Development and Validation of Growth Model for *Yersinia enterocolitica* in Cooked Chicken Meats Packaged under Various Atmosphere Packaging and Stored at Different Temperatures

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ABSTRACT

Mathematical models that can predict the growth of *Yersinia enterocolitica* in chicken meats were evaluated in this study. The growth curves for *Y. enterocolitica* in chicken meats variously packaged (air, vacuum, and modified atmosphere packaging [MAP]) and stored at various temperatures (4, 10, 16, 22, 28, and 34°C) were constructed. The Gompertz model was applied to fit each of the experimental curves for the conditions mentioned above. The variations in the parameters, including lag time (\(\lambda\)) and specific growth rate (\(\mu\)), at various temperatures were then described by the following models: the variations in lag time were described by the Adair and Smith models and the variations in the specific growth rate were described by the Ratkowsky and Zwietering models. The various models were then compared using graphical and mathematical analyses such as mean square error (MSE), regression coefficient (\(r^2\)), bias factor, and accuracy factor. The results indicate that the mean \(r\) values in the Gompertz model for chicken meats packaged in air, vacuum, and MAP were 0.99, 0.99, and 0.95, respectively. The lag time modeled with the Adair and Smith functions exhibited a greater variance and demonstrated larger errors. The MSEs were 0.0015 and 0.0017 for Ratkowsky and Zwietering models, respectively. The \(r^2\) values in the Ratkowsky and Zwietering models were both 0.99. The bias factor was 1.017 for the Ratkowsky model and 1.096 for the Zwietering model. The accuracy factor of the Zwietering model was 1.174, which was lower than that in the Ratkowsky model (1.275), indicating that the former model was more accurate than the latter in predicting the specific growth rate of *Y. enterocolitica* in chicken meats.

Mathematical modeling techniques are becoming recognized and accepted as powerful tools for predicting bacterial growth kinetics. Various predictive mathematical models have been proposed to describe the effect of different factors on the growth of microorganisms (4, 25, 35) and have gained much scientific attention in recent years (5). Models in food microbiology are classified as primary, secondary, and tertiary models. Primary models such as the Gompertz function and secondary models of either the Bélehrádek and square root type have been developed to predict microbial growth rates or lag time as a function of several control factors, such as temperature, acidity (pH), and water activity, that affect microbial growth in foods (29).

Refrigerated storage is one of the most important preservation methods used in the meat industry (20). When coupled with modified atmosphere packaging (MAP), fresh meat spoilage can be delayed by inhibiting spoilage flora and retarding enzymatic degradation (14). Fang and Lin (10–13) developed a MAP/nisin combination system for controlling the growth of microorganisms such as *Pseudomonas fragi* and pathogenic *Listeria monocytogenes* in pork stored at low temperatures. *Yersinia enterocolitica* is a psychrotrophic pathogenic bacterium that was an important public health concern in the food industry (27). This pathogen is well documented as a causative agent of human gastroenteritis (23). *Y. enterocolitica* can grow under anaerobic conditions (17) and at temperatures as low as −2°C (23). Large numbers of *Y. enterocolitica* grew rapidly on vacuum-packaged beef stored at refrigeration temperature (14). It has been shown that *Y. enterocolitica* can also grow at the same rate as spoilage microflora in high pH (>6.0) beef packaged under 100% CO\(_2\) and stored at 5°C (17, 19, 25). Occasional contamination of fowls and broilers in different countries has been documented for *Y. enterocolitica* (7, 16, 21, 26, 37).

The objective of this study was to develop and validate predictive models for the effects of temperature and different packaging methods on the lag time (\(\lambda\)) and specific growth rate (\(\mu\)) of *Y. enterocolitica* in chicken meats.

**MATERIALS AND METHODS**

Organisms and culture conditions. *Y. enterocolitica* CCRC 10807 was obtained from the Culture Collection and Research Center, Food Industry Research and Development Institute (Hsinchu, Taiwan, Republic of China). This microorganism was maintained on tryptic soy agar (Difco Laboratories, Detroit, Mich.) slants at 4°C and transferred monthly. The working culture of *Y. enterocolitica* was subcultured twice in tryptic soy broth (Difco) and incubated at 30°C for 18 to 24 h. After the second incubation,
the strain was inoculated to 250 ml of tryptic soy broth at 1% inoculum. The working culture was prepared by diluting the tryptic soy broth cultures with sterile physiological saline containing 0.1% peptone to produce initial populations of approximately $10^6$ CFU/ml.

**Preparation and inoculation of chicken meats.** Fresh chicken meats were purchased from local markets and transported to our laboratory under low temperature ($<7^\circ$C). After the samples were autoclaved at 121$^\circ$C for 15 min and cooled at 4$^\circ$C for 2 h, the meat was cut into 10-g pieces and surface inoculated with 100 ml of diluted culture to produce an initial population of approximately $10^3$ CFU/g. All samples were placed in plastic barrier bags (PET/Al/PE; permeability for air: 0 to 1 cm$^3$/m$^2$/24 h; permeability for moisture: 0 to 1 ml/m$^2$/24 h; Sun A Enterprises, Corp., Taiwan, Republic of China). Bags containing samples for air storage were heat-sealed as is. Bags containing MAP samples were filled with 100% CO$_2$ (Tong-Yang Gas Co., Taiwan, Republic of China) by creating a vacuum followed by flushing with gas at one bar pressure and heat sealed (Super Vac GK 166 REGM, Busch Co., Korneuburg, Austria). These bags were then stored at 4, 10, 16, 22, 28, and 34$^\circ$C. At sampling time, 90 ml of sterile physiological saline supplemented with 0.1% peptone was added to each bag, which contained 10 g of sample, and the contents were stomached for 1 min (Model 400 BA7221, Seward Medical Co., London, England). The sample was diluted with sterile peptone or plated directly as appropriate on tryptic soy agar using a spiral plater (Model DU, Spiral System, Cincinnati, Ohio) (2). Unless otherwise stated, the results are averages of replication.

**Growth curve fitting.** Growth curves were generated from the experimental data using the Gompertz equation according to Zwietering et al. (41) and defined using the following equations:

$$\frac{N}{N_0} = A \times \exp \left\{ -\exp \left[ \frac{\mu_m \times 2.718}{A} \times (\lambda - t) + 1 \right] \right\}$$  \hspace{1cm} (1)

where $A$ is the asymptote level of $(\ln N_{\text{max}}/N_0)$, i.e., the maximal value reached; $\mu_m$ is the maximum specific growth rate (h$^{-1}$); and $\lambda$ is the lag time (h). The three parameters ($A$, $\mu_m$, and $\lambda$) were optimized using nonlinear regression with a Marquardt algorithm.

The following models were used to describe the decay of $\mu_m$ and $A$ at various temperatures. The Zwietering (41) and Ratkowsky (30) models were used to describe $\mu_m$:

$$\mu_m = \left[ b \times (T - T_{\text{min}}) \right]^2 \times \left[ 1 - \exp[c \times (T - T_{\text{max}})] \right]$$ \hspace{1cm} (2)

$$\sqrt{\mu_m} = \left[ b \times (T - T_{\text{min}}) \right] \times \left[ 1 - \exp[c \times (T - T_{\text{max}})] \right]$$ \hspace{1cm} (3)

where $T_{\text{min}}$ and $T_{\text{max}}$ are the minimum and maximum temperatures at which the growth rate is zero and $b$ and $c$ are constants. For describing $\lambda$, the Adair model (1) was used:

$$\lambda = \frac{p}{q(T - T_{\text{min}})}$$ \hspace{1cm} (4)

where $p$ (°C-h$^{-1}$) is a measure of the decrease in the lag time when the temperature ($T$) increased and $T_{\text{min}}$ (°C) is the temperature at which the lag time is infinite.

$$\lambda = \frac{1}{q(b' \times T - c')^2}$$ \hspace{1cm} (5)

Equation 5 is from the Smith model (18), where $b'$ and $c'$ are proportionality constants.

**Statistical analyses.** Statistical analyses for nonlinear and linear regression were performed using Microsoft Excel version 5.0 and Sigmaplot software version 3.0 (Microsoft Inc., Chicago, Ill.). For the nonlinear regression, the parameters of the regression were estimated using the Marquardt method (32).

**Validation.** Lag time and growth rate predictions were made using the models described above, followed by a validation using graphical and mathematical comparisons (35). For the graphical comparison, observed values for the growth parameters were plotted against the corresponding predictions from a model. For the mathematical comparison, indices such as the mean square error (MSE), regression coefficient ($r^2$), bias factor, and accuracy factor were used to evaluate the performance of the predictive growth models. The MSE is a measure of variability remaining and is the residual sum of the square (RSS) divided by the number of degrees of freedom ($n$); MSE = RSS/n $= \sum \left[ \text{predicted} - \text{observed} \right]^2/n$. Bias factor = $10 \times \log \left[ \sum \text{predicted}/\sum \text{observed} \right]$, where $\text{predicted}$ is the predicted specific growth rate and $\text{observed}$ is the observed specific growth rate. The accuracy factor averages the distance between each point and the line of equivalence as a measure of how close the predictions are to the observations. The accuracy factor can be described as follows: accuracy factor $= 10 \times \log \left[ \sum \frac{\text{predicted}/\text{observed}}{\text{predicted} \times \text{observed}} \right]$, where $\text{predicted}$ is the predicted growth rate and $\text{observed}$ is the observed growth rate.

**RESULTS AND DISCUSSION**

The experiments for *Y. enterocolitica* inoculums on chicken meats incubated at temperatures ranging from 4 to 34°C and packaged with different atmospheres (air, vacuum, and 100% CO$_2$) were found to have a typical sigmoid growth trend. Figure 1 shows the growth of this microorganism on cooked chicken meat stored at 10°C. The Gompertz model adequately described this characteristic. The mean coefficients of determination ($r^2$) for different packages by air, vacuum, and 100% CO$_2$ were 0.99, 0.99, and 0.95, respectively. The parameter for lag time and growth rate was developed from the Gompertz equation (Table 1). The variation in lag time was a function of temperature, which was described by the Adair and Smith models. Both models showed a good correlation coefficient of 0.90 and 0.93, respectively. However, the Smith model described an
inappropriate trend at low temperatures (40). The Ratkowsky and Zwietering models were applied to describe the specific growth rate in this investigation. The specific growth rate revealed a characteristic asymmetric bell shape using the Zwietering and Ratkowsky models. The minimum growth temperature of *Y. enterocolitica*, estimated using the Ratkowsky model, was in the range of 0.11 to 0.2°C. The maximum growth temperature was in the range of 38 to 40°C. The Zwietering model estimated the minimum and maximum growth temperatures of *Y. enterocolitica* to be in the range of −5 to 1°C and 32 to 33°C, respectively. At any temperature, the growth rates of *Y. enterocolitica* in chicken meat were in the order of vacuum > air > 100% CO₂. Similar results were reported in *L. monocytogenes* grown in cooked chicken meat (38). In 100% CO₂-packaged samples, the narrowest range of growth temperature was found when compared with the other two packaged samples. Wanu-Tawian et al. (36) indicated that vacuum packaging had no effect on retarding the growth of *L. monocytogenes* and *Y. enterocolitica* in pork chops. In addition, the presence of a naturally occurring microflora on cooked MAP poultry did not influence the growth of *L. monocytogenes* or *Y. enterocolitica* (3).

We have analyzed the nutritional content, including carbohydrate content, proteins, lipids, and ash, of the raw and cooked chicken meats. No significant difference was found between these two samples regarding carbohydrate content, proteins, and lipids (data not shown). Shineman and Harrison (33) indicated that growth rates of *L. monocytogenes* on cooked beef adjusted to pH 5.7 and 7.4 were similar to those noted on the uncooked tissue. However, different growth rates of *L. monocytogenes* were found on raw and cooked shrimp tissue (33). Since we have not performed the growth pattern study of *Y. enterocolitica* in raw chicken meat, the model developed in this investigation for cook chicken meat needs to be justified if it is intended for use with a raw meat model. Many modified atmospheres contain moderate-to-high concentrations of carbon dioxide (14), which together with proper sanitation and refrigeration extend shelf life by reducing microbial growth and retarding enzymatic spoilage (39). This study showed that normal

<table>
<thead>
<tr>
<th>Parameter of models&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Air</th>
<th>Vacuum</th>
<th>MAP&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>A</td>
<td>μ&lt;sub&gt;m&lt;/sub&gt; (h&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>λ (h)</td>
</tr>
<tr>
<td>4</td>
<td>13.92</td>
<td>0.11</td>
<td>25.62</td>
</tr>
<tr>
<td>10</td>
<td>13.61</td>
<td>0.14</td>
<td>15.85</td>
</tr>
<tr>
<td>16</td>
<td>12.78</td>
<td>0.39</td>
<td>2.58</td>
</tr>
<tr>
<td>22</td>
<td>13.17</td>
<td>0.60</td>
<td>1.98</td>
</tr>
<tr>
<td>28</td>
<td>12.98</td>
<td>0.80</td>
<td>1.56</td>
</tr>
<tr>
<td>34</td>
<td>12.24</td>
<td>0.75</td>
<td>1.75</td>
</tr>
</tbody>
</table>

<sup>a</sup> The r² for samples packed in air, vacuum, and MAP were 0.99, 0.99, and 0.95, respectively.

<sup>b</sup> Modified atmosphere packaging (MAP) of 100% CO₂ was used in this study.

pH chicken meat packaged in 100% CO₂ and stored at 4°C provided an environment that suppresses the growth of *Y. enterocolitica*. Several theories have been presented to account for the inhibiting effects of CO₂ on aerobic spoilage. The mode of action of CO₂, the combination of a normal pH meat environment, and a saturated CO₂ atmosphere resulted in the inhibition of *Y. enterocolitica* growth (6). These include bacterial intracellular pH alteration and the inhibition of nondecarboxylation enzymes by CO₂ (20).

The observed results were then compared with the predicted values for *Y. enterocolitica* given by the various growth models with graphical and mathematical analysis. The plot for the predicted lag time value estimated using the Smith and Adair models was plotted as function of the observed value (Fig. 2). Plots of residuals against predictions for the Smith and Adair models were examined for lag time growth parameters (Fig. 3). These plots suggest that these two models are not suitable since they resulted in a heterogeneous variance. Modeling the specific growth rate for *Y. enterocolitica* with the Zwietering and Ratkowsky models showed good estimations for the obtained experimental data (Fig. 4). Most points fall close to the line of equivalence, i.e., the predicted value is equal to the observed value, indicating that these two models had good predictive ability for *Y. enterocolitica* growth in chicken meat. The residual plot for the specific growth rate described by the Zwietering and Ratkowsky models showed a homogeneous error distribution (Fig. 5). Neumeyer et al. (24) evaluated the reliability of the predictive model for the growth of psychrotrophic pseudomonads in various milks, milk-based products, meat, and meat products. They used the residual plot method for comparison of the growth response of pseudomonads and reported that the residual plots were useful in diagnosing any nonlinearity or nonconstant variance in the predictive models.

The ability of these predictions to provide a good description of bacterial growth in food models should be validated. The mathematical and statistical characteristics of various models were compared for the lag time and specific growth rate of *Y. enterocolitica* in chicken meats (Table 2). The MSEs for lag time for the Smith and Adair models
FIGURE 2. Comparison of observed and predicted lag time for Y. enterocolitica on chicken meats fitted with Adair (A) and Smith (B) models. ● samples packaged with air; ■ samples packaged with vacuum; ▲ samples packaged with 100% CO₂.

FIGURE 3. The residual plot against predicted value for comparisons of the lag time of Y. enterocolitica on chicken meats fitted with Adair (A) and Smith (B) models. ●, samples packaged with air; ■, samples packaged with vacuum; ▲, samples packaged with 100% CO₂.

were 0.021 and 0.0178, respectively. The MSE for the specific growth estimated using the Zwietering model was 0.0015. Specific growth using the Ratkowsky model was 0.0017 (Table 2). It has been shown that the lower the MSE the better the adequacy of the model to describe the data (34). Giffel and Zwietering (35) used the Gamma concept, pathogen modeling program (PMP), and Food MicroModel (FMM) to predict the growth of *L. monocytogenes* on foods. They reported that the MSE comparison for the general models showed that the Gamma concept produced the closest prediction for the growth data and the range of estimated standard errors was 0.003 to 0.25 for the Gamma concept, 0.006 to 0.64 for PMP, and 0.006 to 0.29 for FMM (35).

The $r^2$ statistic is often used as an overall measure of the prediction attained. It represents the fraction of variation that is explained by a model. The higher the $r^2$, the better the data predicted by a model (34). In this study, the $r^2$ value was 0.99 for both specific growth rate models and 0.92 to 0.94 for both models describing lag times (Table 2). The $r^2$ values of the lag time models were lower than those for the specific growth rate models in this investigation. For meat and fish, the $r^2$ values for the Gamma concept, PMP, and FMM models were in the range of 0.62 to 0.87 (35).

The index of bias and accuracy provides an objective indication of the model performance. A bias factor less than 1 indicates a fail-safe model, i.e., observed values were smaller than the predicted values, indicating that predicted values give a margin of safety (31). When considering performance, the most immediate interest is whether the model is fail-dangerous, i.e., whether it produces estimates that underestimate the risk of spoilage or extent of pathogen growth. Models should also predict as closely as possible the observed behavior to avoid product waste (31). The bias factor values for the Adair and Smith models were 0.789 and 0.933, respectively (Table 2). Both models for lag time are fail-dangerous because the predicted data were larger than the observed values. The bias factors for specific growth rate in the Zwietering and Ratkowsky models were 1.096 and 1.017, respectively (Table 2). Both models were...
Validation of growth models for *Yersinia enterocolitica*

Comparison of observed and predicted specific growth rate for *Y. enterocolitica* on chicken meats fitted with Raktowsky (A) and Zwietering (B) models: ■, samples packaged with air; ▲, samples packaged with vacuum; ▲, samples packaged with 100% CO₂.

The residual plot against predicted value for comparisons of the specific growth rate of *Y. enterocolitica* on chicken meats fitted with Raktowsky (A) and Zwietering (B) models: ■, samples packaged with air; ▲, samples packaged with vacuum; ▲, samples packaged with 100% CO₂.

Close to 1, indicating that the prediction was similar to the observation. The Grau and Vanderlinde models (18) and the model described by Patterson et al. (28) also gave fail-safe predictions, whereas the models published by Duffy et al. (9) and Farber et al. (15) reported bias factors more than 1 calculated for most foods.

The accuracy factor is a simple multiplicative factor that indicates the spread of the results for the prediction. The larger the value, the less accurate the average estimate (31). An accuracy factor of 2 indicates that the prediction is, on average, a factor of 2 different from the observed value (35). The accuracy factor values for lag time in the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model</th>
<th>No. of observations</th>
<th>MSE</th>
<th>( r^2 )</th>
<th>Bias</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific growth rate (h⁻¹)</td>
<td>Zwietering</td>
<td>18</td>
<td>0.0015</td>
<td>0.99</td>
<td>1.096</td>
<td>1.174</td>
</tr>
<tr>
<td>Lag time (h)</td>
<td>Ratkowsky</td>
<td>18</td>
<td>0.0017</td>
<td>0.99</td>
<td>1.017</td>
<td>1.275</td>
</tr>
<tr>
<td>Lag time (h)</td>
<td>Smith</td>
<td>18</td>
<td>0.0212</td>
<td>0.94</td>
<td>0.933</td>
<td>1.327</td>
</tr>
<tr>
<td>Lag time (h)</td>
<td>Adair</td>
<td>18</td>
<td>0.0178</td>
<td>0.92</td>
<td>0.789</td>
<td>1.433</td>
</tr>
</tbody>
</table>

\( ^a \text{MSE} = \frac{\text{RSS}}{n} = \sum (\mu_{\text{observed}} - \mu_{\text{predicted}})^2/n \).

\( ^b r^2 = \text{regression coefficient} \).

\( ^c \text{For lag time, bias factor} = 10^{\frac{1}{n}\sum (\log(\mu_{\text{observed}})/\log(\mu_{\text{predicted}}))}; \text{for specific growth rate, bias factor} = 10^{\frac{1}{n}\sum (\log(\mu_{\text{observed}})/\log(\mu_{\text{predicted}}))} \).

\( ^d \text{For lag time, accuracy factor} = 10^{\frac{1}{n}\sum (\log(\mu_{\text{observed}})/\log(\mu_{\text{predicted}}))}; \text{for specific growth rate, accuracy factor} = 10^{\frac{1}{n}\sum (\log(\mu_{\text{observed}})/\log(\mu_{\text{predicted}}))} \).

Table 2. Evaluation of general models predicting the growth of *Y. enterocolitica* on chicken meat according to mathematical or statistical characteristics.
Adair and Smith models were 1.433 and 1.327, respectively (Table 2). These results showed that the accuracy factor of the Adair and Smith model indicates, on average, that the predictions differ from the observations by 30 to 50%. For those models that estimate specific growth rate, the accuracy factors for the Zwiering and Ratkowsky models were 1.174 and 1.275, respectively (Table 2). The difference between the average predicted value and the observations in the Ratkowsky model was higher than the Zwiering model. Giffel and Zwiering (35) showed that the accuracy values for L. monocytogenes in different predictive models depended on the type of products. For instance, the accuracy factors for this pathogen in meat predicted by the Gamma, PMP, and FMM models were 1.78, 1.74, and 1.73, respectively (35). On the other hand, accuracy factors of 2.41, 3.00, and 2.39 were found when L. monocytogenes was grown in dairy products and predicted using the Gamma, PMP, and FMM models, respectively (35). Dalgaard and Jørgensen (8) reported that accuracy factors ranging from 1.4 to 4.0 were found for L. monocytogenes growth rates in various seafoods. Orders of magnitude can be predicted, and in many cases the accuracy of the estimates will be sufficient to make management decisions (35). It has been shown that these factors were valuable tools for evaluation of the performance of the predictive models (24).

In this study, mathematical models for predicting the growth of Y. enterocolitica CCRC 10807 in cooked chicken meats were developed and validated. The models were developed on chickens without the natural flora, although the growth of Y. enterocolitica on cooked MAP poultry was not influenced by the presence of a naturally occurring microflora (3). The application of the models in this study to such conditions needs to be further investigated. The results of bias factors showed that the Adair and Smith models that describe the lag time for Y. enterocolitica in food systems were both fail-safe models, since the bias factors for these two models were smaller than 1. On the other hand, the Zwiering and Ratkowsky models were not fail-safe modes for predicting the specific growth rate of Y. enterocolitica in cooked chicken meat. The accuracy analysis indicated that models predicting the specific growth rate for this microorganism were more accurate than the models describing lag time parameters in cooked chicken meat contaminated with Y. enterocolitica.

REFERENCES