COMPARISON OF PRIMARY GROWTH CURVES OF *LISTERIA MONOCYTOGENES* IN VACUUM-PACKAGED CHILLED PORK AT CONSTANT TEMPERATURE

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Abstract –This study described the growth of a cocktail of strains of *L. monocytogenes* in vacuum-packaged chilled pork during storage at selected temperatures (4, 10, 15, 20 and 25°C). Modified Gompertz, Baranyi, Logistic and Huang models were used for fitting. All the models were validated by model parameters. A sigmoid trend was observed for all growth curves, and four primary growth models could be used to fit the growth curves. The R^2 values were > 0.97 and MSE values were ≤ 0.2198 log cfu/mL in all models. All the Bf and Af values were in the limit of $1.0 < Bf < Af < 1.1$. *F* test showed that the modified Gompertz, Logistic and Baranyi models were sufficient to describe growth curves, and the Huang model was rejected only 1 time out of 10. The application of predictive models can aid to reduce the risk of *L. monocytogenes* to ensure the safety of meat and meat products.

Key Words – *Listeria monocytogenes*, growth curve, vacuum-packaged chilled pork

I. INTRODUCTION

Predictive microbiology has been used to model the population dynamics of a number of pathogenic and spoilage bacteria at any given time and temperature during storage of foods using mathematical models. The steps of predictive modeling were followed by assembling the data into a database and synthesizing the data into a mathematical model to be incorporated into predictive software [1]. With the advancement of predictive modeling and the development of computing [2-3], more detailed models were developed. For example, modified Gompertz, Logistic, Baranyi and Huang models were described by sigmoid functions. A typical microbial growth curve consists of three phases—a lag phase, followed by an exponential phase, and finally a stationary phase [4]. To determine whether predictions provide good description of growth in foods, models should be validated to evaluate their predictive ability. Selection of an appropriate model will obtain more accurate prediction. Therefore, predictive mathematical models can be useful in decision making in food processing operations in HACCP implementation and risk assessment in the event of temperature abuse and process deviation [5].

In this study, the main objective was to develop the four primary growth curves of *L. monocytogenes* in vacuum-packaged chilled pork during storage at selected temperatures. The applicability of predictive models was evaluated to estimate microbial safety of food.

II. MATERIALS AND METHODS

2.1 Bacterial strains and preparation of inoculum

A five-cocktail, consisting of *L. monocytogenes* ATCC 19115, ATCC 19112, ATCC 15313, ATCC 19117, CICC 21583, was used in the study. They were obtained from China Center of Industrial Culture Collection. A mixture containing equal numbers of cells from each of five strains of *L. monocytogenes* was used as the inoculum.

2.2 Preparation of samples

Chilled pork samples were purchased from a local grocery store. The outer pork samples were removed using sterile surgical scissors and discarded. The inner pork samples were divided into 20 g portions (approximately 6 cm × 6 cm × 1 cm) and packaged in sterile plastic bags. Each bag was vacuum-packaged using a vacuum-packaging machine (DZQ500/2SB, Zhejing Baochun Package Equipment Plant, China). The bags containing chilled pork samples were treated with high pressure (300 MPa, 10 min at 20°C) to eradicate all contaminating microorganisms. Each sample was inoculated with 100 μL of *ca.* 10^6 cfu/mL of *L. monocytogenes*, held at room temperature for 20 min, and vacuum-packaged. The initial inoculum

of *L. monocytogenes* in vacuum-packaged pork was *ca*. 10^3-10^4 cfu/mL.

2.3 Storage and growth measurements

Inoculated vacuum-packaged pork samples were incubated isothermally at 4, 10, 15, 20, and 25°C for different times depending on incubation temperature. At frequent intervals, samples were removed for enumeration of *L. monocytogenes*. For each temperature, quintuplicate growth curves were obtained.

2.4 Enumeration of L. monocytogenes

Each inoculated vacuum-packaged pork sample (20 g) was homogenized in 80 mL sterile saline peptone water. After shaking at 230 rpm for 10 min with a stomacher, viable counts were obtained by plating onto selective agar (PALCAM agar base with selective supplements) (Land Bridge, Beijing, China), and plates were incubated at 37°C for 72 h. An average cfu/mL of two platings of each growth curve point were recorded and used to determine estimates of the growth kinetics.

2.5 Mathematical kinetic modeling of growth of L. monocytogenes *in vacuum- packaged chilled pork*

Growth curves were generated as the natural logarithm of cfu/mL vs. time using the average microbial counts at each time from the five trials at all constant temperatures. Various models based on non-linear (modified Gompertz, Logistic, Baranyi, and Huang) equations (see Equation (1) (2) (3) (4)) [6-8] were chosen as growth kinetic models and reparameterized to reflect microbial growth parameters.

$$Y=Y0+(Ymax-Y0)*\exp\{-\exp[-\mu G*(t-M)]\} \quad (1)$$

$$Y=Y0+\frac{(Y_{max}-Y_0)}{1+\exp[-\mu_G*(t-M)]} \quad (2)$$

$$Y=Y0+k*\{t+\ln[\exp(-K*t)+\exp(-h0)+\exp(-K*t-h0)]\} \quad (3)$$

$$Y=Y_0+Y_{max}-\ln\{\exp(Y_0)+[\exp(Y_{max})-\exp(Y_0)]*\exp[-K*(t+\frac{1}{25}\ln\frac{1+\exp[-25*(t-\mu)]}{1+\exp(25\lambda)})]\} \quad (4)$$

2.6 Evaluation of model performance

To evaluate the goodness of fit of the overall models, correlation coeffcients (R^2), the mean square error (MSE), bias factors (Bf) and accuracy factors (Af) were calculated. R^2, RMSE, Af and Bf were defined by the following equations [9-12].

$$Bias\ factor = 10^{\left(\frac{\sum\log(predicted/observed)}{n}\right)} \quad (5)$$

$$Accuracy\ factor = 10^{\left(\frac{\sum|\log(predicted/observed)|}{n}\right)} \quad (6)$$

Where n is the number of observations, p is the number of model parameters. The observed, predicted and mean are the observed values, predicted values and average values, respectively.

Another way to discriminate among models is to compare them statistically using the *F* test [6, 13]. The residual sum of squares (RSS, difference between observed and predicted values) was calculated for each growth curve (modified Gompertz, Logistic, Baranyi and Huang model). The Schnute model is a comprehensive model, the RSS of which was taken as an estimate of the measuring error. The test models were compared with the Schnute model by comparing the f-value against the critical F-table value at the 95% confidence level. If f-values were smaller than *F*-values for any growth curve, the model was considered equivalent to the comprehensive model indicating that the test model is sufficient to describe a growth curve. The following was calculated:

$f=\frac{RSS_{test}}{RSS_{schnute}}$ against $F_{DF_{schnute}}^{DF_{test}}$

2.7 Statistical analysis

L. monocytogenes growth data was fitted to the models using Marquardt iterative method employing a non-linear regression procedure, PROC NLIN, in SAS package (Release 8.1, SAS Institute Inc., Cary, NC).

III. RESULTS AND DISCUSSION

In recent years, a large number of predictive mathematical models have been developed to predict the growth of pathogen organisms during the storage of meat products. In this study, vacuum-packaged chilled pork inoculated with *L. monocytogenes* were incubated at 4, 10, 15, 20 and 25°C. Different kinds of models, such as modified Gompertz, Logistic, Baranyi and Huang models were used for fitting, and were validated to evaluate their feasibility and predictive ability.

3.1 Preliminary fitting and observation of isothermal growth curves

The growth curves of a cocktail of 5 strains of *L. monocytogenes* on vacuum-packaged chilled pork at different constant temperature were constructed. *L. monocytogenes* grew well from 4°C to 25°C, and all growth curves exhibited lag, exponential, and stationary phases (Fig. 1). All four models,

modified Gompertz, Logistic, Baranyi and Huang models, were suitable to describing the growth of *L. monocytogenes* cells inoculated into vacuum-packaged chilled pork incubated at 4, 10, 15, 20, and 25 °C (Fig. 1).The duration of lag phase and exponential growth rate was clearly affected by temperature. The fitting results showed almost the same concentration at the stationary phase in all four models, but a little deviation in the concentration of the initial bacterial value. Compared with modified Gompertz, Logistic and Baranyi models, the Huang model had a more clearly identifiable lag phase, these observations are in agreement with the previous study of Huang [14]. The Logistic model and Baranyi resemble each other in the lag phase, and most of the modified Gompertz models resemble turning into the stationary phase later than other models.

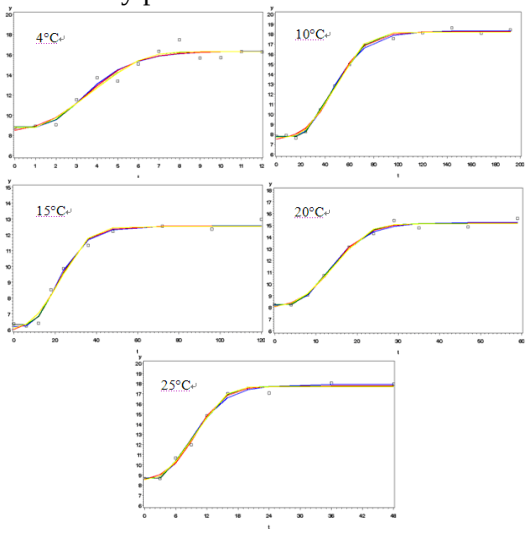

Figure 1 Growth curves of *L. monocytogenes* in vacuum-packaged chilled pork at constant temperatures. Empty squares represent the mean values of quintuplicate trials; blue lines represent the modified Gompertz model; red lines represent the Logistic model; yellow lines represent the Baranyi model; the green lines represent the Huang model

3.3 Estimation of Parameters and comparison of growth curves fitted by different models

All the growth curves were evaluated by correlation coefficient (R^2), the mean square error (MSE), bias factor (Bf) and accuracy factor (Af) values. Parameter values and performance statistics of four fitted equations at each tested temperature are shown in Table 1. The resulting R^2 values were > 0.97 and MSE values were ≤ 0.2198 log cfu/mL in all four models, they all met the requirement. The modified Gompertz model provided a good statistical fit to the observed data, and its R^2 values were closest to 1 in all four models; however, the differences were often small. The estimated MSE values were within the precision of microbial enumeration, indicating that the four models fitted isothermal growth data well. The small MSE values suggest that the model was reasonably accurate describing the effect of time on the cfu of *L. monocytogenes* at constant temperatures.

Table 1 Evaluation of general models (modified Gompertz, Baranyi, Logistic and Huang model) predicting the growth of *L. monocytogenes* on vacuum-packaged chilled pork

T		Model			
		Gompertz	Baranyi	Logistic	Huang
4°C	R^2	0.9994	0.9752	0.9992	0.9982
	MSE	0.0326	0.0329	0.0428	0.2198
	Bf	1.02	1.03	1.03	1.03
	Af	1.05	1.05	1.05	1.06
10°C	R^2	0.9998	0.9965	0.9998	0.9998
	MSE	0.0107	0.0160	0.0202	0.0327
	Bf	1.00	1.01	1.01	1.01
	Af	1.04	1.02	1.03	1.03
15°C	R^2	0.9995	0.9774	0.9990	0.9991
	MSE	0.0211	0.0331	0.0349	0.0607
	Bf	1.01	1.01	1.01	1.01
	Af	1.01	1.02	1.01	1.02
20°C	R^2	0.9993	0.9877	0.9993	0.9996
	MSE	0.0198	0.0260	0.0222	0.0456
	Bf	1.04	1.05	1.04	1.04
	Af	1.05	1.06	1.05	1.04
25°C	R^2	0.9995	0.9889	0.9995	0.9995
	MSE	0.0398	0.0318	0.0329	0.0729
	Bf	1.03	1.04	1.04	1.03
	Af	1.03	1.05	1.04	1.04

Additional five data sets at each temperature were determined to compare with model predictions, and the Bf and Af values between observations and predictions were calculated by Eq. (5) and (6) to assess the performance of the developed models. Table 1 shows that all the Bf and Af values of models were in the limit of $1.0<Bf<Af<1.1$. The average Af of all the models was 1.0375. The results suggested that the predictions were almost no deviation with observations in all the models, and the predicted curves could accurately describe growth of *L. monocytogenes* on vacuum-packaged chilled pork at constant temperature.

The performance of the four models (modified Gompertz, Logistic, Baranyi and Huang model) was also evaluated by the *F*-test. The premise of the *F*-test was that the Schnute model is a

comprehensive model and exactly predicts the microbial counts, the RSS of the Schnute model was considered as an estimate of the measuring error [14]. The results from the *F*-test for the modified Gompertz, Logistic, Baranyi and Huang models are shown in Fig 2. The *f*-values of the modified Gompertz, Logistic and Baranyi models were all smaller than the critical *F*-table values (95% confidence), thus, the three models were sufficient to describe growth curves, but the differences among the three models were very small. It can be seen that the Huang model was rejected only 1 time out of 5.

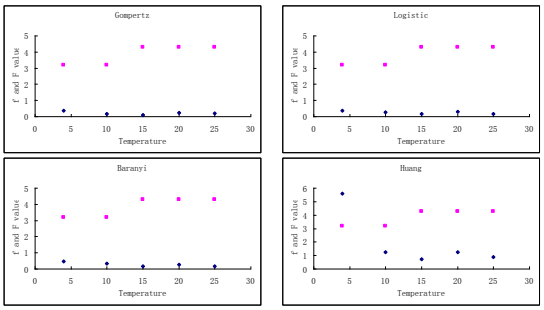

Figure 2 *F*-test on *L. monocytogenes* growth data as fitted by the modified Gompertz, Logistic, Baranyi and Huang models at different constant temperature. The diamonds represent the *f*-testing values and the squares represent the critical *F* table values (95% confidence)

IV. CONCLUSION

This study evaluated and compared the primary growth models of *L. monocytogenes* in vacuum-packaged chilled pork under selected temperature conditions. Pooling over all experiments, the modified Gompertz, Logistic, Baranyi and Huang models can be fitted for this predictive model. More research to apply this model in meat industry is needed to help reduce the risk of *L. monocytogenes* to ensure the safety of meat and meat products.

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