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ORIGINAL RESEARCH PAPER

COMPARISON OF PREDICTIVE GROWTH MODELS FOR *BACILLUS CEREUS* IN COOKED AND FRIED RICE DURING STORAGE

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Abstract

This study aimed to develop and compare growth models of *Bacillus cereus* in cooked and fried rice. A cocktail of *B. cereus* was inoculated into cooked and fried rice to obtain an initial concentration of ca. 2 log CFU g^{-1} and stored at 13, 30, 42, and 60ºC. The cell concentration of *B. cereus* during storage were fitted to the primary models of Baranyi, Huang, and modified Gompertz. The maximum specific growth rates (μ_{max}) were fitted to the Ratkowsky model, while the lag phase was fitted using the first-order polynomial model. There was no significant difference ($p>0.05$) in the μ_{max} and lag phase of *B. cereus* in cooked rice or fried rice at 13 and 30ºC. However, at 42ºC the μ_{max} in fried rice was significantly higher ($p<0.05$) and the lag phase was significantly longer ($p<0.05$) than those in cooked rice. No growth was observed at 60ºC. The Baranyi model showed the best performance in fitting the μ_{max} of *B. cereus* in cooked and fried rice. The validation study confirmed that the Ratkowsky and the first-order polynomial model could satisfactorily fit the μ_{max} and the lag phase, respectively. Modeling of *B. cereus* growth in cooked and fried rice can use the primary model of Baranyi fitted to the secondary model of Ratkowsky and first-order polynomial to predict the growth rate and lag phase, respectively. The model suggests that *B. cereus* grows significantly faster in fried rice at 42ºC.

Keywords: *Bacillus cereus*, fried rice, predictive microbiology, rice, validation

Introduction

Bacillus cereus is one of the bacteria causing food borne disease. It can form spores, have ability to adapt to varying conditions, and produce harmful toxins (Jovanovic *et al.*, 2021). The bacteria produce a collection of virulence factors, including

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cereulide that leads to emesis and enterotoxins that leads to diarrhea (Tuipulotu *et al*., 2021). Cereulide toxin is likely not be destroyed or inactivated by processing, because it is resistant to heat, pH, and proteolysis (Rouzeau-Szynalski *et al*., 2020). The enterotoxins are divided into cytolysin K (CytK), hemolysin BL (HBL), non-hemolytic enterotoxin (NHE), with HBL suggested to be the main virulence factor that leads to diarrhea (Granum and Lund, 1997; Berthold-Pluta *et al*., 2019).

B. cereus is one of the most widely distributed bacteria in the environment (Jovanovic *et al*., 2021). In Poland, 27.1% of rice contaminated with *B. cereus* with average count 1.3± 0.36 log CFU g^{-1} (Berthold-Pluta *et al*., 2019). In Korea, 32.6% of starch food from Chinese restaurant were contaminated with *B. cereus*, which 14% of it was from fried rice (Kim and Chang, 2020). In Eastern China, 8.5% of ready-to-eat rice product were tested positive for *B. cereus*: 6.1% in boiled rice, 0.6% in boiled rice noodles, 1.2% in fried rice noodles, 0.1% in sticky rice roll, 0.3% in boiled sticky rice dishes, and 0.2% in fried rice cake with the level of contamination ranged from 1 to 6 log CFU g^{-1} (Chen *et al*., 2022).

Predictive microbiology models are important tools to estimate microbial behavior in products (Possas *et al*., 2021). The models generated from predictive microbiology may assist in the development of Hazard Analysis and Critical Control Point (HACCP) plan, in the improvement of Food Safety Objectives, and in Quantitative Microbial Risk Assessments (QMRA) (Stavropoulou and Bezirtzoglou, 2019; Martinez-Rios *et al*., 2020). The objective of this study was to develop and compare the predictive models for *B. cereus* growth in cooked and fried rice at various storage temperatures. The novelty of this work relies on the development of growth model for cooked and fried rice based on various primary models and use their parameters to generate the secondary models. Moreover, this work also validates the generated models based on their prediction error.

Materials and methods

Bacterial cultures

Bacteria used in this study were *Bacillus cereus* ATCC 10876 from the IPB Culture Collection (IPBCC), *B. cereus* ATCC 11778 from the National Food and Drug Control Agency, and *B. cereus* InaCC B51 from the Indonesian Institute of Sciences. *B. cereus* ATCC 10876 and ATCC 11778 are known to own the genes encoded for diarrhea toxins (Kim *et al.,* 2015). *B. cereus* InaCC B51 was isolated from soils of the Mekongga Sanctuary Forest, Tinukari, North Kolaka, Southeast Sulawesi, Indonesia but its pathogenicity is unknown. The bacteria were confirmed by Gram stain as well as biochemical tests, including the catalase activity test (BAM, 2020b), lecithinase, and mannitol fermentation (BAM, 2020a).

Preparation of cooked and fried rice

Long-grain rice was obtained from a local retail store. The rice was rinsed twice and cooked using a rice cooker with a water to rice ratio of 2:1. The rice was cooked for approximately 20 min. The internal temperature of rice after cooking was 90.06±0.04°C. The fried rice was made by frying cooked rice and a commercial instant spice mix containing maltodextrin, salt, MSG, sugar, fat, red chilies, garlic, synthetic flavor, chicken extract, and natural flavors (garlic, oleoresin peppers). Ten grams of cooking oil was heated before 400 g of cooked rice, and 20 g of fried rice instant spice mix were added. The mixture was stirred while heating for ±10 min, until the instant spice mix was evenly distributed. The proximate analysis as well as salt content and pH of the rice and fried rice samples were evaluated.

Sample preparation

Fifty grams of cooked and fried rice were weighed separately in thermal-resistant HDPE plastics, placed in jars, and sterilized using ALP/MC 40 autoclave (Japan) for 15 min. Each sterilized sample was cooled to room temperature. Cocktail of *B. cereus* vegetative cells were inoculated into cooked and fried rice to obtain the initial concentrations of ca. 2 log CFU g^{-1}. Afterwards, the samples were mixed aseptically to ensure an even distribution of the bacteria in the samples. The plastic was then gently compressed to remove excess air and stored back in the jar.

Growth of B. cereus under isothermal conditions

Cooked and fried rice samples that had been inoculated with the *B. cereus* cocktail were stored at 13, 30, 42, and 60ºC in an incubator (Gallenkamp cooled incubator (United Kingdom), Heraeus incubator (Germany), and Fisher incubator (USA)). Two replications were carried out at each storage temperature. Sampling was conducted at 0-5 days for samples stored at 13ºC, at 0-24 h for samples stored at 30-42ºC, and at 0-3 h for those stored at 60ºC.

Bacteria enumeration

Bacterial enumeration was carried out based on BAM, 2020. Serial dilutions were conducted appropriately to produce 15-150 colonies on the plates after incubation for 24 h. The enumeration was conducted in duplicate. The total number of bacteria was counted and expressed as the unit of log CFU g^{-1} (BAM, 2020a).

Primary models

The primary models used to fit the *B. cereus* growth curve were the Baranyi (Baranyi and Roberts, 1994), Huang (Huang, 2008), and modified Gompertz models (Gibson, *et al*., 1987). The primary models were fitted using the Integrated Pathogen Modeling Program (IPMP) 2013. The USDA Agricultural Research Service (ARS) developed this software for data analysis and predictive microbiological model development. The software is available to download for free at https://www.ars.usda.gov/.

The Baranyi model was developed based on the physiological phase of the bacteria (h_0) (Baranyi and Roberts, 1994). The Baranyi model formula is as follows:

$$y_t = y_o + \mu_{max} F(t) - \ln\left(1 + \frac{e^{\mu_{max} \cdot F(t)} - 1}{e^{ymax-yo}}\right) \qquad (1)$$

where:

$$F(t) = t + \frac{1}{v}\ln\left(e^{-vt} + e^{-h_0} - e^{(-vt-h_0)}\right) \qquad (2)$$

where y_t is the concentration of bacterial cells at a certain time (t) (log CFU g^{-1}); y_0 is the initial concentration of bacterial cells (log CFU g^{-1}); y_{max} is the maximum concentration of bacterial cells (log CFU g^{-1}); μ_{max} is the maximum specific growth rate (log CFU h^{-1}); v is the rate of increase in a limited substrate assumed to be equal to μ_{max}; λ is the length of lag phase (h); and h_0 is calculated using the equation of $\mu_{max} \cdot \lambda$.

The Huang model can distinguish the lag and exponential phases more clearly than other models (Huang, 2008). The Huang model formula is as follows:

$$y_t = y_o + y_{max} - \ln \{e^{yo} + [e^{ymax} - e^{yo}]\ e^{-\mu\ max \cdot B(t)} \quad (3)$$

where:

$$B = t + \frac{1}{\alpha} \ln \frac{1 + e^{-4(t-\lambda)}}{1 + e^{4\lambda)}} \quad (4)$$

where α is the lag phase transition coefficient. The α value is 4, obtained from the optimization of the model on *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Clostridium perfringens* (Huang, 2013).

The Gompertz model was first used to analyze the growth of *Clostridium botulinum* influenced by sodium chloride content and temperature. The mathematical function of the Gompertz model was then modified and used to describe the sigmoid curve (Gibson *et al.*, 1987). The modified Gompertz model formula is:

$$y_t = A + C \exp \{-\exp - [B \cdot (t - M)]\} \quad (5)$$

where A is equal to log (x_{max}/x_0); x_0 is the number of the initial bacterial population; x_{max} is the maximum number of the population; B is the growth rate (h^{-1}) when t = M, M is the time (h) when the absolute growth rate is maximum; and C is the asymptotic value as t increases. The specific growth rate (μ_{max}) and the lag phase can be calculated equation 6.

$$\mu_{max} = \frac{BC}{e} \quad (6)$$

$$\lambda = M - \frac{1}{B} \quad (7)$$

Secondary models

The secondary model used to determine the maximum specific growth rate (μ_{max}) of *B. cereus* was the Ratkowsky model. The formula for the Ratkowsky model is:

$$\sqrt{\mu_{max}} = a\,(T - T_o) \quad (8)$$

where T is the temperature; and T_o is the minimum growth temperature (notational). The relationship between the lag phase and growth temperature was analyzed using a secondary polynomial model of the first-order, with equation 9.

$$\sqrt{1/\lambda} = aT + b \qquad (9)$$

where a and b are the coefficients and T is the temperature (Ratkowsky *et al.,* 1982). The model was then validated at 35°C, where the predicted values of the kinetic parameters were compared with the laboratory results.

Evaluation of model performance

The model performance was evaluated using the following parameters: the determination coefficient (R^2), accuracy factor (*Af*), bias factor (*Bf*), root mean squared error (*RMSE*), prediction error, and relative error (*RE*). The R^2 was coefficient of determination and ranges from 0 to 1, where the closer to 1, the better the prediction of the model constructed. *Af* describes the model's accuracy; *Bf* assesses whether the prediction is higher or lower than the model, while *RMSE* depict the consistency of the model constructed (Ross, 1996; Zhao *et al.,* 2001). Prediction error was used to measure the absolute error. The formula of model performance evaluation is as follows:

$$Af = 10^{(\Sigma \mid \log (\text{Xpredicted/Xobserved})\mid/n)} \qquad (10)$$

Af equal to 1 is considered as good, the greater the *Af*, the less accurate the prediction is.

$$Bf = 10^{(\Sigma \log (\text{Xpredicted/Xobserved})/n} \qquad (11)$$

A *Bf* value of 0.9-1.05 is considered as good, 0.7-0.9 or 1.06-1.15 is acceptable, whilst 0.5-0.7 is not acceptable.

$$RMSE = \sqrt{(\Sigma \tfrac{1}{n}(\text{predicted} - \text{observed})^2)} \qquad (12)$$

The lower the *RMSE* values, the better the model constructed.

Prediction error = Predicted - Observed

RE for λ = (Predicted – Observed)/ Predicted

RE for $\mu_{\max}$ = (Observed – Predicted)/ Predicted

Statistical analysis

Analysis of variance (*ANOVA*) was used to observe the difference in the values of growth kinetic parameters in different models, while the t-test was used to analyze the effect of rice and fried rice media on the values of growth kinetic parameters. Statistical analysis was performed using Microsoft Excel 365 and SPSS v.16.0.

Results and discussion

Primary models of B. cereus growth in cooked and fried rice

The growth curves of *Bacillus cereus* in cooked and fried rice are presented in Figure 1. *B. cereus* growth was observed at 13-42ºC, while no growth was seen at 60ºC. The results of this study were similar to that conducted by Juneja *et al*. (2019a) who observed the growth of *B. cereus* at temperatures of 13-46ºC in cooked rice. Kim *et al*. (2018b) reported that *B. cereus* was able to grow in fried rice at storage temperatures of 25, 35, and 45ºC. However, no growth was observed at 10ºC. Gilbert *et al*. (1974) concluded that *B. cereus* was able to grow in rice in the temperature range of 4-55ºC, with minimum and maximum growth temperatures reported by Finlay *et al*. (2000) at 12 and 46ºC, respectively.

B. cereus growing in cooked and fried rice at various storage temperatures resulted in differences in the $\mu_{\max}$ and lag phase values. The values of the growth kinetic parameters and performance evaluation of the primary models are presented in Table 1. There was no significant difference ($p>0.05$) in the growth kinetic parameters of the $\mu_{\max}$ and lag phase of *B. cereus* at 13 and 30ºC in cooked rice as well as in fried rice. However, at 42ºC, the $\mu_{\max}$ of fried rice was significantly higher ($p<0.05$) than that of cooked rice.

Increases in the lag time of *B. cereus* in fried rice could be due to the increase in pH from 8.32 in cooked rice to 8.96 in fried rice as well as the addition of seasoning. The fried rice also had a higher salt content which can inhibit bacterial growth. Bae *et al*. (2012) reported that seasoned spinach had a longer lag phase than unseasoned spinach. The addition of garlic in seasoned spinach has been reported to have antibacterial activities that could suppress bacterial growth. Moreover, another study conducted by Kim *et al.* (2018a) revealed that an extended lag phase of *B. cereus* occurred when the pH was increased from 8 to 9. The lag phase of other bacteria such as *E. coli* O157:H7, *L. monocytogenes*, *S. aureus*, and *Salmonella* spp. also decreased as the pH increased from 5 to a pH of 6-8, and then started to increase from pH 9.

The largest $\mu_{\max}$ occurred in fried rice at 42ºC. Data from other studies shows that bacteria such as *B. cereus*, *E. coli* O157:H7, *L. monocytogenes*, *S. aureus*, and *Salmonella* spp. have optimal conditions for growth at a pH of 9 and a storage temperature of 35°C. All of these bacteria had lower growth rates in an acidic environment than in an alkaline environment (Kim *et al*., 2018a).

Table 1 also indicates that all models can be used to fit the growth of *B. cereus* in cooked and fried rice at all storage temperatures. The growth curves of *B. cereus* in cooked and fried rice fitted to the modified Gompertz model have the highest R^2 values at all storage temperatures.

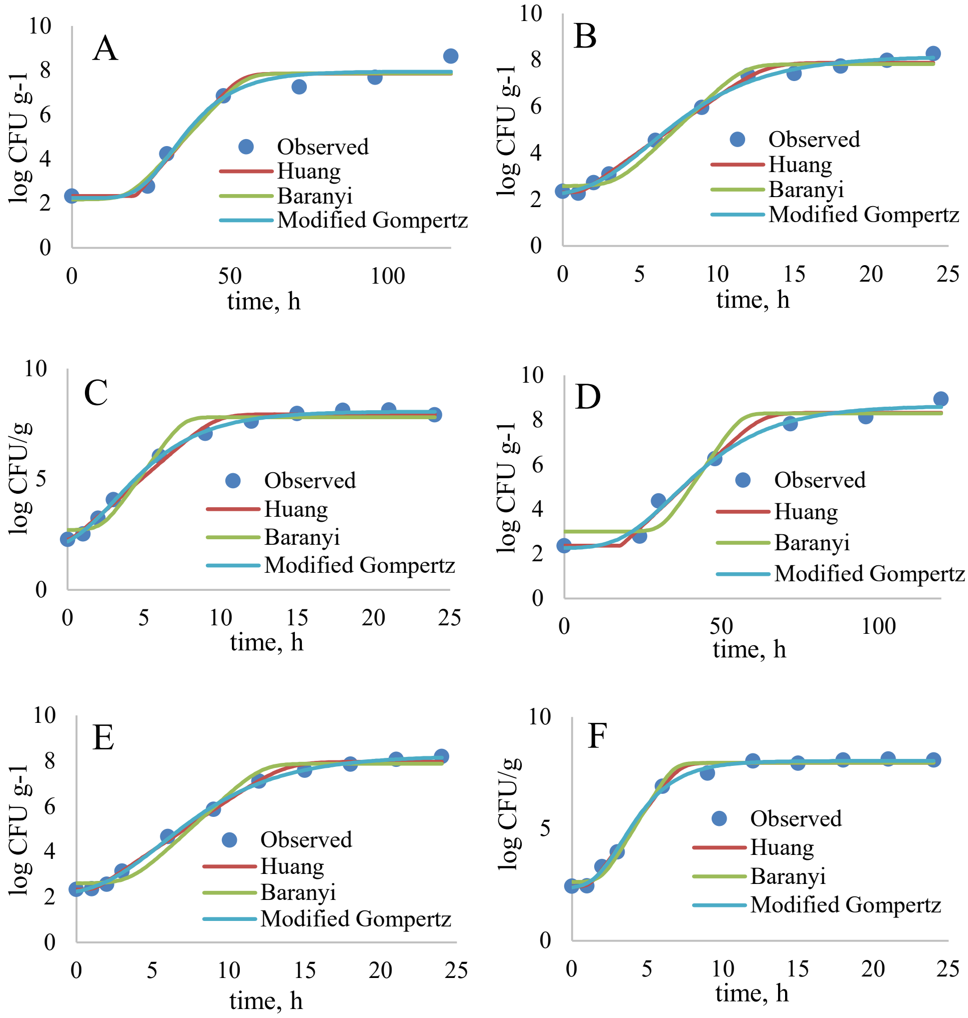

Figure 1. Growth curves of *B. cereus* in cooked rice at storage temperatures of A) 13ºC, B) 30ºC, C) 42ºC and in fried rice at storage temperatures of D) 13ºC, E) 30ºC, and F) 42ºC.

The modified Gompertz model had the lowest *RMSE* values ranging from 0.090 to 0.365 in cooked rice and 0.083-0.294 in fried rice, which were similar to a previous study conducted by Pla *et al.* (2015). It was reported that the modified Gompertz model of *E. coli* CECT 515 growth at 37ºC had a lower *RMSE* value than the Baranyi model. A similar result was also found in Kowalik *et al.* (2018) for an *E. coli* growth model in mascarpone cheese at 3-15ºC.

Table 1. Growth kinetic parameters of *B. cereus* at different growth temperatures.

Temp (°C)	Model	Cooked rice[1,2]				Fried rice[1,2]			
		λ (h)	μ_{max} (log CFU h^{-1})	R^2	*RMSE*	λ (h)	μ_{max} (h^{-1})	R^2	*RMSE*
13	Baranyi	$22.23\pm3.72^{a,a}$	$0.19\pm0.03^{a,a}$	0.968	0.415	$25.51\pm1.55^{a,a}$	$0.18\pm0.04^{a,a}$	0.941	0.598
	Huang	$20.16\pm1.36^{a,a}$	$0.17\pm0.00^{a,a}$	0.971	0.397	$16.98\pm3.54^{a,a}$	$0.13\pm0.04^{a,a}$	0.978	0.367
	Modified Gompertz	$20.77\pm1.00^{a,a}$	$0.20\pm0.01^{a,a}$	0.976	0.365	$17.41\pm3.16^{a,a}$	$0.15\pm0.04^{a,a}$	0.986	0.294
30	Baranyi	$3.43\pm0.03^{b,a}$	$0.64\pm0.01^{b,a}$	0.986	0.279	$3.54\pm0.01^{b,a}$	$0.62\pm0.01^{b,a}$	0.984	0.295
	Huang	$1.24\pm0.17^{a,a}$	$0.47\pm0.02^{a,a}$	0.994	0.182	$1.20\pm0.26^{a,a}$	$0.45\pm0.02^{a,a}$	0.997	0.133
	Modified Gompertz	$1.53\pm0.50^{a,a}$	$0.55\pm0.04^{ab,a}$	0.997	0.138	$1.35\pm0.58^{a,a}$	$0.54\pm0.03^{ab,a}$	0.999	0.083
42	Baranyi	$2.26\pm0,02^{b,a}$	$0.96\pm0.01^{c,a}$	0.974	0.372	$1.96\pm0.01^{b,b}$	$1.12\pm0.01^{b,b}$	0.991	0.221
	Huang	$0.14\pm0.13^{a,a}$	$0.61\pm0.02^{a,a}$	0.992	0.199	$1.17\pm0.05^{a,b}$	$0.93\pm0.01^{a,b}$	0.995	0.169
	Modified Gompertz	$0.16\pm0.00^{a,a}$	$0.74\pm0.02^{b,a}$	0.998	0.090	$1.37\pm0.01^{a,b}$	$1.06\pm0.02^{b,b}$	0.997	0.122

[1] *The figures in the same column followed by the same letter are not significantly different at the 5% test level (Tukey's interval test).*

[2] *The figures in the same row followed by the same letter are not significantly different at the 5% test level (t-test).*

The predicted $\mu_{\max}$ values of *B. cereus* in cooked and fried rice using the modified Gompertz model at 30°C were 0.55 log CFU h^{-1} and 0.54 log CFU h^{-1}, respectively. These values were different from the $\mu_{\max}$ of *B. cereus* in spinach stored at the same temperature, i.e. 0.864 log CFU h^{-1} (Bae *et al*., 2012). The $\mu_{\max}$ values from this study were also different from the $\mu_{\max}$ of *B. cereus* in milk predicted using the modified Gompertz model at a storage temperature of 30°C (0.75 log CFU h^{-1}) (Kim *et al*., 2013). The different types of growth media resulted in different growth rates, as the growth rate was influenced by the concentration of nutrients in the growth media (Allen and Waclaw, 2019). In addition, another study shows that *B. cereus* grows at different rates in different matrixes such as BHI, meat, dairy-based food, and cereal-based food. *B. cereus* in BHI and dairy products has the fastest growth rate, followed by meat and vegetable-based products. *B. cereus* growth in a cereal-based matrix was reported to have the lowest growth rate (Ellouze *et al*., 2021).

The Baranyi model predicted a longer lag phase of *B. cereus* in fried rice at 30 and 42°C than the other models. However, there was no difference in the lag phase predictions for all primary models at 13°C, although the Baranyi model tends to make longer predictions than other models. The $\mu_{\max}$ values predicted by the Baranyi model were not significantly different from the modified Gompertz model but significantly different from the Huang model. The Baranyi model predicted the fastest growth rate. Furthermore, the $\mu_{\max}$ values at 42°C as predicted by the Baranyi model and the modified Gompertz model were also greater than the other models.

Differences in the models' assumptions can result in a difference in the predicted values of the primary models. The Baranyi model generally generates good predictive values due to the dynamic nature of the model. This model assumes that the specific growth rate during the lag phase depends on the need for each cell to synthesize intracellular substances, known as the bottleneck model (Baranyi and Roberts, 1994).

Although the modified Gompertz model provides a good degree of accuracy in the estimation of microbial growth, it only performs well under fully defined growth curves for all phases. Some conditions of the growth curves do not have a lag phase, so the prediction of the lag phase is not accurate. It makes mechanistic models preferable for predicting bacterial growth since they are constructed on a theoretical basis and usually have fewer parameters and better data adjustment and extrapolation (Juneja *et al*., 2009). We observed that at 42°C, the average lag phase of cooked rice as predicted by the modified Gompertz model was negative (-0.19 h, data not shown), hence the data was considered inaccurate and was thus excluded for further analysis.

Secondary models of growth kinetic parameters of B. cereus in cooked rice

The performance evaluation of each secondary models is presented in Table 2.

The data shows that the Baranyi model has the best performance for predicting the $\mu_{\max}$ of *B. cereus* in cooked and fried rice as indicated by the best *RMSE*, *Af*, and *Bf* values.

Table 2. Performance evaluation of μ_{max} by the Ratkowsky model and lag phase by a first-order polynomial model on the growth kinetic parameters of *B. cereus* in cooked rice.

Parameter	**Model**	**T (°C)**	**O**	**P**	***RE***	***RMSE***	R^2	***Af***	***Bf***
$(\mu_{max})^{1/2}$	Baranyi	13	043	0.44	-0.023	0.021	0.992	1.029	1.004
		30	0.80	0.77	0.041				
		42	0.98	1.00	-0.020				
	Huang	13	0.41	0.43	-0.037	0.028	0.968	1.042	1.002
		30	0.69	0.65	0.062				
		42	0.78	0.80	-0.027				
	Modified Gompertz	13	0.44	0.46	-0.043	0.025	0.980	1.035	1.001
		30	0.74	0.70	0.057				
		42	0.86	0.88	-0.023				
$(1/\lambda)^{1/2}$	Baranyi	13	0.21	0.23	0.073	0.029	0.979	1.070	1.016
		30	0.54	0.50	-0.081				
		42	0.67	0.69	0.035				
	Huang	13	0.22	N/A	N/A	N/A	0.758	N/A	N/A
		30	0.90	N/A	N/A				
		42	5.98	N/A	N/A				
	Modified Gompertz	13	0.22	0.02	-7.927	0.336	0.878	2.505	0.538
		30	0.84	1.31	0.357				
		42	2.50	2.22	-0.125				

O: observed value; P: predicted value; RE: relative error; RMSE: root mean squared error; R^2: coefficient of determination; Af: accuracy factor; Bf: bias factor

The results of this study are similar to another study by Juneja *et al.* (2019a) which reported that the Baranyi model has the best compatibility with the Ratkowsky secondary model in cooked rice with a R^2 of 0.992 and *RMSE* of 0.021. Similar results were obtained for modeling the maximum growth rate of *B. cereus* in pasta (Juneja *et al.*, 2019b) and peas (Juneja *et al.*, 2018). The *RE* values obtained in this study vary between -0.037 to 0.062 for $\mu_{\max}$. This means that all models are categorized as fail-safe because they have a value between -0.3 (fail-safe) to 0.15 (fail-dangerous) (Oscar, 2005b).

Table 2 also shown that the best model to be used to fit the lag phase is Baranyi Model, which has the best R^2, *RE*, *RMSE*, *Bf* and *Af* value. Huang Model cannot be used to fit the lag phase because the low R^2 value. Furthermore, the modified Gompertz model cannot be used because RE value fall into fail dangerous zone at low temperature growth.

One of the weaknesses in predicting the lag phase is the effects of history prior to exposure to heat. Bacteria treated with heat shock may have a different lag phase than those without heat shock treatment. Hence, the model could be inaccurate for predicting the lag phase under different bacterial growth scenarios (Juneja *et al*., 2018). This study used bacterial vegetative cells without heat shock treatment; therefore, they may have different lag phases as compared to those inoculated in the form of spores with prior heat shock.

Secondary models of growth kinetics parameters of B. cereus in fried rice

Model performance evaluation shows that all secondary models can be used to fit the growth kinetic parameters of fried rice as presented in Table 3. The data shows that the Baranyi model has the lowest *RMSE* value when predicting $\mu_{\max}$. The R^2 value of the Baranyi model is the best as compared to the other models followed by the modified Gompertz model. This model also has *Af* and *Bf* values closest to 1. The *Bf* value of this model is 1.001 which indicates that the model predicts the maximum specific growth rate faster than the actual growth rate. This can be seen at the temperature observation of 30ºC. All the secondary models' equations constructed based on the above four secondary models have good *RE* values in the range of -0.3 (fail-safe) and 0.15 (fail-dangerous). The models are also categorized as acceptable because the $\mu_{\max}$ deviation values are not greater than 30% in the fail-safe direction (Oscar, 2005a).

Table 3 also shows that the Baranyi model has the best *RMSE*, *Af*, and *Bf* values for predicting the lag phase, followed by the Huang and modified Gompertz models. The Baranyi model generates the lowest *RMSE* value as compared to other models and has the closest *Af* value to1. The *Bf* value of the Baranyi model is categorized as good as and the other models categorized as acceptable as it is within the range of 1.06-1.15. Furthermore, the equations obtained from these four secondary models were validated at a certain temperature within the test temperature range.

Secondary model validation

The validation of the secondary models was carried out at 35°C, which was within the test temperature range. The validation results are presented in Table 4. The data shows that the Baranyi model is the best to be used to fit $\mu_{\max}$ value of *B. cereus* in rice and fried rice at 35ºC as well as lag phase at the same temperature, as shown by the low prediction error values. The Baranyi model has better *RMSE*, *Af*, and *Bf* values than the modified Gompertz model as shown in Table 2 and 3. Hence, the Ratkowsky equation from the primary model of Baranyi is better for predicting the

μ_{max} of *B. cereus*, even though its prediction for growth rate in fried rice at 35°C is slightly lower than the actual growth rate.

Table 3. Performance evaluation on μ_{max} by the Ratkowsky model and lag phase by a first-order polynomial model on the growth kinetic parameters of *B. cereus* in fried rice.

Parameter	Model	T (°C)	O	P	*RE*	*RMSE*	R^2	*Af*	*Bf*
$(\mu_{max})^{1/2}$	Baranyi	13	0.42	0.42	0.000	0.008	0.999	1.007	1.001
		30	0.78	0.79	-0.013				
		42	1.06	1.05	0.010				
	Huang	13	0.36	0.34	0.059	0.022	0.994	1.038	0.992
		30	0.67	0.70	-0.043				
		42	0.96	0.95	0.011				
	Modified Gompertz	13	0.38	0.37	0.027	0.014	0.997	1.021	0.997
		30	0.73	0.75	-0.027				
		42	1.03	1.02	0.010				
$(1/\lambda)^{1/2}$	Baranyi	13	0.20	0.21	0.038	0.014	0,996	1.032	1.004
		30	0.53	0.51	-0,042				
		42	0.72	0.73	0.014				
	Huang	13	0.25	0.32	0.240	0.134	0,827	1.227	1,056
		30	0.93	0.74	-0.253				
		42	0.93	1,04	0,107				
	Modified Gompertz	13	0.24	0.33	0.269	0.154	0,751	1.269	1.063
		30	0.93	0.71	-0,305				
		42	0.86	0.98	0.128				

O: observed value; P: predicted value; RE: relative error; RMSE: root mean squared error; R^2: coefficient of determination; Af: accuracy factor; Bf: bias factor

The lag phase of *B. cereus* in cooked rice can be predicted well by the Baranyi model as indicated by the prediction error values. The modified Gompertz model has lower prediction error for lag phase in fried rice, but the predicted value is shorter than the observed value. It can lead to unsafe prediction. Summarizing this data, it is suggested to use the Baranyi model to fit the *B. cereus* growth curve in both cooked and fried rice and predict the responses of growth kinetic parameters due to temperature changes, by using the Ratkowsky model for the μ_{max} and a first-order polynomial for the lag phase.

Table 4. Validation of growth kinetic parameters *B. cereus* in cooked and fried rice.

Media	**Model**	**μ_{max} (log CFU h^{-1})**		**Prediction error**	**λ (h)**		**Prediction error**
		P	**O**		**P**	**O**	
Cooked rice	Baranyi	0.75	0.73	0.02	2,98	2.98	0.00
	Huang	0.50	0.50	0.00	N/A	0.62	N/A
	Modified Gompertz	0.60	0.65	-0.05	0.34	1.36	-1.02
Fried Rice	Baranyi	0.81	0.83	-0.02	2.78	2.62	0.16
	Huang	0.64	0.61	-0.03	1.33	1.00	0.33
	Modified Gompertz	0.74	0.79	-0.05	1.47	1.52	-0.05

P: predicted value, O: observed value.

Conclusions

In conclusion, we reveal that growth kinetic parameters values of *B. cereus* in cooked and fried rice are significantly different in growth rate and lag phase at 42ºC, where the maximum growth rate occurs. The lag phase of *B. cereus* in fried rice tends to be longer than in cooked rice but has a higher growth rate. The Baranyi model shows good performance for the secondary modelling of Ratkowsky and first-order polynomial of *B. cereus* in cooked and fried rice, validated at 35ºC.

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