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Development of a Predictive Mathematical Model for the Growth Kinetics of *Listeria monocytogenes* in Sesame Leaves

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Abstract Square root models were developed for predicting the kinetics of growth of *Listeria monocytogenes* in sesame leaves as a function of temperature (4, 10, or 25°C). At these storage temperatures, the primary growth curves fit well (R^2=0.898 to 0.980) to a Gompertz equation to obtain lag time (LT) and specific growth rate (SGR). The square root models for natural logarithm transformations of the LT and SGR as a function of temperature were obtained by SAS's regression analysis. As storage temperature (4-25°C) decreased, LT increased and SGR decreased, respectively. Square root models were identified as appropriate secondary models for LT and SGR on the basis of most statistical indices such as coefficient determination (R^2=0.961 for LT, 0.988 for SGR), mean square error (MSE=0.197 for LT, 0.005 for SGR), and accuracy factor (A_f=1.356 for LT, 1.251 for SGR) although the model for LT was partially not appropriate as a secondary model due to the high value of bias factor (B_f=1.572). In general, our secondary model supported predictions of the effects of temperature on both LT and SGR for *L. monocytogenes* in sesame leaves.

Keywords: *Listeria monocytogene*, temperature, sesame leave, Gompertz equation, square root model

Introduction

The demand for raw vegetables and fruits as minimally processed foods has gradually increased worldwide in recent years. Therefore, increasing consumption has resulted in a higher frequency of outbreaks of foodborne illness related to raw produce (1-4). *Listeria monocytogenes* is one of the main causes of most vegetable-related foodborne illness is of great interest in the food industry and public because of its ability to persist and grow under a wide range of unfavorable conditions such as low pH (5, 6), high pH (6, 7), low water activity (8, 9), high osmolarity (6), and at refrigeration temperatures (6, 10). This pathogen can cause listeriosis in human (11).

In recent years, predictive food microbiology (PFM) has become established as an important method of ensuring food safety in the food chain (11). PFM can mathematically predict the changes occurring in spoilage and pathogenic microorganisms by describing the growth, survival, and death of microorganisms in laboratory media and food matrices under specific environmental conditions.

Mathematical quantitative models that predict the growth of *L. monocytogenes* as a function of important environmental factors will improve the shelf life and safety of foods (7, 13-19). However, earlier studies of predictive models describing the effects of environmental factors such as temperature, pH, water activity, CO_2, and preservative agents on the growth, survival, or inactivation of *L. monocytogenes* are still limited in diverse food matrices. Specifically, there were no predictive models on the growth of *L. monocytogenes* on fresh sesame leaves which are widely consumed as in Korea.

The purpose of this study was to investigate the effects of temperature on the growth kinetics of *L. monocytogenes* on sesame leaves with the goal of developing a model that could be used to predict the growth of the pathogen at storage temperatures of 4, 10, and 25°C.

Materials and Methods

Bacterial culture A cocktail of *L. monocytogenes* ATCC 19112 (serotype 1/2c), 19113 (serotype 3a), and 19115 (serotype 4b) was used to determine growth characteristics and develop predictive models for growth. Each strain was maintained at -70°C in tryptic soy broth (TSB, Difco Laboratories, Detroit, MI, USA) containing 50% glycerol. Each culture of *L. monocytogenes* was thawed at room temperature and then 10 μL of a mixed resuspended stock culture were added in 9 mL of sterile TSB containing

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0.6% yeast extract (Difco). The culture was incubated at 37°C for 24 hr. One mL of the culture was added into 100 mL of TSB and the culture was incubated at 37°C for 24 hr. One mL of the starter culture was serially diluted in 9 mL of sterile 0.1% peptone water for inoculation into TSB.

Preparation of sesame leaves Sesame leaves were purchased from a local supermarket. The wilted sesame leaves were discarded. To remove background microorganisms on the sesame leaves, intact sesame leaves were washed twice with running tap water and then rinsed with distilled water. The rinsed sesame leaves were submerged in a solution of 3.6% hydrogen peroxide (H_2O_2) (Roam Cheime NV, Belgium) for 5 min, rinsed with distilled water, and cleaned with sterile water. The sanitized sesame leaves were air-dried under a bio-safety cabinet at room temperature (22±2°C) for about 1 hr before inoculation.

Inoculation and storage temperature Two hundred mL of 0.1% peptone water containing the cocktail of *L. monocytogenes* at a final concentration of 5-6 $\log_{10}$CFU/g were inoculated on 5-10 spots of the surface of 10 g sesame leaves. After inoculation, the leaf samples were air-dried at room temperature (22±2°C) under a bio-safety cabinet for about an hour. After drying, the inoculated leaf samples were aseptically divided into sterile bags (Nasco, Modesto, CA, USA) and stored at 4, 10, and 25°C.

Enumeration of *L. monocytogenes* Each 10 g sample of sesame leaves was taken at each respective sampling interval, combined with 40 mL of 0.1% peptone water in a sterile bag, and evenly mixed for 1 min in a stomacher (SH-IIM; Elmex Ltd., Tokyo, Japan). The sample was then serially diluted in 0.1% peptone water and plated in duplicate on tryptic soy agar (TSA, Difco), which was incubated at 37°C for 48±2 hr. The colonies were counted by standard plates count and expressed as colony-forming units (CFU)/g. The data were converted to values of $\log_{10}$CFU/g. Three independent trials were performed for this study. Duplicate all samples and analyze at least 3 samples at each respective sampling interval.

Primary modeling Growth curves of viable cell count versus sampling time were iteratively generated from the experimental data using the Gompertz equation and fitted to a nonlinear regression model (Prism, Version 4.0; GraphPad Software, San Diego, CA, USA) to determine lag time (LT, in hr) and specific growth rate (SGR, in $\log_{10}$CFU/g per hr) at each incubation temperature. The Gompertz parameter values were described by Gibson *et al.* (20).

$$Y = N_0 + C^*\exp[\exp\{(2.718^*SGR/C)^*(LT-X) + 1\}]$$

Y = Log cell number, X = incubation time, N_0 = log initial number of cells, C = difference between initial and final cell numbers, LT = lag time before growth, same units as X, SGR = maximum specific growth rate.

Secondary modeling A square root model for the effects of temperature was calculated on the LT and SGR. These two Gompertz parameters for *L. monocytogenes* growth data were determined by the least squares analysis of PROC GLM of the SAS version 8.1 (21). The square root model was described by Ratkowsky *et al.* (22).

$$\sqrt{SGR} \text{ or } \sqrt{LT} = b(T-T_{min})$$

b = Regression constant, T = temperature, T_{min} = conceptual minimum temperature for microbial growth.

Evaluation of model performance The coefficient of determination (R^2), which is provided by GraphPad (GraphPad Software) is often used as an overall measure of the prediction attained. It measures the fraction of the variation about the mean that is explained by a model.

The mean square error (MSE), the residual sum of squares divided by the number of degrees of freedom, is a measure of variability remaining that is not accounted for by deliberate changes in factors such as temperature, pH, and a_w.

$$MSE = [\Sigma \log(LT_{predicted}/LT_{observed})2]/n$$
$$= [\Sigma \log(SGR_{predicted}/SGR_{observed})^2]/n$$

$LT_{predicted}$ = the predicted lag time, $LT_{observed}$ = the observed lag time, $SGR_{predicted}$ = the predicted specific growth rate, $SGR_{observed}$ = the observed specific growth rate, n=the number of observations.

The bias factors (B_f) answers the question whether, on average, the observed values lie above or below the line equivalence and, if so, by how much. It gives the structural deviations of a model.

$$B_f = 10^{(\Sigma \log(LT_{predicted}/LT_{observed})/n)}$$
$$= 10^{(\Sigma \log(SGR_{predicted}/SGR_{observed})/n)}$$

The accuracy factor (A_f) averages the distance between each point and the line of equivalence as a measure of how close, on average, predictions are to observe.

$$A_f = 10^{(\Sigma \log|(LT_{predicted}/LT_{observed})|/n)}$$
$$= 10^{(\Sigma \log|(SGR_{predicted}/SGR_{observed})|/n)}$$

Results and Discussion

Primary modeling In a previous research survey for microbial risk assessment (MRA) of fresh sesame leaves, 4(±1) and 10(±1)°C were considered as the main storage temperature in both homes and restaurants as well as the distribution temperature in both supermarkets and traditional markets in winter, respectively (data not shown). Additionally, 25(±1)°C was in a middle range of distribution temperature in supermarkets (16) and traditional markets (32) in summer. Therefore, the current study selected 4, 10, and 25°C as the temperatures at which we monitored *L. monoytogenes* growth on the sesame leaves. Growth of *L. monocytogenes* on the sesame leaves was observed in the storage of 4°C for 25 days, 10°C for 15 days, or 25°C for 5 days (data not shown). Also, the primary model in the current study involved 27 growth curves including three independent trials associated with the temperature effects on the sesame leaves.

The Gompertz equation is typically used to fit bacterial growth curves for estimating LT and SGR by the US Department of Agriculture (USDA) (5, 9, 14, 23). Therefore, the current study used the Gompertz equation to fit growth curves for *L. monocytogenes*. Table 1 shows best-fit values (R^2, coefficient determination) for LT and SGR in the primary model. In general, the data of LT and SGR for the sesame leaves fit a Gompertz equation model well, with a high degree of goodness of fit (R^2=0.898 to 0.980) at 4, 10, and 25°C (Table 1) although these values of R^2 in the current study were somewhat low compared to those of R^2 in the broth study with *L. monocytogenes* (24). From this observation, we speculate that bacterial growth kinetics in a food matrix was less consistent than that in a laboratory media matrix.

Secondary modeling Many bacterial predictive growth models have been reported extensively but few predictive growth models have been constructed with respect to fresh produce (10, 25, 26). Therefore, in the current study, the predictive growth models using the square root analysis of *L. monocytogenes* on fresh sesame leaves have been constructed describing the effects of temperature. The square root analysis has been carried out to determine the relationship between $\sqrt{SGR}$ or $\sqrt{LT}$ and the temperature of *Escherichia coli* O157:H7, *Salmonella* sp. and *L. monocytogenes* (11, 22, 27, 28). LT and SGR from the fitted growth curve were transformed to their natural logarithm to stabilize model variance (20) and regressed against model variables to obtain the square root model. The following equations were given:

$$\text{Ln (LT)} = [-5.02824(T-25.9075)]^2 \quad R^2 = 0.940$$
$$\text{Ln (SGR)} = [0.00109(T+0.0052)]^2 \quad R^2 = 0.900$$

These equations estimated the predicted LT (Fig. 1) and SGR (Fig. 2) of *L. monovytogenes* as a function of temperature.

When the effects of temperature in sesame leaves were compared, the LT of the *L. monocytogenes* increased as temperature decreased (4-25°C). Approximately, a 24-fold increase of the LT of the *L. monocytogenes* was predicted for sesame leaves stored at 4°C compared to 25°C. However, only a 1.4-fold increase of the LT of the *L. monocytogenes* was predicted at 4°C storage than for storage at 10°C. The SGR of the *L. monocytogenes* at storage temperatures of 10 and 25°C was 2.03 and 4.53-fold higher, compared to storage at 4°C. Based on these predictions, the LT and SGR appeared to be generally longer and lower, respectively at the lower storage temperature than at the higher storage temperature.

Table 1. For the primary modeling, best-fit lag time (LT), and specific growth rate (SGR) of *L. monocytogenes* in sesame leaves obtained from the Gompertz equation model

Temperature (°C)	LT (hr)	SGR (log_{10} CFU/hr)	R^2 [1)]
4	118	0.0070	0.980
	112	0.0072	0.974
	144	0.0091	0.898
10	85	0.0097	0.940
	82	0.0096	0.936
	79	0.0095	0.940
25	7	0.0266	0.920
	8	0.0264	0.935
	10	0.0326	0.943

[1)] R^2, coefficient of determination.

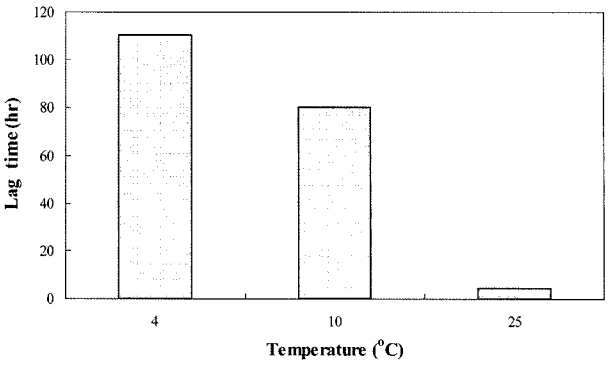

Fig. 1. Square root models for the effects of temperature on lag time (LT) of *L. monocytogenes* in sesame leaves.

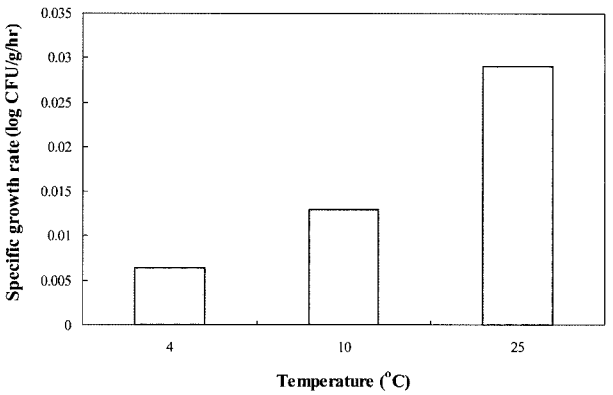

Fig. 2. Square root models for the effects of temperature on specific growth rate (SGR) of *L. monocytogenes* in sesame leaves.

Evaluation of the model performance Table 2 presents four different statistical indices of the secondary modeling step for LT and SGR on the sesame leaves. The R^2, MSE, B_f, and A_f for LT of *L. monocytogenes* were 0.961, 0.197, 1.572, and 1.356, respectively. The R^2, MSE, B_f, and A_f for SGR of *L. monocytogenes* were 0.988, 0.005, 1.106, and 1.251, respectively. Our results indicated that developed square root models for LT and SGR on the sesame leaves were generally considered to be quite good or acceptable. On the other hand, the fits achieved for the secondary model for the LT were not as good as the fits achieved for the secondary model for the SGR because one index, B_f (1.572) for the LT was high. The higher value of R^2 ($0 < R^2 < 1$), the better is the prediction by the model (29-31). The lower value of MSE, the better is the adequacy of the model to describe the data (32, 33). $B_f < 1$

Table 2. Statistical indices of the secondary modeling step for lag time (LT) and specific growth rate (SGR) of *L. monocytogenes* in sesame leaves

Model	R^{2} [1)]	MSE[2)]	B_f[3)]	A_f[4)]
LT	0.961	0.197	1.572	1.356
SGR	0.988	0.005	1.106	1.251

[1)]R^2, coefficient of determination.
[2)]MSE, mean square error.
[3)]B_f, bias factor.
[4)]A_f, accuracy factor.

indicates a 'fail safe' model (34). $B_f > 1$ indicates a 'fail dangerous' model (9). Ross *et al.* (35) also noted that for models describing pathogen growth rate, B_f in the range 0.9-1.05 could be considered good, in the range 0.7-0.9 or 1.06-1.15 considered acceptable, and <0.7 or >1.5 considered unacceptable. The larger value of A_f, the less accurate is the average estimate. Ross (31) and Park *et al.* (24) additionally noted that an acceptable model that predicts the effect of temperature, pH, and water activity on *Listeria* growth rate could be expected to have A_f in the range 1.3-1.5. LT duration has often been considered erratic, and evaluations of predictive models have been shown that LT are less reliably predicted than generation time (31) or growth rate (36-38).

In conclusion, the square root model proved reliable in predicting the effects of the temperature both LT and SGR for *L. monocytogenes* on sesame leaves. However, the extrapolation of model predictions for the LT in the current study must be made cautiously. Predicting the growth of *L. monocytogenes* on sesame leaves will assist in the reduction of the microbial quantitative risk involved in the consumption of the sesame leaves. Moreover, the predictions will provide important information concerning the shelf life of fresh sesame leaves to consumers.

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