introduction to shelf life control (1).

On the other hand, prediction of microbial quality under different conditions has emerged as a useful technique for estimating efficacy of microbial controls and for predicting shelf life (2,3). The microbial growth kinetics applicable to food shelf life management should have the capacity to estimate the microbial number of relevant spoilage organisms over time under a variety of environmental conditions frequently met during food distribution and storage. The microbial growth model, both the primary and secondary model, is adopted to satisfy the purpose and requirements (2,3): primary model refers to the mathematical function describing the microbial quality index as a function of time, and its parameters defined for the designated or relevant conditions represent the rate and degree of microbial growth or spoilage. The secondary model incorporates the effect of environmental and compositional factors (e.g. temperature, water activity, and pH) on the parameters of the primary model. Temperature is the most important independent variable to be considered in microbial growth modeling for shelf life management. Rigorous models of predicted microbiology have been proposed to handle dynamic storage conditions, particularly during fluctuating temperature conditions (4-11).

Because there are uncertainty and variation in microbial growth data used for modeling, the estimations derived from the model should have variability and needs to be provided with confidence intervals for the parameters and microbial growth levels (12,13). The nature of variability is dealt with in the distribution of model parameters, which are obtained by regression or curve fitting procedures. In order to deal with the variability problem, randomization techniques such as the Monte Carlo method and bootstrap are often applied (11,14,15). The stochastic analysis of microbial growth modeling has been conducted at a single constant temperature

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successfully by the Monte Carlo method to predict the microbial counts at any storage time (16). Analysis of temperature effects on the primary model parameters in the secondary model requires another step of regression which also generates uncertainty and variation in its parameters. Therefore, the prediction of microbial growth under different temperature or dynamic storage conditions achieved by two stage modeling has difficulty in dealing with variability and distribution of the model parameters. Even though some prediction models have the capacity to give the confidence band of prediction under several different temperatures, they do not consider the variability of the primary model parameters obtained from experimental data which have variability and uncertainty by nature (6,13,17). Almonacid-Merino et al. (18) obtained the Arrhenius equation parameters from non-isothermal experiments using the bootstrap regression technique, but did not try to estimate the band of estimated microbial growth. To our knowledge, there have been no intensive treatments of the confidence interval of the predicted microbial growth obtained through two stages of primary and secondary modeling.

This study therefore aims to compare different approaches of analyzing temperature effects on the quality of the predicted band of microbial growth and find an appropriate one for practical applications. Fluctuating temperature conditions were tested to validate the proposed method.

MATERIALS AND METHODS

Microbial growth data and primary model parameters

The total aerobic bacterial counts and their primary model parameter distribution reported by Lee et al. (11) for four different temperatures (0, 5, 10, and 15°C) were used for this study (Table 1, Fig. 1). As discussed by Lee et al. (11), total aerobic bacterial growth was assumed to be useful as a general criterion for practical shelf life determination, but a similar approach can be applied to growth of specific strains of spoilage bacteria on defined media which have been used as criteria in many shelf life studies (1). One thousand parameter sets of Baranyi and Roberts (19) microbial growth model (Equations 1 and 2) determined from bootstrapped mean counts at each temperature were used to analyze the temperature effect in three different methods described below.

$$\frac{dq}{dt} = \mu_{max} q \tag{1}$$

$$\frac{dN}{dt} = \mu_{max} \left(\frac{q}{1+q}\right)\left(1 - \frac{N}{N_{max}}\right)N \tag{2}$$

where q is the normalized concentration of an unknown substance critically needed for cell growth and represents the physiological state of the cell population, μ_{max} is the maximum specific growth rate (1/day), N is the microbial count in cfu/g at time t, and N_{max} is the maximum cell density in cfu/g. The microbial growth model of Equations 1 and 2 has two explicit parameters of μ_{max} and N_{max} and two implicit parameters of q_o and N_o representing q and N at the initial time, respectively.

Secondary model parameter estimation

Mathematical functions used by Lee et al. (11) were adopted for the analysis of temperature effect on the primary model parameters (q_o, N_o, μ_{max}, and N_{max}); log q_o was described by quadratic Equation 3, log N_o was indifferent with regard to temperature, μ_{max} was given in a square root model (Equation 4), and log N_{max} was expressed by a simple linear Equation 5 (Fig. 1):

$$\log q_o = a_o + a_1 T + a_2 T^2 \tag{3}$$

$$\sqrt{\mu_{max}} = b_o + b_1 T \tag{4}$$

$$\log N_{max} = c_o + c_1 T \tag{5}$$

where a_o-a_2, b_o-b_1, and c_o-c_1 are coefficients of the respective equations and T is temperature (°C).

Different regression techniques were applied to obtain the coefficients of Equations 3～5 and their corresponding variable distributions in the temperature range of 0～15°C. First, the bootstrapping regression method with randomized resampling of residuals (20) was adopted for all the primary model parameter values at four temper-

Table 1. Primary model parameters for aerobic bacterial growth on seasoned soybean sprouts (11)

Temperature (°C)	Parameters of Baranyi's model (Eqs. 1～2)			
	log q_o	log N_o	μ_{max}	log N_{max}
0	-4.014 (-5.357, -3.135)	4.249 (4.173, 4.328)	2.383 (1.996, 3.031)	7.703 (7.659, 7.754)
5	-1.344 (-1.598, -1.018)	4.245 (4.145, 4.316)	2.955 (2.785, 3.110)	8.719 (8.653, 8.786)
10	4.414 (1.351, 9.960)	4.118 (4.082, 4.153)	4.061 (3.974, 4.142)	9.695 (9.655, 9.732)
15	18.60 (13.05, 23.11)	4.235 (4.151, 4.444)	5.967 (4.982, 6.201)	10.25 (10.15, 10.40)

Values are average with 95% bootstrap confidence interval in bracket.

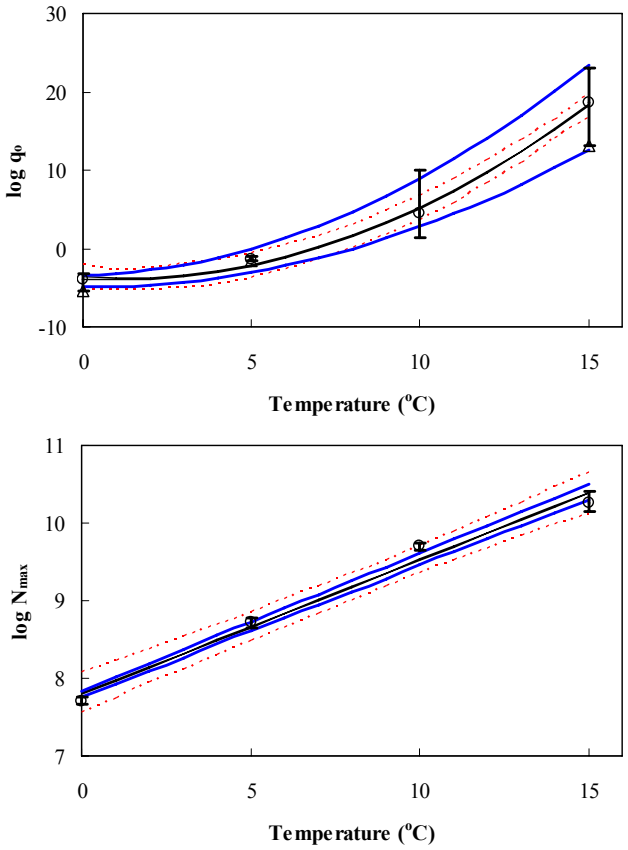

Fig. 1. The primary model parameters of aerobic bacterial growth on seasoned soybean sprouts as a function of temperature with respective 95% confidence intervals. ○: average value; ——: 95% confidence interval from bootstrapped regression using all the individual parameter values (Method 1); ----: 95% confidence interval from bootstrapped regression using average parameter value at each temperature (Method 2); ——: regression line of 2.5% and 97.5% percentile values (Method 3). Vertical bars are 95% bootstrap confidence intervals of the original primary model parameters at each temperature.

atures (1000 values for each temperature) (Method 1). At the first stage of the calculation, the best-fit coefficient estimates were obtained by the least squares method. And the modified residuals were calculated for the n parameter values (n=4000) using the following equation:

$$r_i = \frac{y_i - \hat{y}_i}{\sqrt{1-h_i}} \tag{6}$$

where y_i is the primary model parameter datum (log q_o, $\sqrt{\mu_{max}}$, or log N_{max}), $\hat{y}_i$ is the y value calculated from the best fit model, and h_i is given by i-th diagonal elements of hat matrix H:

$$H = T\ (T^T T)^{-1}\ T^T \tag{7}$$

with T being $\begin{bmatrix} 1 & T_1 & T_1^2 \\ 1 & T_2 & T_2^2 \\ \vdots & \vdots & \vdots \\ 1 & T_n & T_n^2 \end{bmatrix}$ for Equation 3 and $\begin{bmatrix} 1 & T_1 \\ 1 & T_2 \\ \vdots & \vdots \\ 1 & T_n \end{bmatrix}$

for Equations 4 and 5.

Next we set $T_i^* = T_i$ for i=1,2, ….n. And ε_i^* was sampled randomly from $(r_i-\bar{r})$, …. $(r_n-\bar{r})$, with $\bar{r}$ being the average r value, and then used to produce y_i^* as:

$$y_i^* = \hat{y}_i + \varepsilon_i^* \tag{8}$$

Finally least squares regression was fitted to (T_1^*, y_1^*), ……,(T_n^*, y_n^*), giving estimates of another set of coefficients and the y estimate at any temperatures based on the corresponding coefficient set. This procedure was repeated 1000 times to produce 1000 values of log q_o, $\sqrt{\mu_{max}}$, or log N_{max} for any temperature of interest. The ranges from 2.5% percentile value to 97.5% percentile for each y value (log q_o, $\sqrt{\mu_{max}}$, or log N_{max}) for the temperature range of interest were obtained as a measure of confidence interval.

Another version of the bootstrapped regression presented above was tried for average primary model parameter values of log q_o, $\sqrt{\mu_{max}}$, or log N_{max} given at four temperatures (Method 2). Again 1000 values of log q_o, $\sqrt{\mu_{max}}$, or log N_{max} at any temperature were obtained and used to identify the 95% confidence band by the same method as above.

Finally simple linear or polynomial regression according to Equations 3, 4, or 5 was conducted for average

primary model parameter values, 2.5% percentile values and 97.5% percentile values of the primary model parameters at four temperatures (Method 3). The area surrounded by the regression lines of 2.5% and 97.5% percentile values was provided as another measure of the confidence band of the primary model parameters as function of temperature.

Comparison of the different secondary modeling methods

Three different regression methods described above were first compared in estimating the primary model parameters at different temperatures with their confidence bands. The respective secondary models were then used to estimate the microbial counts under static and dynamic temperature conditions: the primary model parameters of log q_o, $\sqrt{\mu_{max}}$, and log N_{max} obtained from the models were supplied to the differential Equations 1 and 2, which were solved for given conditions and then compared with experimental data. As for initial microbial counts of log N_o for the estimation at constant temperature conditions where the primary model parameters were derived, 2.5% percentile and 97.5% percentile values shown in Table 1 were supplied into the solution as lower and higher bounds, respectively. The confidence band of the estimated microbial count was obtained from the substitution of the lower and higher boundary values of the primary model parameters at 95% confidence interval. As a reference for microbial count estimation band at constant temperatures, 1000 sets of the original model parameters of Lee et al. (11) were also supplied to Equations 1 and 2 to produce 1000 growth curves, from which their 95% confidence band was extracted. For the simulation under dynamic temperature conditions, lag time and initial q_o value was calculated by using the method reported by Lee et al. (11). As the initial confidence limit values of log N_o for the estimation at dynamic temperature conditions, 95% percentile values of the experimental bacterial counts were supplied for the solution.

RESULTS AND DISCUSSION

Microbial growth model parameters

The best-fit lines or curves obtained for log q_o, $\sqrt{\mu_{max}}$, or log N_{max} in all three methods were the same and coincided with the very narrow central confidence band lines from Method 1 (bootstrapped regression using all the individual parameter values) in Fig. 1. The regression coefficients of the regression curve or line were the same as given in Table 2, but the R^2 value was different between methods. In regression statistics it is accepted that the same number of data number at each independent variable gives the same regression curve for different data treatments but with different R^2 (21). Fig. 1 also compares the 95% confidence intervals for the primary model parameters of log q_o, $\sqrt{\mu_{max}}$, and log N_{max} from three regression methods. The bootstrapped regression using all the individual primary parameter values (Method 1) gave the narrowest band merging to almost a single line. The bootstrapped regression using the average parameter values (Method 2) resulted in a much wider confidence band. This bootstrapped regression using average parameter values does not take into consideration the variability of the model parameters given for each temperature, but assumes their constant distribution for calculating the regression coefficient and confidence interval: this seems to be a drawback of this method.

Another confidence band between two regression lines of 2.5% and 97.5% percentile values (Method 3) shows a wider range than that of Method 1. The band of log q_o was the widest among the methods while the bands for $\sqrt{\mu_{max}}$ and log N_{max} values were narrower compared

Table 2. Regression equations for describing the 2.5% and 97.5% percentile values of the primary model parameters

Parameter	Equations with coefficients (Eqs. 3～5)	R^2
log q_o		
Regression curve	log q_o = -3.7468 – 0.2554 T + 0.1152 T^2	0.995
2.5% percentile curve	log q_o = -4.8787 – 0.0275 T + 0.0794 T^2	0.976
97.5% percentile curve	log q_o = -3.4693 + 0.1392 T + 0.1103 T^2	0.995
$\mu_{max}^{1/2}$		
Regression line	$\mu_{max}^{1/2}$ = 1.5607 + 0.0541 T	0.908
2.5% percentile line	$\mu_{max}^{1/2}$ = 1.4094 + 0.0556 T	0.997
97.5% percentile line	$\mu_{max}^{1/2}$ = 1.6294 + 0.0504 T	0.871
log N_{max}		
Regression line	log N_{max} = 7.7985 + 0.1726 T	0.984
2.5% percentile line	log N_{max} = 7.7573 + 0.1697 T	0.980
97.5% percentile line	log N_{max} = 7.8356 + 0.1777 T	0.991

to those from Method 2. These bands were shown to be highly dependent on the spread or variability of the primary model parameters and seem to comprise most of the average primary model parameter values as shown in Fig. 1. This type confidence band has the advantage to being able to be described as a mathematical function (Table 2), which makes easy its application to dynamically changing temperature conditions.

Estimated microbial growth band under constant and fluctuating temperatures

The difference in confidence bands of the primary model parameters in the secondary model is expected to result in different confidence intervals of estimated microbial growth at their substitution into Equations 1 and 2, which may be useful for evaluating the regression method for applicability to the microbial quality and shelf life estimation under various temperature conditions. Therefore the primary model parameters at the limits of the confidence bands were adopted for simulation of microbial growth under constant temperature conditions, whose original data were used for determining the primary model parameters (Table 1) reported by Lee et al. (11). Fig. 2 compares the different methods for estimating microbial growth at 0, 5, 10, and 15°C. As a reference for comparison, the 95% percentile of the microbial counts estimated from 1000 sets of the original primary model parameters was also given in the same figure.

A general overview on Fig. 2 tells that there is a much narrow band of confidence when the primary model parameters are obtained from Method 1 (bootstrapped regression with all the individual parameter values) while the broadest band results from Method 2 (bootstrapped regression with four average values at four different temperatures). The difference between these two methods is greater at low temperatures of 0 and 5°C. Even though the use of the bootstrapped regression with all the individual parameter values can give the estimated band close to the experimental data of average microbial counts, the band does not comprise some experimental data at 5 and 10°C. It does not overlap either with 95% percentile band of the microbial growth which was estimated with 1000 sets of the original primary model parameters at 5°C.

On the other hand, when the regression lines of 2.5% and 97.5% percentile values (Method 3) were used as bounds of the primary model parameters for the simulation, the estimated microbial count bands were much broader than those both from Method 1 and from 1000

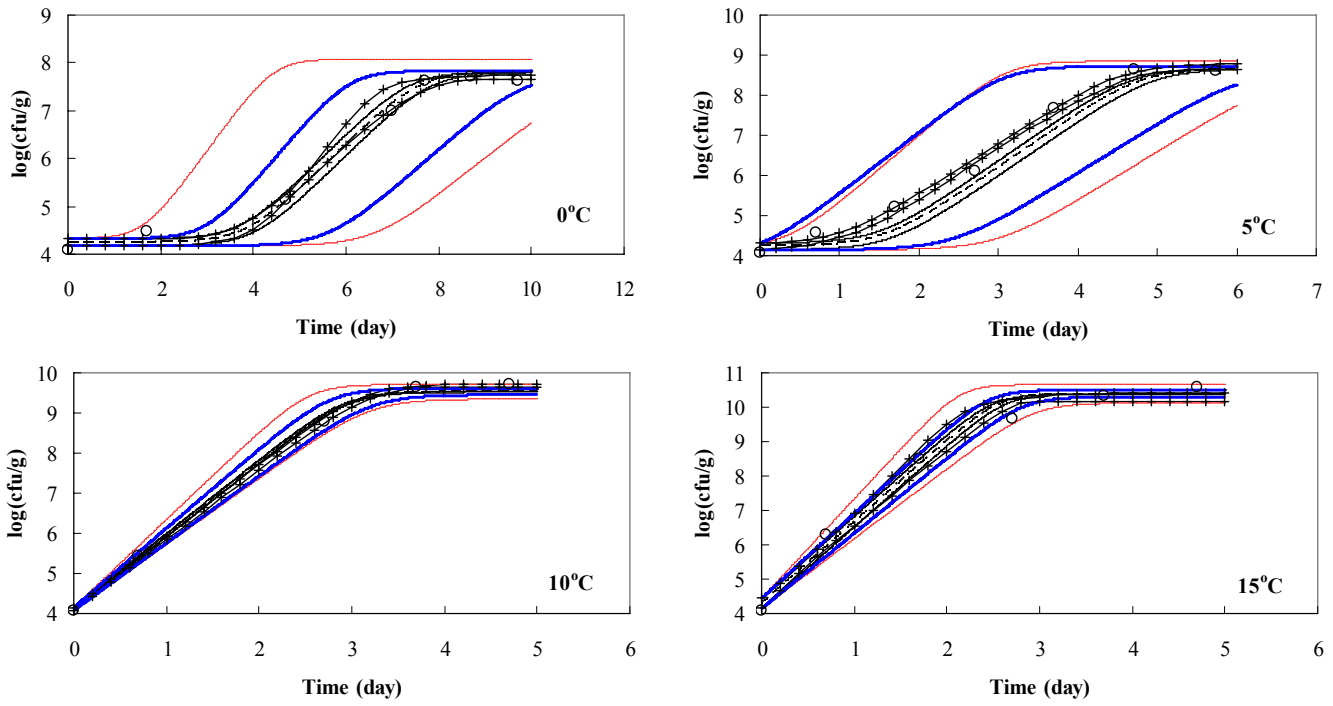

Fig. 2. The estimated 95% confidence band of aerobic bacterial counts on seasoned soybean sprouts at 0, 5, 10, and 15°C. ○: average value; —+—: 95% percentile of the microbial counts estimated with 1000 sets of the original primary model parameters; ——: average estimation based on the best-fit primary model parameters; ——: use of the parameters from bootstrapped regression with all the individual parameter values (Method 1); ----: use of the parameters from bootstrapped regression with average parameter value at each temperature (Method 2); ——: use of the parameters from regression line of 2.5% and 97.5% percentile values (Method 3).

growth curve sets given by the original primary model parameters, but were generally thinner than those from Method 2. The bands also covered all the experimental data points for four temperatures, which would provide a safe margin in estimating the shelf life.

The simple parametric regression with average values at experimental temperatures (Method 2) is the most usual practice in secondary modeling, which has also been widely used for estimating the confidence intervals for the secondary model parameters (9,22). The procedure is simple but does not consider the variability of the microbial count data in the primary modeling. Nonparametric bootstrapping in the regression of the secondary model with average values at experimental temperatures does not take into account the variability of the primary modeling procedure, and thus still has the same limitation as the simple parametric regression. As shown in Fig. 2 its confidence in the estimation was very limited with a wide band. On the other hand, the bootstrapped regression with all the primary model parameter values at different temperatures (Method 1) does consider the variation of primary model parameters and produces higher confidence on the secondary model parameters, resulting in a narrow band of estimation. However, it does have the limitation of not fully covering the variability in the actual experimental data. Method 3 using the regression lines of 2.5% and 97.5% percentile values as bounds of the primary model parameters is a simplified approach for handling the apparent variation of primary model parameters through the temperature range of interest, and is understood as an empirical treatment. Being different from two other nonparametric bootstrapping methods of regression (Methods 1 and 2), it can be easily adopted in simulation of dynamic temperature condition.

The confidence bands of microbial estimation from regression procedures mean the region having a 95% chance of containing the true estimation (21), and are often understood as confidence intervals of the estimated means (12). In attaining the lowest limit of confidence band, this study applied the lower bound values (2.5% percentile) simultaneously for log q_o, log N_o, $\sqrt{\mu_{max}}$, and log N_{max} values, and also the upper bound values (97.5% percentile) for the highest confidence limit of all the parameters, which is an exaggeration and would have resulted in a wider band. Similar approaches have been made for estimating microbial spoilage of fish exposed to non-isothermal condition by Koutsoumanis (17). For true confidence band, correlation matrix would be required but it cannot be determined exactly for this kind of complex system. Thus the attained band of the estimated microbial growth is somewhat wider, and is understood as a 95% confidence band having some safe margin.

While narrower bands may give more confidence on the estimation, there are high possibilities that the estimation does not cover the actual data of occurrence. To our current knowledge, there is no prefect method for estimating the microbial count with its confidence limit for diverse temperature conditions. More appropriate or improved stepwise regression methods in the primary and secondary modeling need to be developed taking account of theoretical validity and practical application. As shown above, Method 3 using regression lines of 95% band values of the original primary model parameters could give the reliable and consistent prediction covering the real outcome of the event. Lower temperatures of 0 and 5°C with longer lag time resulted in a much wider confidence band: accurate prediction of the lag time would contribute to narrowing the band and improving the estimation, but it is not easily made at present due to its complicated dependence on several factors such as environmental conditions, cell growth stage, initial microbial load, etc. (23). Use of the regression line bands expressed as mathematical functions (Table 2) has an additional advantage of being easily applied to the dynamic temperature condition.

When regression lines of 2.5% and 97.5% percentile values given in Table 2 were used for predicting microbial growth under dynamic temperature conditions, we obtained confidence bands covering or close to the experimental data (Fig. 3). As shown in Fig. 3A, having a long lag time based on the estimation by using best-fit parameters (due to initial low temperature period) resulted in a wide confidence band. Some deviations in Fig. 3B might have been caused by sample variability and simplified model assumptions, as was discussed by Lee et al. (11). Even with some deviation, the estimation band is more useful compared to point estimation providing a safe margin. While the accuracy and distribution of the microbial model parameters primary and secondary are influenced by the consistency in the measurement locations, scattering of experimental data, and regression method (16,24), their ability to represent or describe the variability and uncertainty in the real world is thought to be strongly dependent on consistency among the samples and environmental variables. Projection of modeling onto the real world should be possible only on the basis of close proximity between experimental conditions and practices of the food storage and distribution. More data and experience are required to

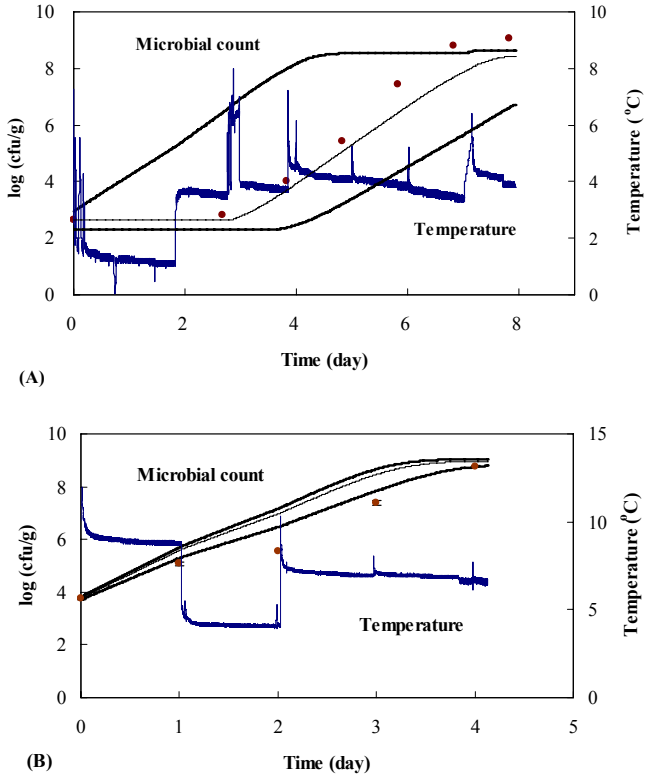

Fig. 3. Two sets (A & B) of estimated confidence bands of microbial growth on seasoned soybean sprouts under dynamically changing food temperatures. ●: experimental microbial data. Thick solid lines for microbial count show estimated microbial growth band of 95% confidence while thin line is the estimation based on the best-fit parameter set. Vertical bars indicate standard deviations of microbial count data.

produce a solid and reproducible picture of the microbial spoilage of perishable foods.

CONCLUSION

From the comparison of several different regression methods for describing the temperature effect on microbial spoilage, regression lines of 2.5% and 97.5% percentile values showed the capability to account for experimental data of microbial growth under constant and fluctuating temperature conditions. The method has the advantage of extracting the primary model parameters instantaneously at any temperature, which is required for predicting the band of microbial growth under dynamic temperature conditions.

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