Establishment of a Predictive Growth Model for Foodborne Escherichia coli in Fresh-Cut Lettuce

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***Abstract*—In order to study the growth characteristics of foodborne *Escherichia coli*, we established a prediction model of foodborne *Escherichia coli* in fresh-cut lettuce based on Matlab7.0. In this experiment, the prediction model can well forecast the dynamic growth of *Escherichia coli 1.1187* in fresh-cut lettuce, and it provides a rapid and effective method for safety assessment of fresh-cut lettuce during storage. The growth state of *Escherichia coli 1.1187* was analyzed at different temperatures (4 ℃, 13 ℃, 21 ℃, 29 ℃ and 37 ℃). The primary prediction model was established by modified the Gompertz equation. The secondary prediction model was established by square root equation. Then the model was verified through the actual data at different temperatures (22 ℃ and 34 ℃). The results showed that the primary prediction model fitting correlation coefficient is above 0.99, the bias factor and accuracy factor of secondary model is in the range of 0.90 and 1.05. At low temperature 4 ℃, *Escherichia coli 1.1187* growth was restrained dramatically, so it is impossible for fitting by modified the Gompertz equation.**

***Index Terms*—*Escherichia coli*, fresh-cut lettuce, predictive growth model**

I. Introduction

Pathogen contamination in food is one of the main factors that cause foodborne illness. National foodborne disease monitoring network statistics show that over the past decade, in the event of domestic foodborne diseases caused by microorganisms, *Escherichia coli* accounted for 5.6%, is one of the main pathogens [1]. Fresh-cut lettuce maintaining the in vivo characteristics of fresh vegetables, but cut the lead to a deterioration of a series of physiological and biochemical reactions occur. In addition, due to the internal outflow of nutrients on the microbial provide a favorable living conditions, increase the types and quantities of microbial cross-contamination of fruits and vegetables, the internal organization has also been microbial infection. Internal and external factors affecting the growth of microorganisms in fresh-cut lettuce, temperature is one of the most important external factors. Analysis of *Escherichia coli* under different temperature conditions of growth and reproduction of the law, not only conducive to the health and safety of fresh-cut lettuce and provide a reliable basis.

Predict food microbiology built up a microbiology mathematical statistics and applied computer science on the basis of a new discipline [2]. It is also an important tool for managing food safety, its main research direction is to design a series of models to describe and predict the growth and survival of microorganisms under certain conditions. It provides a scientific basis for quantitative microbiological risk assessment (QM-RA) and Critical Control Point Hazard Analysis (HACCP) [3]. The experiment under laboratory conditions, the choice of pollution sterile raw juice, *Escherichia coli K12* strain of *Escherichia coli 1.1187*. Using the same inoculum at a constant temperature of 4 ℃, 13 ℃, 21 ℃, 29 ℃ and 37 ℃ for the major indexes, the total number of bacteria measured temperature changes of *Escherichia coli 1.1187* growth law. Through a combination of the predictive microbiology-related knowledge, and use Matlab7.0 [4] software to build the microbial prediction model.

II. Materials and Methods

A. Materials

Lettuce purchased from the northern suburb market in Beijing; *Escherichia coli 1.1187* purchased from the Institute of Microbiology of the Chinese Academy of Sciences; Nutrient agar (NA) was purchased from Beijing Lanyi Chemical Co., Ltd..

B. Methods

1) Bacterial suspension and sample preparation

Preparation of the bacterial suspension: *Escherichia coli 1.1187* on nutrient agar crossed activation, cultured 18h at 37 ℃, single colony was picked into a conical flask containing 100mL of physiological saline to shake uniform;

Fresh lettuce - selection - remove the skin one or two layers - crushing juice - fresh lettuce juice - distribute into triangular flask - sterilization – sterile lettuce juice;

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Inoculated with *Escherichia coli 1.1187*: suck up the quantitative bacterial suspension in accordance with the proportion of 1mL/100g to inoculate into the sterile lettuce juice.

2) Bacterial culture and detection

Based on fruit and vegetable cold chain temperature and bacterial culture temperature, 4 ℃, 13 ℃, 21 ℃, 29 ℃ and 37 ℃ were selected as the constant culture temperature. The sterile lettuce juices after inoculated with *E.coli 1.1187* were respectively bottled into 250mL triangular flasks, and cultivate them under different temperature conditions. Every 12h check once the total number of bacterias. Each trial fetch 1mL of lettuce juices under different temperature conditions with the aseptic technique, and diluted to ten folds. Under each different temperature condition separately taken right gradient, by hybrid method pour it into the nutrient agar media which cooled to about 45 ℃. Check the total number of bacterias after 48h culture under 37 ℃. Do 3 sets of parallel experiments for each sample, finally take the average of the counting results.

3) Colony counts method and data processing and analysis

Referencing the method of National Standard GB 4789.2-2010(Food microbiological testing determining bacterial colony number), count the colony of lettuce juices in 1CFU/g; The experimental datas are analyzed and plotted by Matlab 7.0 software.

4) Growth curve and the fitting of two order equations

The datas at different constant temperature are fitted with the modified Gompertz equation [5] in order to describe the growth dynamics at different temperature. Kinetic parameters were calculated based on the growth curve, using (2) and (3) to fit the relationship between microbial growth rate and microbial growth delay time and temperature in order to describe the influence of temperature on the parameters of growth kinetics, then do nonlinear regression method using the least squares method by Matlab7.0 software.

Modified Gompertz model function expression:

$$\log(N_t)=\log(N_0)+\log\left(\frac{N_{\max}}{N_0}\right)*\exp\left(-\exp\left(\frac{e*\mu_{\max}}{\log\left(\frac{N_{\max}}{N_0}\right)}*(\lambda-t)+1\right)\right) \quad (1)$$

In the expression, e = 2.718281828459, t is the time (h), N_t is the bacterial counts of microorganisms at time t (cfu/g), $\log(N_{\max}/N_0)$ is the difference of the logarithm of the maximum bacterial counts $\log(N_{\max})$ in stable stage and the logarithm of the initial bacterial counts $\log(N_0)$, λ is lag phase (h) in Microbiology sense, $\mu_{\max}$ is the maximum specific growth rate of microorganisms (h^{-1}).

This is a nonlinear microbial growth model. The microorganism quantities N of at different time t and different conditions in the experiment are fitted respectively by the equations, in order to find the four parameters, namely $\log(N_0)$, $\log(N_{\max})$, $\log(N_{\max}/N_0)$ and $\mu_{\max}$.

The model of square root is the major model used to describe the impact of environmental factors, the simple expression:

$$\sqrt{U}=b\left(T-T_{\min}\right) \quad (2)$$

$$\sqrt{\mu_{\max}}=b_1\left(T-T_{\min}\right) \quad (3)$$

$$\sqrt{1/\lambda}=b_2\left(T-T_{\min}\right) \quad (4)$$

where λ is lag phase (h) in Microbiology sense, b_1 and b_2 are coefficients ($℃^{-1}h^{-0.5}$), T is the culture temperature (℃), $\mu_{\max}$ is the maximum specific growth rate of microorganisms (h^{-1}), $T_{\min}$ is a hypothetical concept and refers to the temperature at which microorganism is no metabolic activity. $T_{\min}$ is the temperature at which is obtained by intersecting the extrapolation regression line and temperature axis.

5) Model validation

By the comparison of measurement datas under 22 ℃ and 34 ℃ storage conditions and forecast datas of the model, Bias factor (B_f) and Accuracy factor (A_f) are calculated with (5) and (6) to validate the reliability of the model. Bias factor (B_f) is used to measure the fluctuations up and down the range, Accuracy factor (A_f) is used to measure the difference between the predicted and measured datas. When the caculated results is 1.0, which means that the predicted value has no error, when the caculated results is 1.1 and 0.9, which means that the predicted has respectively error of 10% up and down. Meanwhile the square of the correlation coefficient (R^2) between the predicted and measured values can be used to determine the accuracy between the predicted and measured values.

Bias factor and Accuracy factor are expressed by the following formula:

Bias factor:

$$B_f=10^{\frac{\sum\log\left(\frac{N_{prd}}{N_{obs}}\right)}{n}} \quad (5)$$

Accuracy factor:

$$A_f=10^{\frac{\sum\left|\log\left(\frac{N_{prd}}{N_{obs}}\right)\right|}{n}} \quad (6)$$

where N_{prd} is forecast value, N_{obs} is the measured value, n is the time of experiments. When Bias factor and accuracy factor are:

- 0.90-1.05, the model has high precision and with little error.
- 0.70-0.90 or 1.06-1.15, the precision of the model can be accepted, but the error is larger.
- <0.70 or > 1.15, the model is unreliable, so it cannot be used to simulate to describe the growth of microorganisms [6].

III. Results and Analysis

A. Growth Curve for Escherichia Coli 1.1187 in Different Constant Temperature

The sterile lettuce juices were inoculated with *Escherichia coli 1.1187*, the initial bacteria amount are all 6.0×10^2 cfu/g, and were respectively placed in 4 ℃, 13 ℃, 21 ℃, 29 ℃ and 37 ℃ conditions, every 12h check once the total bacterias counts, its growth curve as in Fig. 1.

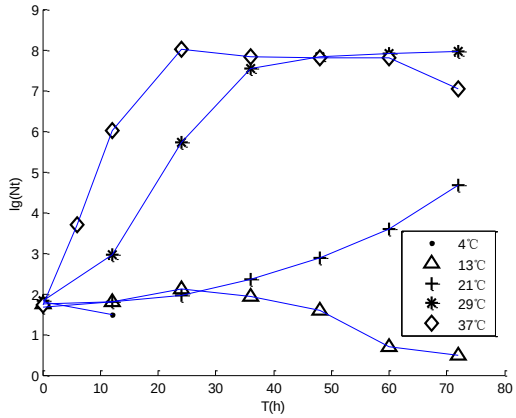


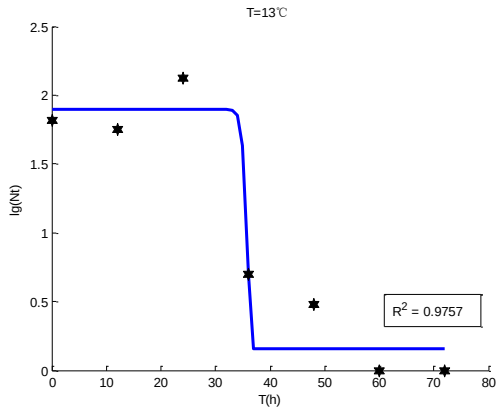
Figure 1. *Escherichia coli 1.1187* growth curve under different constant temperature.

B. The Fitting of the Primary Prediction Model

Use Matlab7.0 software to process the data of the bacteria counts which were measured in the stored sterile lettuce juices were inoculated with *Escherichia coli 1.1187* under different temperature conditions by the method of nonlinear regression, to get the growth characteristic parameters of the modified Gompertz equation as in Table I.

TABLE I. KINETIC GROWTH PARAMETERS OF ESCHERICHIA COLI 1.1187 IN FRESH-CUT LETTUCE AT DIFFERENT TEMPERATURES

Temperature (℃)	Growth characteristic parameters				Correlation coefficient R^2
	$\log(N_0)$	$\log(N_{max}/N_0)$	μ_{max}	λ	
13	0.1590	1.7359	-1.2501	36.4316	0.9757
21	4.4354	11.7015	0.2425	42.0305	0.9942
29	4.2529	13.9950	0.6163	8.3688	0.9988
37	3.7988	13.9318	1.1237	1.9184	0.9908



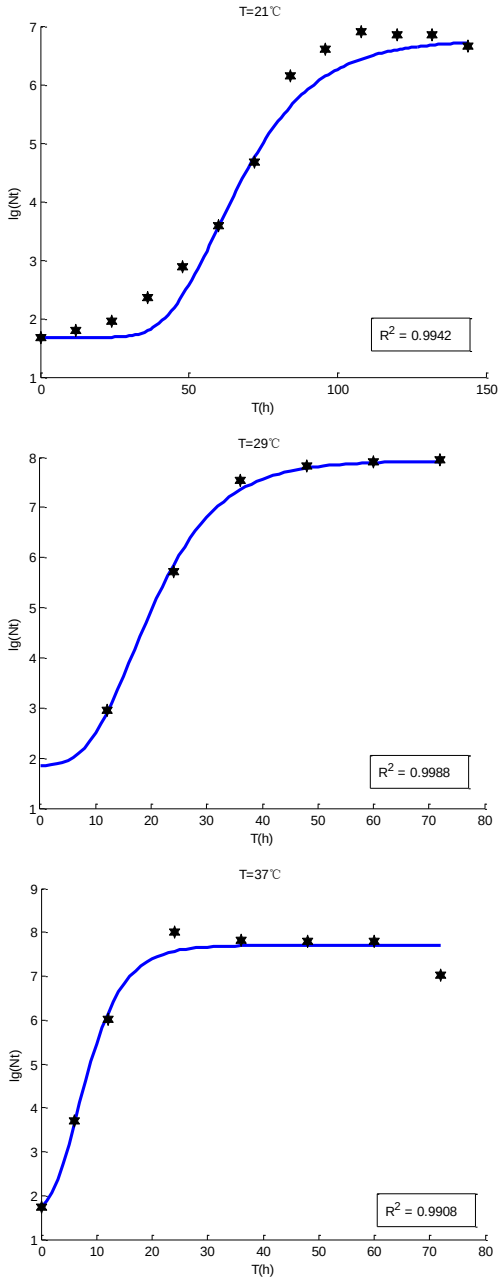


Figure 2. Fitted growth curves of *Escherichia coli 1.1187* in Fresh-cut Lettuce juice at different temperatures

Fig. 2 shows the data of the bacteria counts which were measured in the stored sterile lettuce juices were inoculated with *Escherichia coli 1*.1187 under 13 ℃, 21 ℃, 29 ℃ and 37 ℃ conditions. According to the measured data, the growth model of *Escherichia coli 1.1187* under different temperature conditions is established by the modified Gompertz equation using the least-square linear fitting, as follows:

C. The Fitting of the Secondary Prediction Model

Use Matlab7.0 software to analysis the effects of temperature on the growth characteristic parameters of *Escherichia coli 1.1187* in the sterile lettuce juices, to establish the square root equation [7] according to (2). The relationship of temperature with μ_{max} and $1/\lambda$ were shown in Fig. 3.

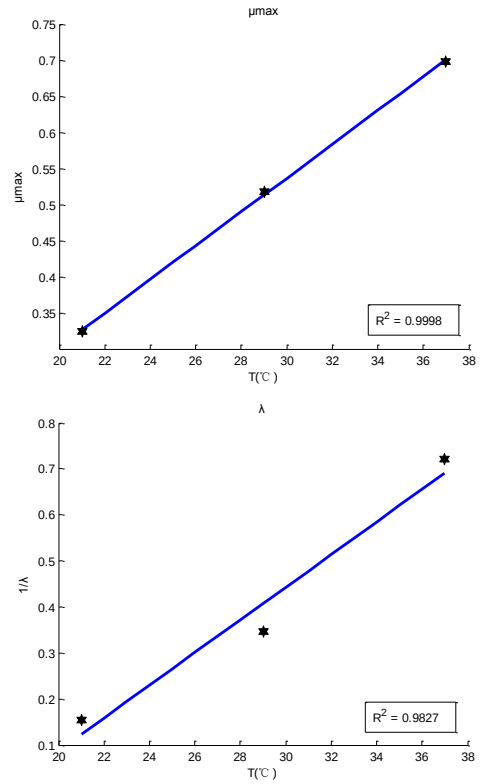


Figure 3. Relationship of temperature with μ_{max} and Lag time

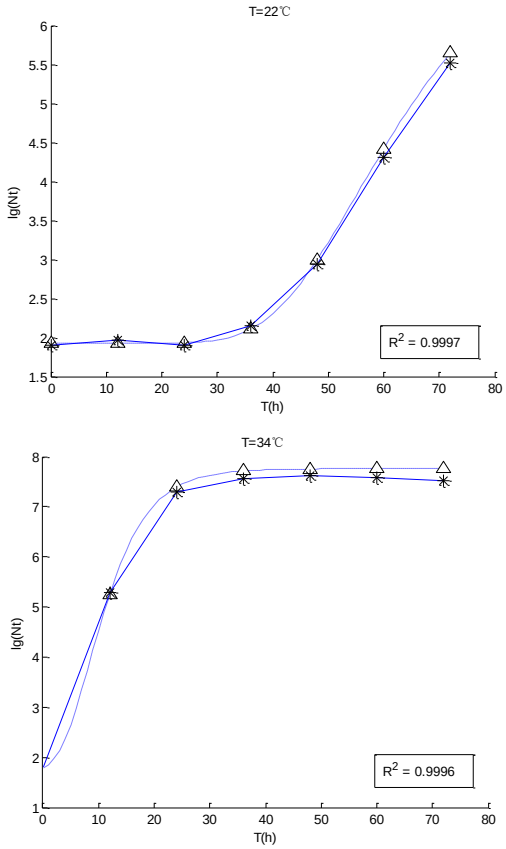


Figure 4. Predicted and observed growth curves of *Escherichia coli 1.1187* in Sterile lettuce juice at 22 ℃ and 34 ℃

As can be learned from Fig. 3, there was a good linear relationship between temperature and μ_{max} and $1/\lambda$. The Belehradek equation between temperature and $1/\lambda$ and μ_{max}:

$$\sqrt{\mu_{max}} = 0.0355(T - 7.0360) \quad (7)$$

$$\sqrt{1/\lambda} = 0.0355(T - 17.5214) \quad (8)$$

D. The Validation of the Model

The forecast and the measured growth curve of *Escherichia coli 1.1187* in the sterile lettuce juices under 22 ℃ and 34 ℃ conditions are shown in Fig. 4.

IV. Discussion

Pathogenic microorganisms in food in the throughout exposure assessment is dynamic, if you can not grasp the dynamic changes of pathogenic microorganisms, then the quantitative assessment of the hazards is almost impossible. But the model of predictive microbiology is just the most powerful tool to solve this problem. The predictive model which is established by the experiment describes the growth changes of *Escherichia coli 1.1187* in different temperatures, and if combined with other environmental factors, you can predict the growth changes of *Escherichia coli 1.1187* in the entire exposure process, and ultimately, to estimate the levels of pathogenic bacteria in various stages and when lettuce is eaten. Then input this result into the dose-response model, can be concluded that the distribution of *Escherichia coli 1.1187* in fresh-cut lettuce of consumption and the dosage of consumers, and bring these quantitative and qualitative information into together by risk characterization, can be concluded a safety evaluation of microorganisms in fresh-cut lettuce [8].

Used in this experiment *E. coli K12* strains of *E.coli 1.1187* inoculated into sterile raw juice to simulate pathogenic microorganisms in food contamination of fresh-cut lettuce. A branch of the Department of the coliform, *E. coli K12* strains (*Escherichia coli K-12*) at Stanford University in 1922 diphtheria patient's body separated from the convalescent, widely used in various studies, the biosphere, a chromosome map is the most detailed grasp biological, widely used to replace pathogenic *E. coli*-related research as a common type strain [9].

This test used the modified Gompertz equation. At 4 ℃, 13 ℃, 21 ℃, 29 ℃ and 37 ℃ storage conditions *E. coli 1.1187* in fresh-cut lettuce growth conditions, temperature return to the square of the correlation coefficient (R^2) as shown in Table I all above 0.99, indicating that the equation can describe *E. coli 1.1187* dynamic growth rhythm in the fresh-cut lettuce. However, at 4 ℃ *E. coli 1.1187* basically no growth, so the equation does not apply to 4 ℃ .

The study confirmed that the square root of the model was one of the best model for description of temperature on microbial growth affect. Thus, this experiment and the influence of different temperatures on the square root of the model will be described by a linear regression curve, R^2, respectively,was 0.9998 and 0.9827, showed a good

linear relationship, so as to create the second model has a good reliable sex. Wherein according to (7) and (8) to obtain *E.coli 1.1187* Low growth temperature and optimum growth temperature of 7.03 ℃ and 17.52 ℃ respectively.

Sterile raw juice stored under conditions at 22 ℃ and 34 ℃ in the growth of *E. coli 1.1187* measured and predicted values is verified by comparing the model, the Bias factor (B_f) was 0.998, 0.990, the Accuracy factor (A_f) was 1.0037, 1.0022, the Bias factor (B_f) is used to check the predictive value of the amplitude fluctuations and the Accuracy factor (A_f) is used to measure between the predicted and measured values, the two values between 0.90-1.05 verified constant temperature of the validity of the model. The R^2 were 0.9897 and 0.9796 at 22 ℃ and 34 ℃, indicating a good correlation between the two.

In summary, the experiment established prediction model can effectively predict sterile raw juice *E. coli 1.1187* dynamic growth rhythm of growth model established in this experiment, rapid microbiological testing conditions on product safety and shelf life prediction, the prediction model can thus be used to obtain the *E. coli 1.1187* growth or inactivation at different temperatures in different time, and be able to predict the situation in *Escherichia coli* contamination in fresh-cut lettuce to develop critical control points, to take preventive control measures to ensure food safety.

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