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Predictive Modeling for Microbial Risk Assessment (MRA) from the Literature Experimental Data

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Abstract One of the most important aspects of conducting this microbial risk assessment (MRA) is determining the model in microbial behaviors in food systems. However, to fully these modeling, large expenditures or newly laboratory experiments will be spent to do it. To overcome these problems, it has to be considered to develop the new strategies that can be used data in the published literatures. This study is to show whether or not the data set from the published experimental data has more value for modeling for MRA. To illustrate this suggestion, as example of data set, 4 published *Salmonella* survival in Cheddar cheese reports were used. Finally, using the GInaFiT tool, survival was modeled by nonlinear polynomial regression model describing the effect of temperature on Weibull model parameters. This model used data in the literatures is useful in describing behavior of *Salmonella* during different time and temperature conditions of cheese ripening.

Keywords: microbial risk assessment (MRA), predictive modeling, experimental data, *Salmonella* spp., survival

Introduction

Microbial risk assessment (MRA) in food safety is a scientific tool that can be used to evaluate the qualitative or quantitative estimation of potential adverse health effects associated with consumption by foods of individuals or populations regarding pathogenic microorganisms (1,2). The fast development within World Trade Organization (WTO) has encouraged Codex Alimentarius to establish quantitative MRA and develop tools to do so (1,3). The most important purpose of MRA is an answer the risk management questions for some special foods and processing standards etc. To do so, of 4 components of MRA, exposure assessment methodologies have been rapidly developed to take into consideration the complexity of the food production, processing, and storage including the partitioning and mixing of the food (4) and microbial community dynamics (5). One of the most important aspects of this exposure assessment is determining the modeling of food chain. The aim of the modeling is to describe the change in prevalence and number of pathogenic microorganisms per processing unit. However, to fully complete MRA modeling, in particular for model in microbial behaviors in food systems, large sums of money or newly laboratory experiments will be spent to do it. There were some developed predictive modelings on the subjective of Korean foods through laboratory experiment (6,7). The data needs for MRA or modeling are considerable, and sometimes it is suggested that these may be prohibitive to carrying out MRA. If data are insufficient for modeling, they may likewise be insufficient for risk management decisions. To overcome these problems, there is usually can be used the data on available in the literature or in publicly available database. However, there is generally a large gap between these data because they were collected in a different system or because they do not cover all model requirements (8). Once the literature data was used to validation or comparing to developed predictive model (9,10). However, recently the models for the specific growth, reduction, or survival are based on data generated in themselves laboratory and/or obtained from published literature data were performed under well-defined conditions that do not differ markedly from those used to develop the model (11,12). Therefore, there is a need today to develop the new strategies or methods using the data from other sources i.e., previous published literature experimental data because there were large amount of data available on the modeling. To do this, it was proposed a database protocol that encompasses observations of various bacterial responses to various food environments (13), resulted in database 'ComBase' (http://www.combase.cc) for predictive microbiology purpose. The data included in this database were obtained from research institutes and from the literature.

The main purpose of this study was to show whether or not the data set from the previous published experimental data has more value for mathematical modeling or complications of modeling data such as ComBase and it can be used in a stochastic model for some special food processing input into MRA. To illustrate this suggestion, as example of data set, 4 published *Salmonella* survival in Cheddar cheese reports (14-17) were used.

Materials and Methods

Data and curve fitting The data for survival curves used were taken from the literatures where milk was inoculated with *Salmonella* spp. to have 3.78 to 6.04 log CFU after pressing (Table 1). The data reported in figures were scanned and plotted using digitization software program Ungraph 4.0 (Biosoft, Ferguson, MO, USA). Seventeen curves were created and each curve fitted with Weibull

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Table 1. Survival of artificially inoculated *Salmonella* spp. during Cheddar cheese ripening

Curve no.	Species	Used milk[1)]	Temp. (°C)	pH		Log CFU/g or mL		Time (day)	Data source
				Initial	Final[2)]	Initial	Final[2)]		
1	*S. enteritidis*	*P*	8.0	5.20-5.40[3)]		5.11	3.77 (1.34)[4)]	99<	(9)
2	*S. enteritidis*	*R*	8.0	5.20-5.40[3)]		5.76	3.81 (1.95)	99<	(9)
3	*S. typhimurium*	*P*	7.0	5.82	5.30 (0.52)[4)]	4.96	0.60 (4.36)	90	(10)
4	*S. typhimurium*	*P*	7.0	5.80	5.23 (0.57)	4.18	1.23 (2.95)	210	(10)
5	*S. typhimurium*	*P*	7.0	5.65	5.31 (0.34)	6.04	0.30 (5.74)	180	(10)
6	*S. typhimurium*	*P*	7.0	5.71	5.40 (0.31)	5.04	0.35 (4.69)	300	(10)
7	*S. typhimurium*	*P*	7.0	5.78	5.35 (0.43)	4.91	0.80 (4.11)	180	(10)
8	*S. typhimurium*	*P*	13.0	5.82	5.07 (0.75)	4.92	1.31 (3.61)	90	(10)
9	*S. typhimurium*	*P*	13.0	5.80	5.58 (0.22)	4.18	0.90 (3.28)	210	(10)
10	*S. typhimurium*	*P*	13.0	5.65	4.96 (0.69)	6.04	1.12 (4.92)	60	(10)
11	*S. typhimurium*	*P*	13.0	5.71	5.18 (0.53)	5.04	1.92 (3.12)	60	(10)
12	*S. typhimurium*	*P*	13.0	5.78	5.05 (0.73)	4.91	0.90 (4.01)	60	(10)
13	*Salmonella* spp.[5)]	*P*	0.0	5.52	5.22 (0.30)	5.79	1.00 (4.79)	180	(11)
14	*Salmonella* spp.[5)]	*P*	4.5	5.52	5.15 (0.37)	5.79	0.30 (5.49)	150	(11)
15	*Salmonella* spp.[5)]	*P*	10.0	5.52	5.10 (0.42)	5.79	1.78 (4.01)	90	(11)
16	*S. typhimurium*	*P*	7.5	5.10	-[6)]	3.78	-0.40[7)] (4.18)	112	(12)
17	*S. typhimurium*	*P*	13.0	5.10	-	3.78	-0.52[8)] (4.30)	84	(12)

[1)]*P*, pasteurized milk; *R*, raw milk.
[2)]Initial count is after pressing, and final is after ripening.
[3)]Range.
[4)]The difference between initial and final pH values.
[5)]Mixed culture of *S. senftenberg, S. typhimurium, S. new brunswick,* and *S. Newport.*
[6)]The final pH not indicated in published article.
[7)]0.398 CFU/g.
[8)]0.302 CFU/g.

model of the GInaFiT 1.4 (Geeraerd and Van Impe Inactivation Model Fitting Tool, downloaded via the KULeuven/BioTec-hompage http://cit.kuleuven.be/biotec) (18). In GInaFiT, the Weibull model is used as proposed by Mafart *et al.* (19), which can be formulated as follows:

$$\text{Log } (N)=\log (N_0)-((t/\alpha)\text{^}\beta) \quad (1)$$

where, t is time, α is a scale parameter and can be denoted as the time for the first decimal reduction of surviving cells if $\beta=1$, p is a curve shape parameter, concave upward survival curve if $\beta<1$ and concave downward if $\beta>1$. To evaluate the curve fitting and prediction accuracy of each Weibull models, the coefficient of determination (R^2), sum of square error (SSE), and root mean squares error (RMSE) (18) were used.

Polynomial regression modeling and survival rate According to the result of best fit and prediction accuracy, proper curves were selected and modeled by a nonlinear polynomial regression model. The equation was developed by RSREG statistic function using SAS system for Windows V8 (Statistical Analysis System, version 8.1, Cary, NC, USA) to model the effect of temperature on α and β of Weibull model parameters. The survival rate (S_X) calculated follows equation:

$$S_X=N_i/N_0 \quad (2)$$

where, S_X means the proportion of salmonellae surviving to day interval X. N_i is the number (CFU/g) of salmonellae alive (estimated from formula 1) during the initial ripening day (i=0, N_0) and each subsequent day. S_X is daily specific survival rate (1/day) and ranges from 0.0 to 1.0, where 0=all died or at least none detectable, and 1=all survived and capable of growing. Low survival rate means fast inactivation or fewer survivors; high survival rate means slow inactivation or more survivors.

Model validation In order to evaluate the predictive capacity of the developed survival model, bias factor (*Bf*), and accuracy factors (*Af*) (20), together with SSE and RMSE were calculated. The *Bf* 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.

$$Bf=10^{[\Sigma \log (\text{predicted/observed})/n]} \quad (3)$$

The *Af* averages the distance between each point and the line of equivalence as a measure of how close, on average, predictions are to observe.

$$Af=10^{[\Sigma|\log(\text{predicted/observed})|/n]} \quad (4)$$

Results and Discussion

When Cheddar cheeses were ripened at 0-13°C over different time periods, the pH of cheese fell from 0.30 to 0.75 (mean, 0.48), and the number of salmonellae decreased by 1.34-5.74 log CFU/g (mean, 3.93 log CFU/

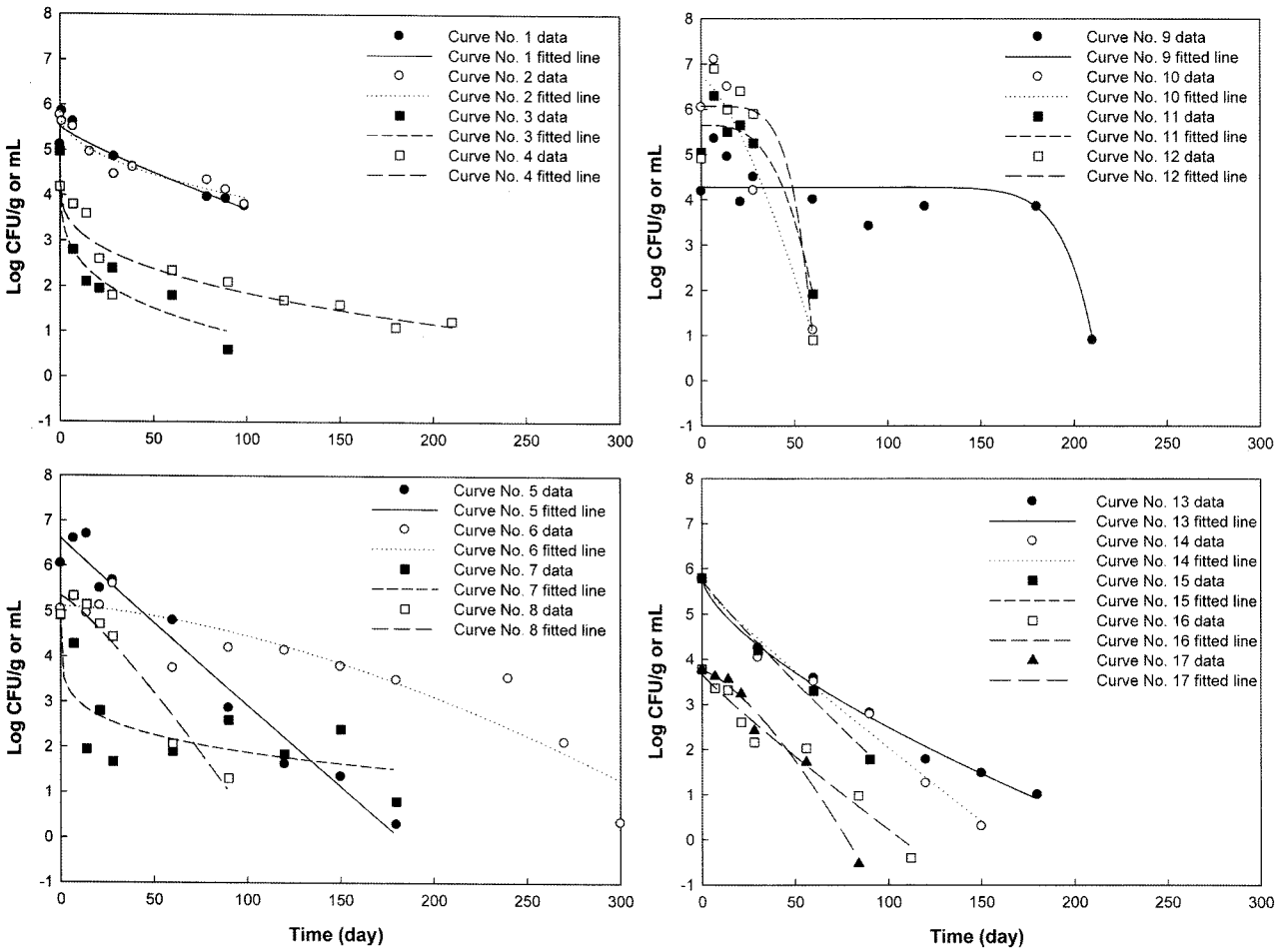

Fig. 1. Seventeen curves fitted with Weibull model of the GInaFiT tool of *Salmonella* spp. survival data from Table 1 for various ripening temperature. Among these curves, 7 curves (curve no. 2, 8, 13, 14, 15, 16, and 17) were selected for polynomial regression modeling.

g), but there were survivors up to 300 days (Table 1).

It was tested and compared with 8 models (log-linear classical, log-linear+shoulder, log-lonear+tail, log-linear +shoulder+tail, Weibull model, Weibull+tail, Biphasic model, and Biphasic+shoulder) included in the GInaFiT tool (18). The results show that only log-linear classical and Weibull model fitted well with all curves derived from data. It is clear that classical log-linear modeling fails to assess accurately the majority of these survival curves (18), since it is known that the typical behavior of survivor curves is non-log-linear with or without tailing/shoulder. Therefore, it was decided that the Weibull model was the most suitable for expression of survival of salmonellae during Cheddar cheese ripening. Figure 1 shows 17 curves fitted with Weibull model of the GInaFiT tool. The Weibull model had already been shown to be suitable for describing inactivation or survival of vegetative cells (21) and spores (19).

According to statistical measure and parameter values (R^2, RMSE, and SSE) obtained from Weibull model, it was selected only 7 curves (curve no. 2, 8, 13,14,15,16, and 17) for polynomial regression modeling (Table 2). The other curves not chosen since firstly they have low R^2 (<0.90, curve no. 1, 4, 6, 7, and 9) and then have high RMSE (>0.5, curve no. 10, 11, and 12) and SSE (>1.0, curve no. 5), secondly curve no. 3 have inappropriate, too low, α and β values. However, β (0.46) and α (20.86) of selected curve no. 2 and 15, respectively, were not used to RSREG because these values are too low in proportion to temperatures.

Survival was modeled by a nonlinear polynomial regression. This equation described the effect of temperature on α and β of Weibull model parameters of reduction of *Salmonella* spp. during Cheddar cheese ripening. The estimated regression coefficients for Weibull parameter (α and β) obtained response surface analysis are showed in Table 3. The regression equation is as follows:

$$\alpha = 17.122286 + 1.634135\mathbf{T} - 0.0527270\mathbf{T}^2 \quad (5)$$

$$\beta = 0.711601 + 0.007036\mathbf{T} + 0.002823\mathbf{T}^2 \quad (6)$$

where **T** is temperature (°C), α is a scale parameter, and β is a curve shape parameter.

A comparison of the predicted values (calculated from Equation 1 and the polynomial regression model (Equation 5 and 6) and the observed data described in Table 1 show some agreement (Table 4). The *Bf* ranges were from 0.93 to 1.09, which compares some with the acceptable range (*Bf* 0.7-1.15) noted by Ross (22). Therefore, this comparison indicates this model might be used to some accurately predict the decline of *Salmonella* spp. in Cheddar cheese

Table 2. Statistical measure and parameter values obtained from the Weibull model in GInaFiT tool using the experimental data described in Table 1[1)]

Curve No.	R^2	RMSE	SSE	α [day]	β [-]	Curves chosen
1	0.8989	0.2700	0.44	49.02	0.84	
2	0.9423	0.1938	0.23	25.85	0.46	*
3	0.9353	0.4142	0.69	0.27	0.24	
4	0.8615	0.4412	1.56	8.31	0.36	
5	0.9670	0.4917	1.69	26.45	0.98	
6	0.8456	0.6205	3.85	131.34	1.62	
7	0.6675	0.8081	4.57	0.29	0.19	
8	0.9493	0.4451	0.79	26.82	1.21	*
9	0.7857	0.6282	2.76	191.57	13.23	
10	0.9363	0.8637	1.49	17.95	1.44	
11	0.9298	0.5276	0.84	39.75	3.20	
12	0.9098	0.8498	2.17	43.92	5.27	
13	0.9929	0.1769	0.13	16.79	0.67	*
14	0.9797	0.3627	0.39	24.52	0.92	*
15	0.9909	0.2773	0.08	20.82	0.93	*
16	0.9399	0.3409	0.58	25.70	0.91	*
17	0.9762	0.2917	0.34	30.81	1.42	*

[1)]R^2, coefficient of determination; RMSE, root mean squares error; SSE, sum of square error; α is a scale parameter, β is a shape parameter.

Table 3. Coefficients values of Weibull parameter (α and β) estimated by RSREG statistic function[1)]

	Coefficients	
	a	b
Intercept	17.122286***	0.711601**
T	1.634135*	0.007036
T^2	- 0.0527270	0.002823
R^2	0.9031	0.8314

[1)]T is temperature (°C); α is a scale parameter, and β is a curve shape parameter. *, **, *** Significant at p=0.10, 0.05, and 0.01 level, respectively. R^2, coefficient of determination.

during a variety of ripening temperature conditions. However, more experimental or observational data are required to validate the model since these should be different from those used to construct the original model (23).

Figure 2 shows the result of polynomial model from regression statistics for the survival of *Salmonella* spp. during ripening at various temperatures and times in Cheddar cheese production. Temperature ranges are from 0 to 13°C. In ripening, the maximum duration was decided at 60 days since there had been much variation in experimental results after 60 days among the literature data source. At 60 days, the decreased salmonellae numbers were the higher at 10-13°C, the lower at 4-8°C. These facts show that *Salmonella* spp. die more readily at higher storage temperatures during Cheddar cheese ripening. This result confirmed the results of the White and Custer (24) study to evaluate *Salmonella* survival in Cheddar cheese. They added *Salmonella* to milk and aged the subsequent cheese for 9 months at 4.5 and 10°C. Of 48 lots of cheese inoculated with the salmonellae, detectable numbers of *Salmonella* appeared in 16 lots (33%) at 4.5°C and in 6 lots

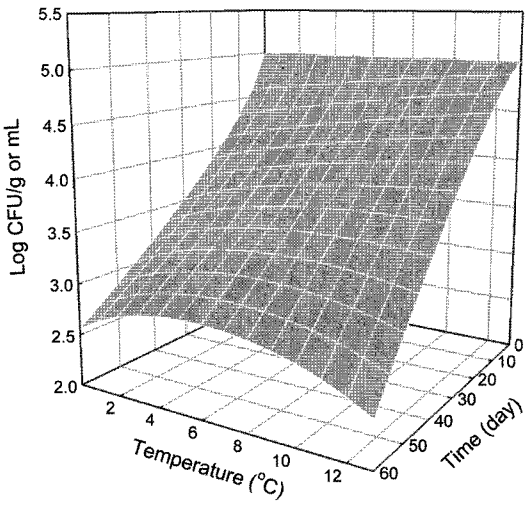

Fig. 2. The result of polynomial regression model from regression statistics for the survival of *Salmonella* spp. during ripening at various temperatures (0-13°C) and storage time (0-60 days) in Cheddar cheese production where the initial inoculum was assumed to be 5 log CFU/g. Result from Equation 1 using Weibull parameter (α and β, Equation 5 and 6) developed by RSREG statistic function.

(12.5%) at 10°C, but no quantization was done. The similar result also reported by Goepfert *et al.* (17); the number of salmonellae decreased by factor of 10,000 during 10 to 12 weeks at 13°C and a similar decrease required 14 to 16 weeks at 7.5°C.

The polynomial regression model was then used to estimate the survival rate (Fig. 3). There is a significant relationship between time and temperature for the survival rate, with a lower survival rate at the lower temperatures

Table 4. Comparison of reported (observed) from the data in Table 1 and estimated (predicted) values (log CFU/g) by response surface model of *Salmonella* spp. reduction during Cheddar cheese ripening

Curve No.	Temperature (°C)	Time (day)	Initial inoculum level[1)] (log CFU/g or mL)	Observed	Predicted
2	8	1	5.76	5.62	5.72
	8	7	5.76	5.51	5.48
	8	16	5.76	4.95	5.14
	8	29	5.76	4.46	4.67
	8	39	5.76	4.62	4.32
				Bf: 1.01, *Af*: 1.04, SSE: 0.18, RMSE: 0.19	
8	13	7	4.92	5.34	4.76
	13	14	4.92	5.14	4.52
	13	21	4.92	4.72	4.25
	13	28	4.92	4.44	3.95
	13	60	4.92	2.07	2.35
				Bf: 0.93, *Af*: 1.13, SSE: 1.26, RMSE: 0.50	
13	0	30	5.79	4.26	4.29
	0	60	5.79	3.59	3.34
				Bf: 0.97, *Af*: 1.04, SSE: 0.06, RMSE: 0.18	
14	4.5	30	5.79	4.04	4.56
	4.5	60	5.79	3.51	3.65
				Bf: 1.08, *Af*: 1.08, SSE: 0.29, RMSE: 0.38	
15	10	30	5.79	4.20	4.70
	10	60	5.79	3.30	3.51
				Bf: 1.09, *Af*: 1.09, SSE: 0.29, RMSE: 0.38	
16	7.5	7	3.78	3.36	3.48
	7.5	14	3.78	3.32	3.22
	7.5	21	3.78	2.60	2.96
	7.5	28	3.78	2.16	2.71
	7.5	56	3.78	2.03	1.76
				Bf: 1.04, *Af*: 1.12, SSE: 0.53, RMSE: 0.33	
17	13	7	3.78	3.63	3.61
	13	14	3.78	3.56	3.38
	13	21	3.78	3.24	3.11
	13	28	3.78	2.42	2.81
	13	56	3.78	1.72	1.42
				Bf: 0.97, *Af*: 1.09, SSE: 0.29, RMSE: 0.24	

[1)]The initial inoculum levels from Table 1; SSE, sum of squares error; RMSE, root mean squares error; *Bf*, bias factor; *Af*, accuracy factor.

(0, 1.67, and 4°C) and a higher survival rate at the higher temperatures (7.5, 10, and 13°C) from the initial ripening stage to about 35 days. However, after about this day, the survival rates were reversed with a little better survival at the lower temperatures. Finally, at 60 days, the survival rate was the lowest at 13°C. This is why, as shown in Fig. 2, salmonellae declined more rapidly at the higher temperatures and but persisted longer at the lower temperatures when the ripening periods were longer.

In conclusion, this study has shown that time and temperature are the main factors influencing *Salmonella* survival in ripened Cheddar cheese, but also there are subtle changes in the dynamics of the rate of decline during ripening over different storage times. It is clear that *Salmonella* can survive in cheese aged for 60 days or more in experimental studies where the inocula are relatively high. The developed Weibull model is useful in describing behavior of *Salmonella* spp. during different time and temperature conditions of ripening. From this model a MRA can be developed, although additional experimental work would be useful for more precise validation. This study has also shown that the GInaFiT tool is useful for developing non-thermal inactivation models. The GInaFiT tool is able to use data from the literature or from compilations of modeling data such as ComBase and thus reduce the requirement for new microbiological studies. It

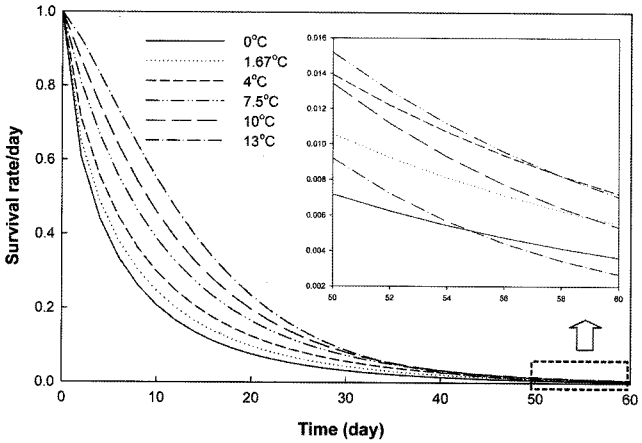

Fig. 3. The comparing of estimated survival rate on the various ripening temperatures. The lower temperature (0, 1.67, and 4°C) the lower survival rate and the higher temperature (7.5, 10, and 13°C) the higher survival rate from the initial ripening stage to about 35 days, but at 60 days, the survival rate was the lowest at 13°C. Result from Equation 2 using Weibull parameter (α and β, Equation 5 and 6) developed by RSREG statistic function.

was also found to valuable to rank different types models for the best fit to experimental data. Such a model will be valuable for conducting MRA in the future.

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