J Korean Soc Appl Biol Chem (2013) 56, 731–738
DOI 10.1007/s13765-013-3122-9

ORIGINAL ARTICLE

Predictive Modeling of *Staphylococcus aureus* Growth on Gwamegi (semidry Pacific saury) as a Function of Temperature

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Received: 31 May 2013 / Accepted: 20 November 2013 / Published Online: 31 December 2013

Abstract Gwamegi (semidry Pacific saury [*Cololabis saira*]) is a Korean food made by a traditional method of repeated freezing and de-freezing during winter. The present study aimed at developing predictive modeling of *S. aureus* growth on Gwamegi as a function of temperature (10–35°C). Modified Gompertz, Baranyi, and logistic primary models were fitted to experimental values. Polynomial quadratic, nonlinear Arrhenius and square root models were selected as secondary models and analyzed using specific growth rate (μ_{max}) and lag time (λ) values obtained from the primary models. Based on the optimized models derived from the Baranyi and square root equations for μ_{max}, its r^2 and mean square error (MSE) were 0.991 and 0.00058, and bias factor (B_f) and accuracy factor (A_f) were 1.0087 and 1.0801, respectively. The logistic and polynomial quadratic equations for λ, its r^2 and MSE were 0.989 and 0.22834, B_f and A_f were 0.9742 and 1.0271, respectively. These predictive models can provide basic information for quantitative microbial risk assessment of Gwamegi and other processed semidried seafood.

Keywords Gwamegi · predictive model · *Staphylococcus aureus* · temperature · validation

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Introduction

Gwamegi, a semidried seafood, is traditionally prepared by wind drying blueback fish (Pacific saury [*Cololabis saira*] or Pacific herring [*Clupea pallasi*]) in the shade for more than 2 weeks during winter until the moisture content reaches approximately 30%. However, the natural drying method may deteriorate the product quality, because it facilitates the growth of pathogens not only during production but also during storage (Oh et al., 1998; Kim and Kim, 2005). Furthermore, because the manufacturing and drying processes by handwork is required to make Gwamegi; however, it has very high potential for the food to become contaminated with *S. aureus* due to its transfer from workers' hands during the process of making Gwamegi. In a recent study, *S. aureus* was detected on about 27% (2–4 $\log_{10}$ cfu/g) of 22 kinds of commercial Gwamegi samples (Kang et al., 2011), indicating that it is an important pathogen of Gwamegi.

Staphylococcus aureus is the most common cause of staphylococcal food poisoning, especially in meat products, poultry, and egg products, salad, bakery products, and milk and dairy products. According to the Korea Food and Drug Administration (KFDA, 2011), consumption of food contaminated by *S. aureus* resulted in 172 outbreaks and 7363 hospitalizations between 2002 and 2010. Furthermore, the true incidence of staphylococcal food poisoning may be much higher, because many cases are not reported to the organization of health (Valero et al., 2009).

Predictive microbiology is a branch of food microbiology that combines mathematics, statistics, and microbiology to predict microbial behavior in specific food products under defined conditions. It can quantitatively assess microbial risks, because it predicts changes in the existence and growth of pathogenic and decomposing microorganisms during all processes including manufacturing, distribution, sale, and consumption of foods by using mathematical models (Yoon, 2008), which is a valuable

means to predict the growth of microorganisms in food depending upon internal factors such as pH, water activity (a_w), NaCl content, and external factors such as temperature, time, and humidity (Karl and Da-Wen, 1999; Bahk et al., 2006).

Several studies have been conducted for predictive modeling of *S. aureus* growth in various foods, such as ready-to-eat foods (Ding et al., 2010), meat products (Kaban and Kaya, 2006; Park et al., 2010), and milk and dairy products (Fujikawa and Morozumi, 2006; Le et al., 2009). Its growth rate was used to develop a predictive model of its generation time in broth on the basis of temperature, pH, NaCl content, and a_w (Sutherland et al., 1993; Mccann et al., 2003). However, optimized models for predicting *S. aureus* growth on Gwamegi under varying range of temperatures have not been developed, thereby limiting the information on *S. aureus* contamination of Gwamegi and other processed semidried seafood.

Recently, climate is growing warmer in east costal area during winter, thus increasing the likelihood of microbial growth. Against the backdrop, the measurement of pH and concentration of sodium chloride in Gwamegi revealed that the food has become more favorable for *S. aureus* growth. We developed primary models to determine specific growth rate (μ_{max}) and lag time (λ) values of *S. aureus* and their temperature effects on Gwamegi. Based on the primary models, secondary models were developed on μ_{max} and λ. These models were statistically compared to obtain an optimized model, which was validated by using experimental data obtained under isothermal conditions to assess the performance of the proposed models.

Material and Methods

Bacterial strains and culture condition. Three strains of *S. aureus* (ATCC 6538, ATCC 12600, and ATCC 25923) were obtained from the Korean Culture Center of Microorganisms (Korea). They were stored at −80°C in tryptic soy broth (TSB; Difco, BD Diagnostics, USA) with 15% sterile glycerol as a cryoprotector. The stock cultures were transferred to tubes containing 10 mL of TSB and incubated at 37°C for 24 h.

Sample preparation. Gwamegis were purchased from the JungWon Fishery Union Corporation (Korea). Raw fishes (Pacific saury) were from the North Pacific, where they were caught, frozen, processed, and stored at lower than −18°C. Samples were kept on ice and transported in an ice pack to the laboratory. Samples were processed at HACCP-certified plants to prevent *S. aureus* contamination. Random-sampling prior to the experiment confirmed no presence of *S. aureus*. They were then cut into 3.5×2.5×0.5 cm pieces (length × width × height, ~5 g) by using a sterile surgical knife.

Inoculation and packaging. Three strains were centrifuged at 5,000×g for 10 min and then gently washed three times using buffered peptone water (UK). Subsequently, an *S. aureus* cocktail was prepared by mixing equal volumes of the three bacterial suspensions. One milliliter of this starter culture was mixed with 9 mL of buffered peptone water and then serially diluted before inoculation into the Gwamegi samples. The Gwamegi samples (160 g) were immersed in 250 mL buffered peptone water containing *S. aureus* at a final concentration of 5 $\log_{10}$ cfu/mL and shaken at 10°C for 10 min to ensure even distribution of bacterial cells using the shaking incubator. The inoculated samples were then aseptically divided into sterile plastic bags, with four pieces (19–20 g) per bag.

Incubation and enumeration. The inoculated samples were incubated at constant temperatures of 10, 15, 20, 25, 30 or 35°C. Sampling was performed at 2-h intervals for the 30 samples at 35°C, 3-h intervals for the 20 samples at 25°C, and 6-h intervals for 10 samples at 15°C. At each interval, 0.1 mL aliquots of the appropriately diluted samples were spread onto Baird-Parker agar (Difco) plates and incubated at 37°C for 48 h, and colonies were counted. Each sampling experiment was repeated twice per trial and three plates were counted at each interval.

Primary models. The primary model is a sigmoidal function that describes bacterial growth curve exclusively as a function of time under constant environmental conditions (e.g., temperature, pH, a_w). The modified Gompertz model (Eq. 1), logistic model (Eq. 2), and the Baranyi model (Eq. 3) are the most frequently used to describe growth curves of bacteria on food (Gibson, 1987; 1988):

$$N_t = N_0 + C \times \exp\{-\exp[(2.718 \times \mu_{max}/C) \times (\lambda - t) + 1]\} \quad (1)$$

$$N_t = N_0 + C/\{1 + \exp[(-2.718\mu_{max}/N_0) \times (t - \lambda + N_0/2.718 \times \mu_{max})]\} \quad (2)$$

Equations 1 and 2: N_t is the bacterial count ($\log_{10}$ cfu/g) at time t (h), C is the population density (difference between the initial and the final bacterial counts), N_0 is initial bacterial count ($\log_{10}$ cfu/g), μ_{max} is the specific growth rate ($\log_{10}$ cfu/g), and λ is the lag time (h).

Baranyi and Roberts (1994) also introduced a model (Eq. 3) that describes sigmoidal bacterial growth curves under constant environmental conditions:

$$N_t = N_0 + \mu_{max} f_t - \ln\left[1 + \frac{e^{\mu_{max} f(t)} - 1}{e^{(y_{max} - y_0)}}\right] \quad (3)$$

$$f_t = t + \frac{1}{\nu}\ln[e^{-\nu t} + e^{-h_0} - e^{(-\nu t - h_0)}]$$

In the equation, N_t is the cell density [ln(cfu/g)] at time t (h), N_0 is the initial cell density [ln(cfu/g)], y_{max} is the maximum cell density [ln(cfu/g)], μ_{max} is the specific growth rate [ln(cfu/g], v is the rate of increase in the limiting substrate (assumed to be equal to μ_{max}), h_0 is the lag time, and λ is the lag-phase duration (h).

Secondary models. The secondary model describes the effects of temperature on parameters of a primary model. The square root model (Eq. 4), polynomial quadratic model (Eq. 5), and nonlinear Arrhenius model (Eq. 6) were used to analyze the effects of temperature on the specific growth rate (μ_{max}) and lag time (λ),

respectively, calculated from the primary models by using SPSS 18.0 software (IBM-SPSS, Chicago, IL, USA), as follows:

$$\sqrt{\mu_{max}} = b(T - T_{min}) \quad (4)$$

where T is the temperature, T_{min} is the theoretical minimum temperature for bacterial growth, and b is a regression constant. T_{min} is a model parameter and can vary by 0–10°C from the minimum temperature at which growth is actually observed.

$$\sqrt{\mu_{max}} \text{ and } \lambda = b_0 + (b_1 \times T) + (b_2 \times T^2) \quad (5)$$

$$\sqrt{\mu_{max}} \text{ and } \lambda = b_0 + (b_1 / T) + (b_2 / T^2) \quad (6)$$

where b_0, b_1, and b_2 are regression constants and T represents temperature.

Model Validation. The primary and secondary models were validated by using coefficients of determination (r^2) in GraphPad Prism v4.0 (GraphPad Software, USA). The r^2 statistic is often used for measuring the goodness of fit: a higher r^2 value indicates better predictability. It measures the fraction variation of the mean. As the value of r^2 approaches 1, the relationship between the model and its values improves (Duffy et al., 1994).

The mean square error (MSE), bias factor (B_f), and accuracy factor (A_f) were defined by the following equations (Yoon et al., 2008; Yang et al., 2009; Zhou et al., 2009):

$$\text{MSE} = \frac{RSS}{n} = \frac{\Sigma(\mu_{observed} - \mu_{predicted})^2}{n} \quad (7)$$

MSE is a measure of residual variability between predicted and observed values that are not accounted for by deliberate changes in factors such as temperature, pH, and a_w. As no parameters are estimated, the degrees of freedom equal the number of data points. This remaining variability may originate from several sources including natural variability and systematic errors. A lower MSE indicates better adequacy of the model to describe the data (Sutherl and Bayliss; 1994).

$$B_f \text{ for } \lambda = 10^{\Sigma \log(X_{predicted}/X_{observed})/n}$$

$$B_f \text{ for } \mu_{max} = 10^{\Sigma \log(X_{observed}/X_{predicted})/n} \quad (8)$$

$$A_f \text{ for } \lambda = 10^{\Sigma |\log(X_{predicted}/X_{observed})|/n}$$

$$A_f \text{ for } \mu_{max} = 10^{\Sigma |\log(X_{observed}/X_{predicted})|/n} \quad (9)$$

where n is the number of predictions, and X represents the μ_{max} and λ values. The mean values of B_f and A_f were used as the overall measures of prediction bias and accuracy, respectively, and were also quantified by using the equation described by Ross (1996). B_f and A_f are valuable indices for evaluating the performance of predictive models (Dalgaard and Jorgensen, 1998). Different values of μ_{max} and λ were used so that B_f and A_f <1 would represent fail-safe predictions and B_f and A_f >1 would represent fail-dangerous predictions (Abou-Zeid, 2006). B_f >1 indicates overprediction, B_f <1 indicates underprediction, and B_f = 0.9–1.05 indicates high suitability (Ross, 1996). According to Yang (2009) predictions exceeding the observed values and less than 10% on average in terms of $\log_{10}$ cfu/g were considered accurate: that is, $1.0 < B_f < A_f < 1.1$ was defined as the proper limit. Additional two sets of data were used to evaluate the predictive model. The samples were incubated at 27.5 and 32.5°C to compare optimized model as fitted curve.

Result and Discussion

Growth characteristics. The growth curves of *S. aureus* on Gwamegi under isothermal conditions are presented in Fig. 1; the growth characteristics were observed on Gwamegi samples inoculated with *S. aureus* for 180 h. *S. aureus* growth increased typically at higher storage temperatures and reached the maximum (8.5 $\log_{10}$ cfu/g) when Gwamegi was stored for 20 h at 35°C. In addition, the bacteria survived even at the lowest temperature (10°C), but showed restrained growth at less than 10°C. Valero et al. (2009) reported that *S. aureus* growth is restrained at temperatures less than 8°C under optimal a_w and pH conditions.

Comparison of the primary models. All experimental data obtained under isothermal conditions were fitted into the three primary models. Fig. 1 illustrates that the selected models can be used to fit the data generally. The μ_{max} values increased in proportion to the temperature (15–35°C), whereas the λ values tended to decrease in proportion to the temperature (20–35°C).

The parametric values and performance statistics of the fitted equations at each tested temperature are shown in Table 1. These models provided a good statistical fit for the observed data, with average r^2 values of 0.974, 0.963, and 0.972 in the modified Gompertz, Baranyi, and logistic models, respectively. The modified Gompertz model provided the best fit for the growth data, followed by the Baranyi and logistic models. Although these primary models showed a similar fit for growth curves, the Baranyi model describes linear phases directly, whereas the modified Gompertz and logistic models describe these phases in a more complex way (Xiong et al., 1999).

Secondary modeling of the effects of temperature on *S. aureus* growth on Gwamegi. The secondary models were used to determine the relationships between the growth parameters of *S. aureus* and the storage temperature of Gwamegi. The effects of temperature on the μ_{max} and λ values estimated from the primary models are shown in Tables 2 and 3.

The square root model was evaluated for its ability to predict μ_{max} as a function of temperature as described by Ratkowsky (1982). The secondary models derived from the primary models, the goodness of fit of the Baranyi model to the square root equation was found to be the best. A previous study (Fujikawa

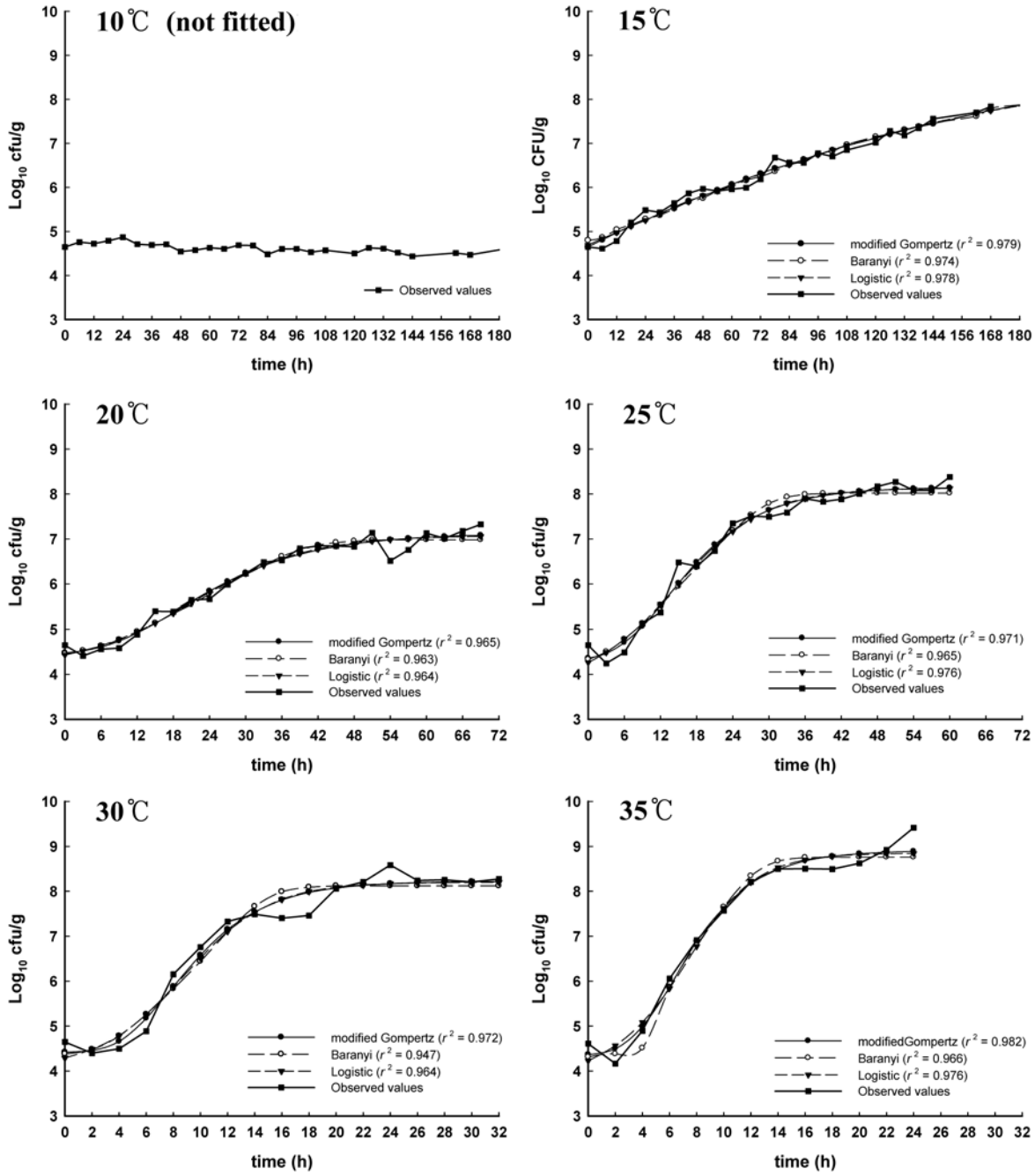

Fig. 1 Comparison of the growth curves of *S. aureus* on Gwamegi as a function of temperature calculated from the three Primary models.

and Morozumi, 2006) showed similar results. In terms of B_f and A_f values as well as statistical characteristics, the Baranyi model provided better values than others (Zhou et al., 2009). Furthermore, it provides the best fit and has the lowest MSE values (Juneja et al., 2007). Graphically, the square root model derived from the Baranyi model provided the best fit, and had the highest r^2 and lowest MSE values.

The polynomial quadratic model was used for predicting λ. The logistic model previously used for thermal inactivation data may also be useful for describing the slow death of bacteria and sensitivity to adverse conditions (Little et al., 1994). In the present study, the polynomial quadratic model derived from the logistic model showed the best fit.

Statistical evaluation of the models. Statistical data from the modified Gompertz, Baranyi, and logistic models for predicting *S. aureus* growth on Gwamegi are presented in Tables 2 and 3. For

Table 1 Parametric values of the three Primary models of *S. aureus* growth on Gwamegi

Temp. (°C)	Modified Gompertz model (Eq. 1)				Logistic model (Eq. 2)				Baranyi model (Eq. 3)			
	C[1)]	μ_{max}[2)]	λ[3)]	r^2	C	μ_{max}	λ	r^2	C	μ_{max}	λ	r^2
35	4.55	0.503	2.960	0.982	4.84	0.577	8.074	0.976	4.44	0.461	2.771	0.966
30	3.80	0.370	4.048	0.972	4.15	0.476	9.519	0.964	3.74	0.329	3.629	0.947
25	3.84	0.163	4.549	0.971	3.71	0.184	14.75	0.976	3.70	0.143	3.770	0.965
20	2.67	0.080	6.602	0.965	4.16	0.160	21.63	0.964	2.53	0.074	6.117	0.963
15	NO[4)]	0.042	NO	0.979	NO	0.001	NO	0.978	NO	0.020	NO	0.974
10	-	-	-	-	-	-	-	-	-	-	-	-

[1)]C, population density ($\log_{10}$ cfu/g).
[2)]μ_{max}, specific growth rate ($\log_{10}$ cfu/g).
[3)]λ, lag time (h).
[4)]NO, no observation.

Table 2 Various statistical characteristics of Secondary models for specific growth rate obtained from three Primary models

Primary model	Secondary model	r^2	P value	MSE[1)]	B_f[2)]	A_f[3)]
	Square root model (Eq. 4)[4)]					
Modified Gompertz	$\sqrt{\mu_{max}}=0.02668(T-8.441)$	0.978	0.001	0.00136	1.0152	1.1668
Baranyi	$\sqrt{\mu_{max}}=0.02756(T-10.168)$	0.991	0.001	0.00058	1.0087	1.0801
Logistic	$\sqrt{\mu_{max}}=0.03492(T-11.767)$	0.922	0.009	0.00862	0.5576	2.4919
	Polynomial quadratic model (Eq. 5)					
Modified Gompertz	$\sqrt{\mu_{max}}=0.14-0.02T+0.009T^2$	0.984	0.016	0.00124	0.6790	1.4728
Baranyi	$\sqrt{\mu_{max}}=0.084-0.016T+0.001T^2$	0.989	0.011	0.00076	0.4746	2.1070
Logistic	$\sqrt{\mu_{max}}=-0.208-0.008T+0.0004T^2$	0.952	0.048	0.00547	0.7702	1.6360
	Nonlinear Arrhenius model (Eq. 6)					
Modified Gompertz	$\sqrt{\mu_{max}}=1.855-62.73/T+534.2/T^2$	0.985	0.015	0.00115	1.0237	2.1514
Baranyi	$\sqrt{\mu_{max}}=1.669-56.03/T+470.9/T^2$	0.982	0.018	0.00122	1.0096	2.8703
Logistic	$\sqrt{\mu_{max}}=1.828-56.32/T+436.0/T^2$	0.943	0.057	0. 10749	0.5213	75.524

[1)]MSE, mean square error.
[2)]B_f, bias factor.
[3)]A_f, accuracy factor.
[4)]T, temperature (°C).

Table 3 Various statistical characteristics of Secondary models for lag time obtained from three Primary models

Primary model	Secondary model	r^2	P value	MSE[1)]	B_f[2)]	A_f[3)]
	Polynomial quadratic model (Eq. 5)[4)]					
Modified Gompertz	$\lambda=17.8-0.759T+0.01T^2$	0.967	0.181	0.22877	1.0750	1.0750
Baranyi	$\lambda=20.5-1.023+0.015T^2$	0.931	0.263	0.42720	1.0236	1.0875
Logistic	$\lambda=0.208-0.008T+0.0004T^2$	0.998	0.045	0.22834	0.9742	1.0271
	Nonlinear Arrhenius model(Eq. 6)					
Modified Gompertz	$\lambda=0.755+36.75/T+1586/T^2$	0.977	0.152	0.16062	1.0015	1.0453
Baranyi	$\lambda=5.9-245.7/T+4976/T^2$	0.959	0.203	0.25469	1.0020	1.0650
Logistic	$\lambda=0.911-4.136/T+8246/T^2$	0.993	0.085	0. 81814	1.0005	1.0376

[1)]MSE, mean square error.
[2)]B_f, bias factor.
[3)]A_f, accuracy factor.
[4)]T, temperature (°C).

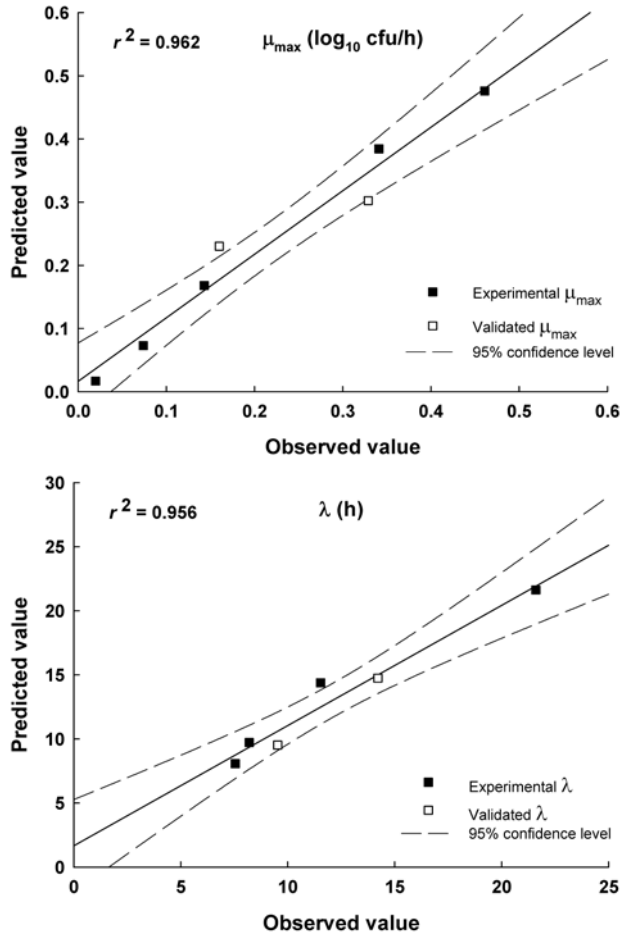

Fig. 2 Comparisons of observed and predicted values of *S. aureus* growth on Gwamegi obtained from the Optimized models. Specific growth rate (μ_{max}) values derived from the Baranyi and Square root models, and lag time (λ) values derived from the Logistic and Polynomial quadratic models are shown.

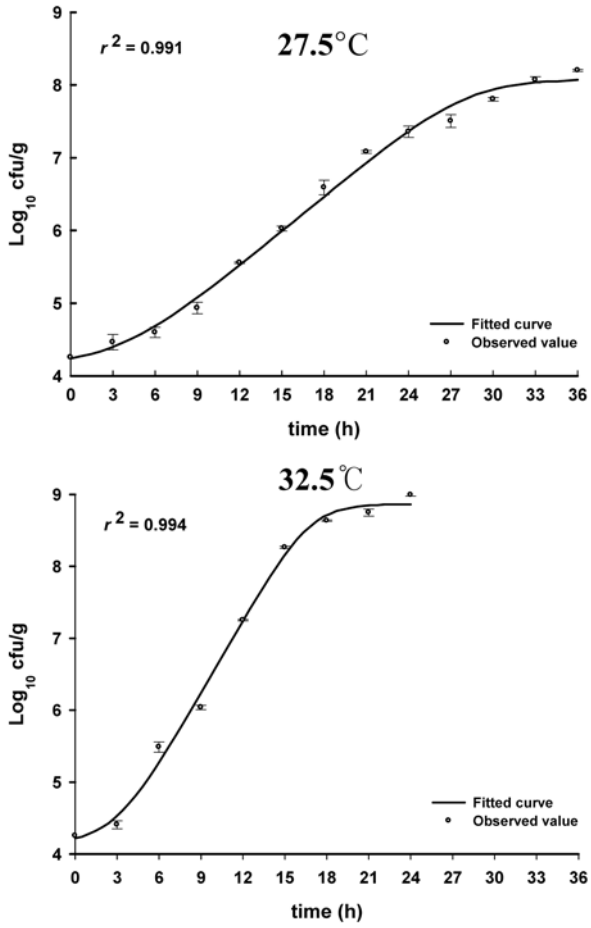

Fig. 3 Validation of the Optimized predictive model derived from the Baranyi equation. The observed data were obtained from *S. aureu*-contaminated Gwamegi samples stored at 27.5 and 32.5°C and are mean (standard deviation) values of triplicate trials.

the optimized secondary models, the predicted and observed values were compared in terms of r^2, MSE, B_f, and A_f, and their statistical suitabilities were also evaluated.

The μ_{max} value derived from the Baranyi and square root models was associated with an r^2 value of 0.991 (p <0.01), which indicates high suitability, and the λ value derived from the logistic and polynomial quadratic models was associated with an r^2 value of 0.998 (p <0.05), which represents high goodness of fit for λ. Comparison of the MSE values showed that the Baranyi model provided the closest prediction of the observed growth data.

In the case of the B_f and A_f values for μ_{max}, the Baranyi model showed the best performance and for λ, the logistic model performed better than the others. Among the secondary models, the square root model was more accurate and had lower prediction bias than the polynomial quadratic model. Taken together, the Baranyi and square root models, the logistic and polynomial quadratic models had better performance than other models.

From Fig. 2, the observed μ_{max} and λ values agreed well with the corresponding predicted values, indicating high goodness-of-fit of the predictive models. The r^2 values of observed μ_{max} and λ obtained from the square root and polynomial quadratic models were 0.962 and 0.956, respectively. Comparison of prediction value with observation value showed the linear regression may predict values closer to the experimental ones (Sheen et al., 2011).

The optimized models were validated by using growth data obtained from inoculated Gwamegi samples stored at 27.5 and 32.5°C. The growth curves from the experimental data were fitted in the optimized models and are presented in Figs 3 and 4. The r^2 values of the optimized models were 0.991 and 0.994 under the given temperatures, indicating that the models described the experimental data well (Fig. 3). In Fig. 4, the predicted and observed values were 27.5 and 32.5°C and close to 99% confidence levels. Therefore, these models can reliably predict *S. aureus* growth on Gwamegi.

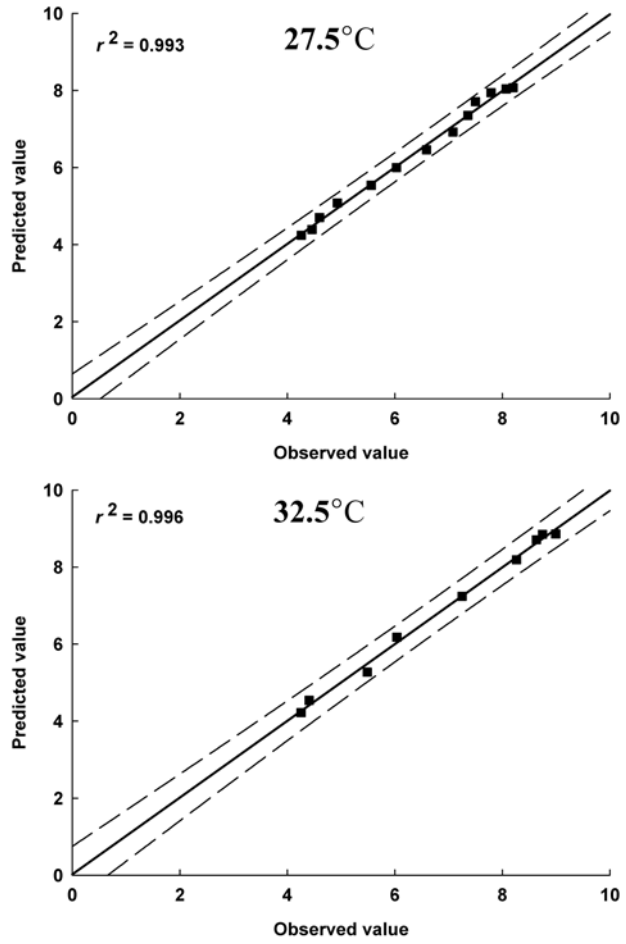

Fig. 4 Validation of the predicted vs. observed values derived from the Baranyi model. The observed values were obtained from *S. aureus*-contaminated Gwamegi samples stored at 27.5 and 32.5°C and are mean (standard deviation and 99% confidential interval) values of triplicate trials.

Comparison of each predictive models of *S. aureus* growth on Gwamegi. Predictive models were developed to describe the growth of *S. aureus* on Gwamegi as a function of temperature (10–35°C). Among the analyzed primary models, the modified Gompertz model provided the best fit for growth data. The optimized square root model derived from the Baranyi model for μ_{max} and the polynomial quadratic model derived from the logistic model for λ have the best predictability. Furthermore, the evaluation results indicate that the Baranyi and logistic models have better performance than the modified Gompertz model for μ_{max} and λ, respectively. These predictive models can provide basic information for quantitative microbial risk assessment of Gwamegi and other processed seafood. In the future, extensive growth kinetics data obtained under nonisothermal conditions are needed to develop the optimal model of *S. aureus* growth in the standard storage environment.

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