MODELING GROWTH KINETIC PARAMETERS OF
Salmonella spp. ON LETTUCE UNDER ISOTHERMAL AND
NON-ISOTHERMAL CONDITIONS

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ABSTRACT – This study aimed to model the growth of Salmonella on lettuce at different temperatures. Pathogen was inoculated on lettuce and stored at 5, 10, 25, 37 and 40°C. Growth curves were built by fitting the data to the Baranyi’s DMFit model and Ratkowsky equation was used as secondary model under isothermal condition. The models were able to assess the growth of Salmonella (R² > 0.98) and data showed that bacteria did not grow for 24 hours at 10°C, what can be a suitable temperature for lettuce distribution on food services. However, prolonged periods demonstrated growth at every temperature examined. Under non-isothermal conditions the model was able to predict the behavior of Salmonella on lettuce showing the model applicability (Bias = 0.90 and Accuracy = 1.17).

KEYWORDS: microbial pathogen; predictive modeling; food safety; leafy greens; temperature.

1. INTRODUCTION

Foodborne pathogens are a major concern in the global food market today and salmonellosis is the bacterial foodborne illness with the major incidence worldwide (Scallan et al., 2011). Currently, the production of fresh produce is associated with a healthy lifestyle, however it can be contaminated throughout the production chain and consumption. Consequently, a high percentage of the population is exposed to foodborne illnesses due to the consumption of fresh products, if appropriate control measures were not applied (FAO 2008).

Lettuce is one of the most consumed leafy green cultivated around the world (Herman et al 2008). In Brazil, lettuces are responsible for approximately 40% of the total volume of fresh produce traded; this high consumption is attributed due to the availability, cost, and nutritional factors (Rodrigues et al 2014). Besides this, independently the way lettuces are served, it is always consumed raw, being possibly to be contaminated by pathogens as Salmonella spp.

The storage temperatures of lettuces before eating are highly variable, depending on the available equipment and environmental conditions. Temperature is an important extrinsic factor affecting microbial growth and, consequently, the safety of lettuces (Ratkowsky et al, 1982). The recommended temperature for storage of ready-to-eat fresh produce in Brazilian food services is <5°C (RDC nº 216/2004). However, it is hardly reached and maintained on buffets, because of equipment...
used are frequently open, allowing temperature changes. Moreover, the environmental temperature in Brazil is much higher than 5°C, increasing the probability of microbial growth.

To model the influence of the temperature on the growth of *Salmonella* spp. on lettuce primary and secondary models can be used. With these models, the behavior of pathogens can be simulated and predicted on lettuce, giving important information on the safety of these leafy greens.

2. MATERIALS AND METHODS

2.1. Strains


2.2. Lettuce

The lettuces were bought in a local supermarket of Porto Alegre city, Brazil. The outer leaves of the lettuces and the core were removed, as well as all visible dirtiness. The intact leaves were cut into 4 X 4 cm² pieces, using a sterile surgical knife and a disinfected metallic template.

2.3. Pathogen inoculation on lettuce

Each *Salmonella* strain was grown in 5 mL of Brain Heart Infusion broth (BHI), at 37°C for 24h. The cultures were centrifuged, separately, at 4°C, for 10 min, at 2810g, the supernatants were discharged and pellets were washed with 0.1% peptone water. This procedure was repeated 3 times and then, after the third repetition, cells were re-suspended with 0.1% peptone water and all *Salmonella* strains were mixed in a pool.

The final cell concentration of 10⁸ CFU/mL was adjusted through optical density (OD₆₃₀nm) and confirmed by plating and counting on BHI agar. Decimal serial dilutions using 0.1% peptone water were prepared, and a *Salmonella* pool was inoculated on lettuce in order to obtain a final cell concentration of nearly 2 log CFU/g.

2.4. Storage conditions and enumeration of pathogen on lettuce

Inoculated portions (10 g) of lettuces were stored at 5, 10, 25, 37 and 40°C for different periods in stomacher bags. These temperatures were chosen because they simulate the following scenarios: recommended temperature of Brazilian regulation (<5°C), suitable fridge temperature (10°C), environmental temperature (25°C) in Brazil, the ideal growth temperature (37°C) for the bacteria (Madigan et al 2014) and summer temperature (40°C) in Brazil.

Analysis was carried out at varied time intervals, depending on the storage temperature. At each time point, 10 g of sample were homogenized with 90 mL of 0.1% peptone water, followed by decimal dilution in 0.1% peptone water. Then, aliquots were plated onto Plate Count Agar and on Xylose Lysine Deoxycholate (XLD) agar and incubation at 37°C for 24h. All the bacterial counts were carried out in triplicate. The experiments were repeated three times and the results were expressed as log CFU/g.

2.5. Modeling of pathogen growth on lettuce under isothermal conditions

The predictive primary model described by Baranyi and Roberts (1994) was used in order to calculate the growth kinetic parameters of pathogens on lettuce. The growth curves for each temperature were built by fitting the experimental data to the Baranyi’s DMFit version 2.1 Excel® add-in (www.ifr.ac.uk/safety/DMfit). The following parameters were obtained: 1) maximum growth
rate (µ), 2) lag time (λ), and 3) maximum population density by the Baranyi and Roberts (1994) model (Equation 1-3)

\[
\ln(N(t)) = \ln(N_0) + \mu_{\text{max}} A(t) - \ln \left[ 1 + \frac{e^{\mu_{\text{max}} A(t)} - 1}{e^{(N_{\text{max}} - N_0)}} \right]
\]

\[
A(t) = t + \frac{1}{\mu_{\text{max}}} \ln(e^{-\mu_{\text{max}} t + q_0})
\]

\[
\lambda = \frac{\ln \left( 1 + \frac{1}{q_0} \right)}{\mu_{\text{max}}}
\]

where: \( \ln(N(t)) \) = log of cell concentration at time \( t \) [h] (CFU/g); \( \ln(N_0) \) = log of initial cell concentration (CFU/g); \( \mu_{\text{max}} \) = exponential growth rate (log CFU/g/h); \( \ln(N_{\text{max}}) \) = log of maximum cell concentration; \( q_0 \) [-] = parameter expressing the physiological state of cells when \( t = t_0 \); \( \lambda \) = lag time (h).

The predictive secondary model was built using the square root model described by Ratkowsky et al. (1982) to describe \( \mu \) and \( \lambda \) as a function of storage temperature (Equation 4).

\[
\sqrt{\tau} = b(T - T_0)
\]

where: \( \sqrt{\tau} \) is the square root of maximum growth rate or of lag time, \( b \) is the slope of the regression line, \( T \) (°C) is temperature and \( T_0 \) (°C) is a conceptual minimum temperature for microbial growth.

2.6. Modeling the growth of pathogen on lettuce under non-isothermal conditions

Scenarios for lettuce storage and lettuce inoculation with *Salmonella*: a scenario to simulate abuse temperature conditions during harvest until purchase of lettuce on supermarkets was used to assess the prediction capabilities of the secondary model for *Salmonella* on lettuce under non-isothermal conditions. Lettuce was inoculated with *Salmonella* as described in 2.3.

The contaminated lettuce was stored under non-isothermal conditions based on Ascal and Tondo (2015) study: I) storage at 30°C for 3 h; II) storage at 25°C for 9 h; III) storage at 35°C for 2 h; IV) storage at 15°C for 8h and V) a final storage at 20°C for 8 h. The first period of storage (I) was used to simulate summer temperature during lettuce harvest. The second period of storage (II) was used in order to simulate storage in the farm at room temperature. The third period of storage (III) was set to simulate transportation from the farm to the distribution center on a sunny day in an open truck. The fourth period of storage (IV) was used to simulate waiting time at the distribution center. The last period of storage (V) was set to simulate the condition on the supermarket.

Throughout lettuce storage, counts of *Salmonella* were done as described in section 2.4, using XLD agar. Counts of *Salmonella* were done in different intervals to allow the capture of *Salmonella* during the whole storage period. At the same time points in which samples were collected for *Salmonella* enumeration, temperature of lettuce was also recorded.

Mathematical model for *Salmonella* growth under non-isothermal conditions: *Salmonella* growth under non-isothermal conditions was modeled through the application of a set of differential equations as described by Baranyi et al. (1995):
where: \( \mu_{\text{max}} \) is the maximum specific growth rate of *Salmonella*, \( x_{\text{max}} \) is the maximum population density, \( x_0 \) is the initial population concentration, \( x(t) \) is the natural logarithm of cell concentration, \( q_0 \) and \( q_t \) (dimensionless) are the amounts related to the critical compounds needed for growth and depict the physiological state of the cells in the instant of inoculation and later period, respectively.

The model for growth rate shown represented by Equation 4 was substituted in the Equation 5, with all parameters of the differential equations being temperature dependent. The differential equation was solved through the fourth order Runge-Kutta method available in MATLAB® version R2016a (Mathworks, Natick, USA). This allowed to be estimated *Salmonella* population concentration under non-isothermal storage of lettuce.

### 2.7. Statistical analysis applied to isothermal and non-isothermal models

Measures of coefficient of determination (R²) and root mean square error (RMSE) were used to evaluate the performance of the models built in this study. The R² (Equation 6) is generally considered as an overall measure of the prediction calculated by developed model, and the closer to 1 the better the model’s performance. The RMSE (Equation 7) is used to offer a standard measurement of goodness-of-fit of a model to the data used to produce it. A RMSE of 0 indicates the best possible fit between predicted and observed values.

\[
R^2 = 1 - \frac{\sum_{i=1}^{\hat{N}} (\hat{y}_i - \hat{y}_0)^2}{\sum_{i=1}^{\hat{N}} y_i^2}
\]

where: \( \hat{N} \) is the number of the points in the data set, \( y_i \) is the observed value, and \( \hat{y}_i \) is the prediction value.

\[
\text{RMSE} = \frac{\sqrt{\sum (\mu - \mu_0)^2}}{n}
\]

where: \( n \) is the number of observations, \( \mu \) is the observed value, and \( \mu_0 \) is the predicted value.

Bias and Accuracy factors were calculated through the Equations 8 and 9 shown below, where \( \mu_{\text{max predicted}} \) and \( \mu_{\text{max observed}} \) are the values of growth rate estimated by the predictive model and obtained in the experiments, respectively, and \( n \) is the number of data point used for the calculations of both factors. The closer to 1 the better the model’s performance, considering both factors.

\[
\text{Bias factor} = 10 \left( \frac{\sum \log(\mu_{\text{max predicted}}/\mu_{\text{max observed}})}{n} \right)
\]

\[
\text{Accuracy factor} = 10 \left( \frac{\sum \log([\mu_{\text{max predicted}}/(\mu_{\text{max observed}})])}{n} \right)
\]

The determination of R² was only done for the assessment of the isothermal growth models, while RMSE was calculated for both isothermal and non-isothermal models. Bias and Accuracy factors were only calculated for non-isothermal models.

### 3. RESULTS AND DISCUSSION

*Salmonella* spp growth curves started with an initial concentration of nearly 2 log CFU/g. and reached a final concentration of 8 log CFU/g. after 10 h at 37°C and 12 h at 40°C (Figure 1B). For the other temperatures, approximately 6 log CFU/g were reached after 12, 130 and 350 h at 25, 10 and 5°C, respectively (Figure 1A and 1B).
It can be observed that the temperature had a considerable influence on the microbial behavior, because final concentrations were different. This may be explained by the enzymatic leaf browning of lettuce that has antimicrobial compounds, causing a reduction of bacteria or by the lettuce microbiota that may cause competition for nutrients and space, resulting in less growth of pathogen mainly at low temperatures (Degl’innocenti et al, 2007).

Table 1 – Primary growth parameters (growth rate, lag time and maximum population density) of pathogen on lettuce stored at different temperatures and $R^2$.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Growth rate (log CFU/h)</th>
<th>Lag time (h)</th>
<th>MPD (log CFU/g)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5°C</td>
<td>0.02 ±0.03</td>
<td>142.4 ±9.3</td>
<td>5.77 ±0.04</td>
<td>0.99</td>
</tr>
<tr>
<td>10°C</td>
<td>0.04 ±0.008</td>
<td>32.9 ±8.3</td>
<td>6.03 ±0.07</td>
<td>0.98</td>
</tr>
<tr>
<td>25°C</td>
<td>0.48 ±0.07</td>
<td>1.26 ±0.6</td>
<td>5.78 ±0.07</td>
<td>0.98</td>
</tr>
<tr>
<td>37°C</td>
<td>0.74 ±0.03</td>
<td>0.32 ±0.3</td>
<td>8.23 ±0.1</td>
<td>0.99</td>
</tr>
<tr>
<td>40°C</td>
<td>0.6 ±0.03</td>
<td>2.19 ±0.3</td>
<td>8.52 ±0.09</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Based on the results of Table 1, it can be observed that there is a good fit between the experimental data and the primary model ($R^2$). Therefore, the data obtained in primary model (the values of $\mu$ and $\lambda$) can be used to elaborate a secondary model (Equation 10), which allowed the prediction of the parameters growth rate described on the basis of the temperature variation. For secondary model the $R^2$ was 0.98 and the RMSE was 0.05, then this developed model is able to assess the growth of *Salmonella* spp. on lettuce under various temperatures, ranging from 5 to 40°C.

$$\sqrt{\mu} = 0.020(\alpha T + 2.01)$$  \hspace{1cm} (10)

Under non-isothermal conditions the model was able to predict the behavior of *Salmonella* on lettuce (Figure 2). Then this model showed applicability (RMSE = 0.5, Bias = 0.90 and Accuracy = 1.17).
4. CONCLUSION

The developed models were suitable to assess the growth of Salmonella spp. on lettuce stored at 5 to 40°C under isothermal and non-isothermal conditions. Besides this, the results of this study indicated that lettuces exposed to 10°C for until 24h did not supported the growth of pathogen investigated, suggesting that this temperature is adequate to keep lettuce exposed on buffets of restaurants and supermarkets.

5. REFERENCES