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Predictive Modeling of the Growth and Survival of *Listeria monocytogenes* Using a Response Surface Model

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Abstract This study was performed to develop a predictive model for the growth kinetics of *Listeria monocytogenes* in tryptic soy broth (TSB) using a response surface model with a combination of potassium lactate (PL), temperature, and pH. The growth parameters, specific growth rate (SGR), and lag time (LT) were obtained by fitting the data into the Gompertz equation and showed high fitness with a correlation coefficient of $R^2 \geq 0.9192$. The polynomial model was identified as an appropriate secondary model for SGR and LT based on the coefficient of determination for the developed model ($R^2 = 0.97$ for SGR and $R^2 = 0.86$ for LT). The induced values that were calculated using the developed secondary model indicated that the growth kinetics of *L. monocytogenes* were dependent on storage temperature, pH, and PL. Finally, the predicted model was validated using statistical indicators, such as coefficient of determination, mean square error, bias factor, and accuracy factor. Validation of the model demonstrates that the overall prediction agreed well with the observed data. However, the model developed for SGR showed better predictive ability than the model developed for LT, which can be seen from its statistical validation indices, with the exception of the bias factor (B_f was 0.6 for SGR and 0.97 for LT).

Keywords: *Listeria monocytogenes*, predictive model, temperature, specific growth rate, lag time

Introduction

Listeria monocytogenes is a widely dispersed foodborne bacteria. Due to its high mortality rate, especially for young, old, pregnant, or immunocompromised patients, and the recent increase in consumer interest (1), risk analysis for *L. monocytogenes* is currently an important topic in this research field. Furthermore, the psychotropic character of the bacteria creates additional importance for *L. monocytogenes* contamination of food in foodborne infections (2). Due to the high frequency and severity of listeriosis, the social and economic impacts of this disease are among the highest of the foodborne diseases (3). Moreover, the European Union is preparing regulation concerning microbial criteria for foods and food production (SANCO/4198/2001) and in the near future will reportedly mandate a tolerance level of 100 CFU/g *L. monocytogenes* at the end of shelf life for chilled ready-to-eat (RTE) foods (4). On the other hand, the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) has already instituted a 'zero-tolerance' policy for *L. monocytogenes* in RTE food (5). With all the concerns associated with *L. monocytogenes* outbreaks in food and food products, modeling of the growth of *L. monocytogenes* in broth as a function of temperature, pH, and potassium lactate was performed. Mathematical models that predict pathogen growth, survival, and inactivation have several benefits including their ability to account for changes in microbial load as a result of environment and handling, their use of predictive microbiology in management of foodborne hazards, and their application in the preparation of Hazard Analysis and Critical Control Point (HACCP) plans. As a result, the magnitude of predictive microbiology in conjunction with mathematical derivations has been more cosmopolitan in scope recently. The current model will add more integrated information and provide a supportive framework to make acceptable exposure risk assessments and to simplify the HACCP regulations for food-based industries concerning the use of potassium lactate (PL) to enhance shelf-life and to provide adequate food protection.

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Materials and Methods

Bacterial strains Cocktails of three strains of *L. monocytogenes* (Scott A, ATCC 19111, ATCC 19116) were used in the study. Strains were stored at -70°C in tryptic soy broth (Difco Laboratories, Detroit, MI, USA) with 0.6% yeast extract (Difco) containing 20% glycerol. Stock culture (0.1 mL) was sub-cultured twice by inoculating into 10 mL tryptic soy broth (TSB), which was then incubated at 35°C for a day successively. Cocktails of these strains were prepared by mixing equal volumes of bacterial suspensions of the inoculums diluted with 90 mL of sterile peptone (0.1%) water to obtain 10^2-10^4 CFU/mL.

Experimental procedure The procedure followed is as outlined in the previous paper concerning *L. monocytogenes* (1). PL volumes (PURASAL P HiPure 60; Purac America Inc., Lincolnshire, IL, USA) of 1.6, 3.2, 4.8, or 6.6 mL of were added to 98.4, 96.8, 95.2, or 93.4 mL of TSB to formulate the PL concentrations of 1, 2, 3, or 4%,

respectively. The broth media without PL was used as a control. The pH of the media was adjusted to 5.0, 5.5, 6.0, 6.5, or 7.0 with 0.1 N HCl or 0.1 N NaOH using the Orion pH meter (model 420A; Orion research Inc., Beverly, MA, USA). The media was autoclaved at 121°C for 15 min and cooled to room temperature before inoculation. Erlenmeyer flasks (500 mL) containing 100 mL of sterile TSB with various concentrations of PL were inoculated with 1 mL of the cocktail of bacterial strains. After inoculation, flasks were incubated at 4, 7, 10, 13, 16, or 25°C for 27, 18, 10, 4.625, 2.375, or 1 day, in all possible combinations. Sampling was done at appropriate time intervals. 0.1 mL aliquots of the appropriate dilution were spread on the surface of duplicate plates of modified oxford medium base agar (Difco) and incubated at 35 °C for 1 day. Colonies were counted using the standard plate count (SPC) method.

Growth model Growth models were constructed from the 150 combinations (using an average value of triplicate experiments for each) as a function of temperature, pH, and PL. For each storage temperature the experimental data were fitted to the Gompertz equation as described by Gibson *et al.* (6).

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

where, Y is the logarithm of microbial counts (Log $_{10}$CFU/mL), X is time (day), N_0 is the initial number of cells with units the same as Y, C is the difference between initial and final cell numbers, LT is the lag time before growth in the same units as X, and SGR is the maximum specific growth rate (Log CFU/mL/day).
The effects of temperature, pH, and PL can be described by the response surface model given by Gibson *et al.* (7).

$$SGR \text{ or } LT = EXP\ (b_0+b_1S+b_2T+b_3P+b_4S^2+b_5T^2+b_6P^2+b_7ST+b_8SP+b_9TP)$$

where, the units for SGR and LT are the same as those previously defined. T is the temperature (°C), P is the pH value, S is the potassium lactate concentration (%), and b_0 -b_9 are the coefficients to be estimated.

Model validation The following indices were used for model validation: coefficient of determination (R^2), mean square error (MSE), bias factor (B_f), and accuracy factor (A_f).

The regression coefficient measures the fraction of the variation about the mean that is explained by a model. It is often used as an overall measure of the prediction attained. The higher the value ($0<R^2<1$), the better is the prediction by the model (8).

Mean square error shows the average of the square of the difference between the desired response and the actual system output (the error). Any measure of the center of a distribution should be associated with some measure of error. The lower the MSE of the model is, the better the adequacy of the model to describe the data will be (9).

$$MSE = \{\Sigma \log (Y_p/ Y_o)^2\}/n$$

where, Y_p is the predicted SGR or LT, Y_o is the value of the observed SGR or LT, and n is the number of observations.

Bias factor is a multiplicative estimate for the mean difference between observed and predicted values. It gives structural deviations with the interface of over- or under-prediction where perfect agreement between predictions and observations would lead to a bias factor of 1.0.

$$B_f = 10^{\{\Sigma \log (Yo/Yp)/n\}}$$

Accuracy factor is the measure of the mean absolute difference between the predicted and observed values and gives overall model error. The acceptability and performance of the model depends on an accuracy factor being close to 1. The model has high performance and is highly accurate if this value is 1 (10). It can be calculated from

$$A_f = 10^{[\Sigma |\{\log(Yp/Yo)\}|/n]}$$

Statistical analysis Each experiment featured two replications per treatment. Growth parameters such as specific growth rate (SGR) and lag time (LT) were obtained from the growth curves that were generated using GraphPad PRISM 4 (Graphic Pad Software San Diego, CA, USA). Transformation of the Gompertz parameters (SGR, LT) was conducted using SAS General Linear Model procedure (SAS Institute, Cary, NC, USA).

Results and Discussion

The present study expands on earlier work designed to demonstrate predictive modeling and to develop a response surface model (1). A total of 150 combinations were generated as a function of temperature, pH, and PL in TSB. The obtained growth data were analyzed using regression analysis to generate the 'best fit' Gompertz equation, which was then used to calculate LT and SGR to develop the secondary response surface model. The data obtained by fitting the Gompertz equation showed high fitness, and the curves showed a high correlation coefficient at all tested conditions ($R^2 \geq 0.9192$) for SGR and LT (data not shown). The data indicated that the growth kinetics of *L. monocytogenes* were dependent on storage temperature, pH, and PL. Figures 1 and 2 represent SGR and LT obtained from the predicted response surface model in TSB. As storage temperature increased, LT decreased and SGR increased. The antimicrobial nature of PL was found to be more efficacious at lower pH and lower temperature compared to higher values. The efficacy of PL was highest at 4°C and pH 5. Possible reasons for efficacy of PL under stressful pH and temperatures were discussed in the previous paper concerning the effects of temperature, pH, and PL on the growth of *L. monocytogenes* in broth (1).

The polynomial model developed for the SGR and LT, along with the mean square error and coefficient of determination (R^2) for the model, is described in Table 1. The error of prediction that is given by MSE provides a measure to judge the quality of the fit to the model. Authors have mentioned that the lower the value of MSE, the better the adequacy of the model to describe the data (9, 11). MSE observed for SGR was 0.06 and that for the

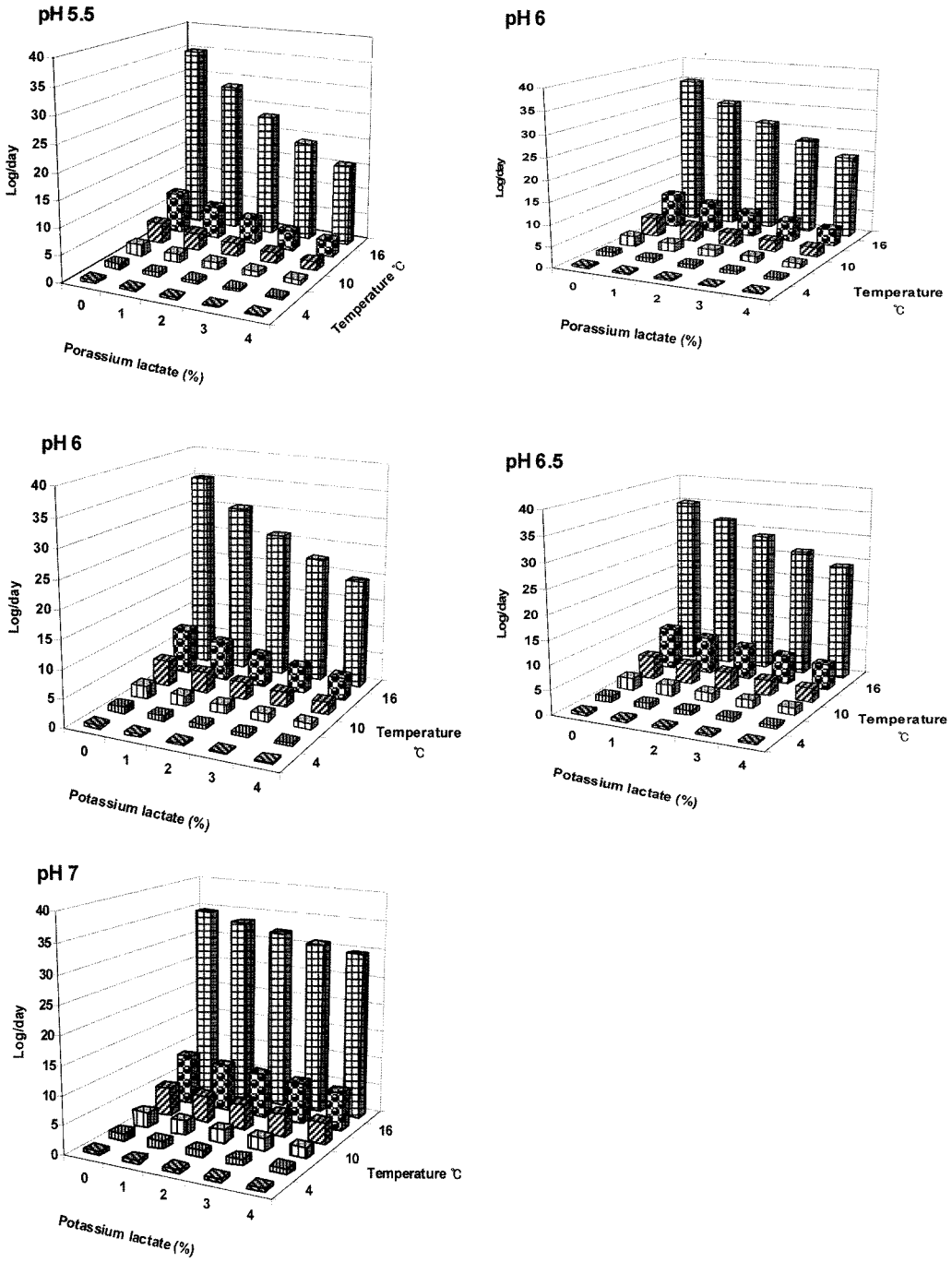

Fig. 1. Specific growth rate obtained from predicted response surface model in tryptic soy broth (TSB).

Table 1. Induced polynomial models for specific growth rate and lag time[1)]

Model	MSE	R^2
SGR=EXP(-4.165+0.358*T+0.516*pH-0.884*PL-0.003*T^2-0.018*pH^2+0.000015*PL^2-0.016*T*pH+0.003*T*PL+0.11*PL*pH)	0.06	0.97
LT = EXP (22.772-0.334*T-6.104*pH+0.86*PL+0.000094*T^2 +0.443*pH^2-0.057*PL^2+0.007*T*pH+0.01*T*PL-0.051*PL*pH)	0.11	0.86

[1)]Specific growth rate (SGR); Lag time (LT); Temperature (T); Potassium lactate (PL); Mean square error (MSE); Correlation coefficient (R^2).

LT was 0.11. Moreover, the variation accounted for by the developed model for SGR and LT was 0.97 and 0.86, respectively. From the scatter plot diagram of observed data versus predicted SGR and LT in Fig. 3 and 4, the data points lie near the line of unity, indicating that the model satisfactorily predicted the growth under the studied

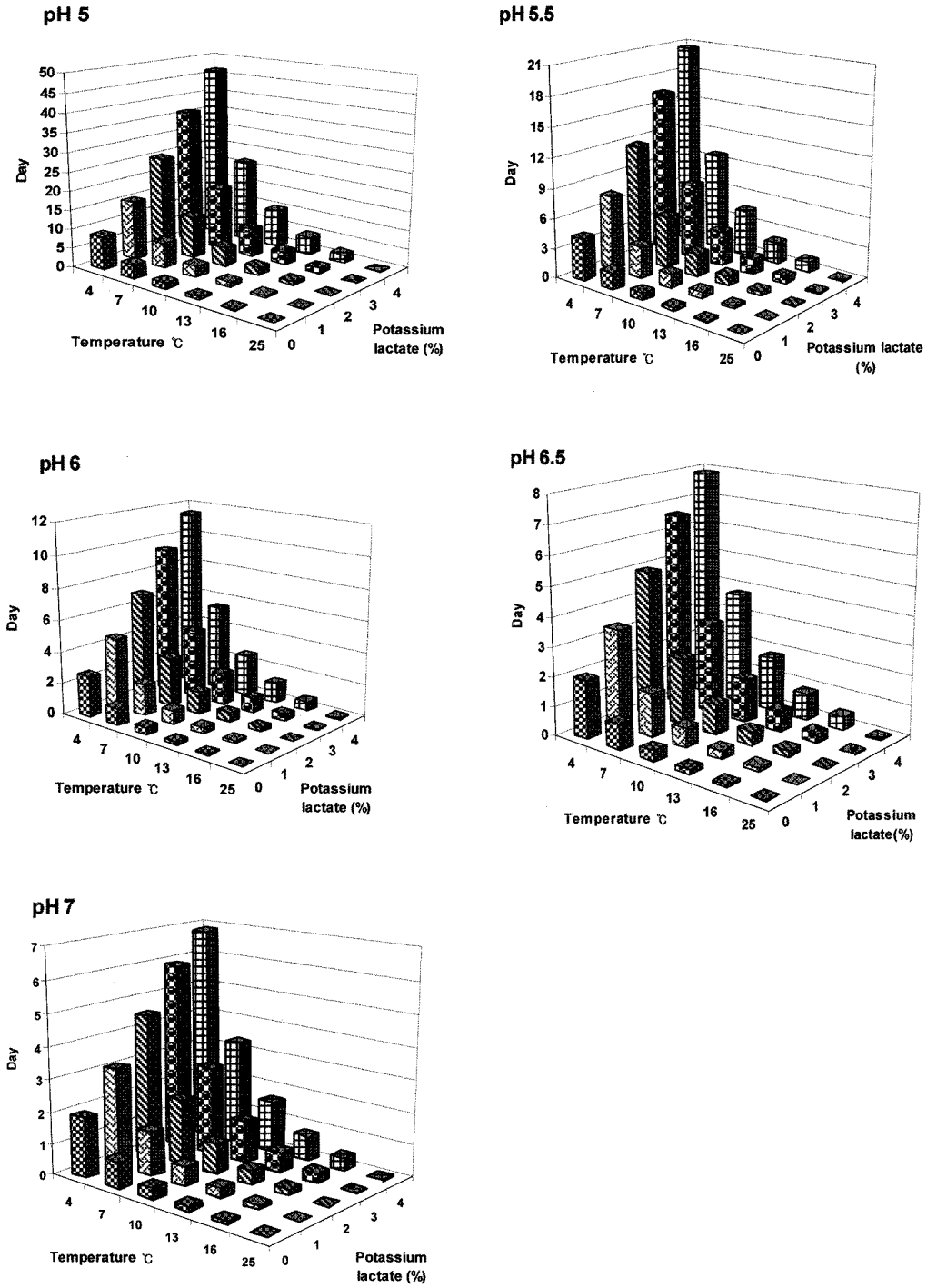

Fig. 2. Lag time obtained from predicted response surface model in tryptic soy broth (TSB).

conditions. However, one of the points fell far from the line of unity (Fig. 4). This might be due to differences in the growth patterns of different strains of same species with successive generation time or to the lag phase differing two or three times depending on the strain as described by Olmez and Aran (12). Most of the points are below the line of unity, signifying that the model provided a good description of the effect of a combination of environmental factors. Overall, a good statistical fit was obtained from the scatter plot diagram. However, the estimated values of correlation coefficient showed that the data used to generate for SGR had better predictive ability than that for the LT. Many authors report a good prediction of SGR with their models (13-15), but the precision on predicted LT was often described as low (6, 16-18). One possible reason for this apparent discrepancy might be that SGR is an autonomous feature, characterizing only the bacteria and the actual environment, whereas lag phase is a non-autonomous feature and depends on the physiological state of the bacterial cells, which cannot be

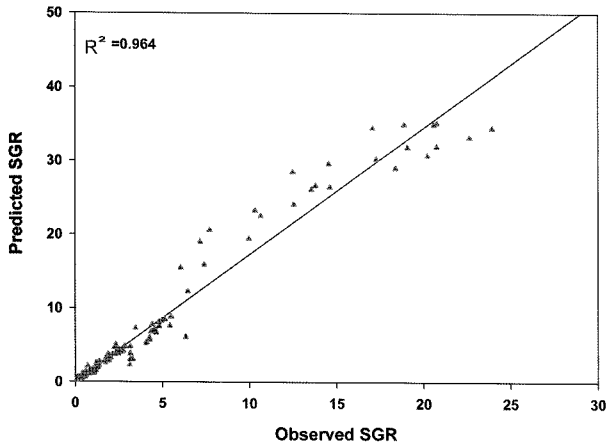

Fig. 3. Observed versus predicted specific growth rate (SGR).

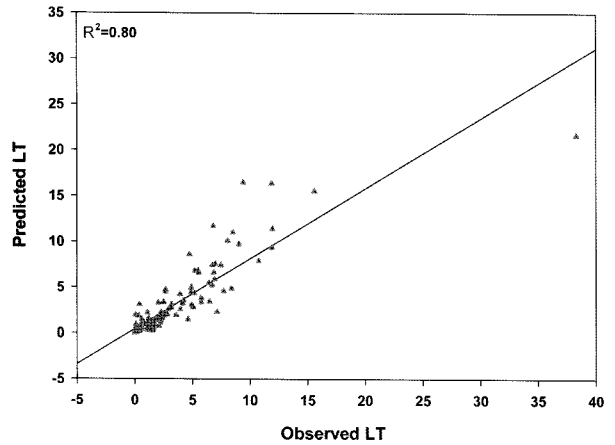

Fig. 4. Observed versus predicted lag time (LT).

predicted from other independent data and is inherently more difficult to predict than the growth rate (15, 19, 20). Nevertheless, Baranyi (21) indicated a need for the development of a stochastic model for the dynamics of individual cells to predict bacterial lag time and growth more accurately. Recently, a study performed by Francois *et al.* (4) on the modeling of individual cell lag phase as a function of temperature and pH explained that the longer lag phase of individual cells was the result of more stressful environmental conditions. The author emphasized that, in the temperature range between 7 and 2°C, LT increased exponentially with increasing temperature stress. These inferences reflect the difficulties in predictive modeling of the LT of bacterial cells.

Thus, our model was validated using the indices, bias and accuracy factor, as they provide an objective indication of model performance. These factors were shown to be valuable tools for the evaluation of the performance of other predictive models (10, 22-24). The B_f and A_f for the model developed for SGR and LT are shown in Table 2. From the results, B_f was unsafe for both SGR and LT, which is an important parameter for the model under consideration. Results with a B_f of less than 1 indicate that the predicted value is higher than the observed value. Similarly, a B_f of more than 1 indicates that the prediction resulted in a lower level of confidence

Table 2. Comparison of bias and accuracy factor for specific growth rate and lag time

Growth parameters[1)]	Bias factor (B_f)	Accuracy factor (A_f)
SGR	0.6	1.67
LT	0.97	1.72

[1)]Specific growth rate (SGR) and lag time (LT).

and is lower than that of the observed value, which is also dangerous and needs to be avoided. On the other hand, a B_f of 1 indicates that the model is neither over- nor under-predicting the growth parameters. Overall, the bias factor indicates a systematic over- or under-prediction. Dalgaard and Jorgensen (22) suggested that bias factor values for seafood spoilage micro-organisms should be between 0.75 and 1.25 in order for a microbial spoilage model to be successfully validated. Moreover, Ross (10) noted that, for models describing pathogen growth kinetics, B_f should be in the range of 0.9-1.05 to be considered good and in the range of 0.7-0.9 or 1.06-1.15 to be considered acceptable.

The B_f provides no indication of the average accuracy of estimates because under- and over-predictions tend to cancel out; therefore, the accuracy factor is calculated to assess the reliability of the model performance. The model has high performance and accuracy if the value of A_f is 1. As shown, accuracy factors of 1.67 and 1.72 for the developed models indicates that, on average, the predictions differed from observations by 67 and 72%, respectively, which implies that the observed SGR and LT were within 67 and 72% of the predicted values. Dalgaard and Jorgensen (22) calculated A_f values ranging from 1.4 to 4.0 for the growth rates of *L. monocytogenes* in various types of seafood, while an A_f of 1.53 was reported by Ross for *Staphylococcus aureus* based on literature data (10). te-Giffel and Zwietering (24) mentioned that using non-sterile, inhomogeneous foods or literature data results in lower levels of confidence. The authors reported that the values of A_f for the different models depended on the type of product analyzed and ranged from 1.7 to 3.5 for the general models and from 1.4 to 4.3 for the specific models. The low agreement between the predicted growth rates and measured values are most likely due to the presence of natural antimicrobial components, low pH value of the food matrix, etc. as discussed by Jay (25).

In conclusion, the developed secondary response surface model for SGR showed slightly better performance than the one developed for LT, which is expressed by its R^2, MSE, and A_f factor. However, the values of the coefficient of correlation from the developed model showed that the developed model had good overall fitness. Further work regarding the predictive ability of growth models for *L. monocytogenes* in food products will help ensure food safety and provide confirmative information on risk hazards for risk management planning.

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