Development of growth and survival models for *Salmonella* and *Listeria monocytogenes* during non-isothermal time-temperature profiles in leafy greens

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Abstract

Leafy greens contaminated with *Salmonella enterica* have been linked to large number of illnesses in many countries in recent years. *Listeria monocytogenes* is also a pathogen of concern for leafy greens because of its prevalence in the growing and processing environment and its ability to grow at refrigeration temperatures. Experimental data for the growth and survival of *S. enterica* and *L. monocytogenes* under different conditions and storage temperatures were retrieved from published studies. Predictive models were developed using the three-phase linear model as a primary growth model and square-root model to calculate specific growth rate at different temperatures. The square-root model for *S. enterica* was calculated as $\mu = (0.020(\text{Temperature}+0.57))^2$. The square-root model for *L. monocytogenes* was fitted as $\mu = (0.023(\text{Temperature}-0.60))^2$. The growth-survival model for *S. enterica* and growth model for *L. monocytogenes* were validated using several dynamic time-temperature profiles during the production and supply chain of leafy greens. The models from this study will be useful for future microbial risk assessments and predictions of behavior of *S. enterica* and *L. monocytogenes* in the leafy greens production and supply chain.

1. Introduction

Production and consumption of leafy greens has increased dramatically in the U.S. over the last few decades (USDA, 2011). Total production of Iceberg, Romaine, and leaf lettuce in the U.S. was 72,103,000 cwt (3.66 billion kg) in 1984, and increased to 90,488,000 cwt (4.59 billion kg) in 2009 (USDA, 2011). The convenience and benefits of cut, prewashed, and packaged leafy greens have created a demand for high quality products (Brecht, 1995; Oliveira et al., 2010). Leafy vegetables can be contaminated during production from many sources, such as contaminated manure, irrigation water, animals, birds, and insects (Beuchat & Ryu, 1997; Mandrell, 2009; Talley et al., 2009). Processes such as harvesting, washing, cutting, packaging, and shipping can create additional contamination (Buchholz, Davidson, Marks, Todd, & Ryser, 2012a, 2012b; Mandrell, 2009). There are fewer barriers protecting the consumer if contamination occurs in the field or during processing and handling because leafy greens are minimally processed and are generally eaten uncooked (Fröder et al., 2007).

The incidence of foodborne infections caused by bacterial pathogens in leafy greens continues to be a problem around the world (Fang, 2005). Surveys have indicated the presence of human enteric pathogens (including *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes*) on produce (Beuchat, 1996; Harris et al., 2003; Sivapalasingam, Friedman, Cohen, & Tauxe, 2004). According to the Centers for Disease Control and Prevention (CDC), almost 400 foodborne illness outbreaks were linked with the consumption of leafy greens in the U.S., causing 14,046 illnesses and 978 hospitalizations between 1973 and 2012 (Herman, Hall, & Gould, 2015). CDC has reported that *S. enterica* was specifically linked to at least 10 U.S. leafy greens outbreaks from 1998-2008 (Jackson, Griffin, Cole, Walsh, & Chai, 2013). Cut leafy greens were added to the list of food requiring time-temperature control for safety in the Food Code (FDA, 2013) in

partial response to at least 24 leafy greens outbreaks between 1998 and 2008. Although animal products have traditionally been considered to be the predominant vehicle for salmonellosis, leafy greens have emerged as a major contributor in recent years (Robertson et al., 2016). Food products were not recognized as a vehicle for *Listeria* until an outbreak was reported in 1981 due to coleslaw contaminated with *L. monocytogenes*, resulting in 41 cases of illnesses (Schlech III et al., 1983). Since then, *L. monocytogenes* has been associated with several leafy greens outbreaks and recalls worldwide. In recent years, an outbreak of ready-to-eat salad contaminated with *L. monocytogenes* was reported in Switzerland in 2013-2014 (Stephan et al., 2015). In 2016, a multi-state outbreak was reported in the U.S. due to packaged salad contaminated with *L. monocytogenes*, causing 19 cases of listeriosis (FDA, 2016). These outbreaks indicate that *L. monocytogenes* contaminated leafy greens can enter the supply chain and might eventually cause a large scale outbreak (Brown, Ryser, Gorman, Steinmaus, & Vorst, 2016).

Predictive microbiology models are used to predict bacterial population changes based on the initial contamination level and the characteristics of the food environment (Ross & McMeekin, 2003). Many predictive models for growth of *S. enterica* and *L. monocytogenes* in leafy greens have been developed and reported in the past few years (Ding, Jin, & Oh, 2010; Koseki & Isobe, 2005a, 2005b; Puerta-Gomez, Moreira, Kim, & Castell-Perez, 2013; Sant'Ana, Franco, & Schaffner, 2012). These models are based on temperature data much higher than the temperatures reported in the surveys for leafy greens during transportation and storage (Brown et al., 2016; Zeng et al., 2014). These models show considerable variability in predicting the growth rate of bacteria during exponential phase at a given temperature. Koseki & Isobe (2005a) and Koseki & Isobe (2005b) fitted the growth data of *S. enterica* and *L. monocytogenes* on packaged leafy greens at isothermal temperatures (5-25°C) using Baranyi model (Baranyi,

Roberts, & McClure, 1993). These studies seem to over-predict the growth rate of these pathogens at the temperature 5-25°C, as compared to other models reported for similar temperatures (Ding et al., 2010; Puerta-Gomez et al., 2013; Sant’Ana, Franco, et al., 2012).

Collecting data generated through different experimental studies from published literature and using those data for development of predictive microbial models is an effective way to generalize microbial kinetics in various environmental conditions from several individual studies. McKellar and Delaquis (2011) and Danyluk & Schaffner (2011) developed models for *E. coli* O157:H7 in leafy greens based on available growth and survival data in literature. The model of McKellar & Delaquis (2011) predicted the growth and survival of *E. coli* O157:H7 at temperatures $>5^{\circ}\text{C}$ and $\leq 5^{\circ}\text{C}$, respectively. Similar studies for *S. enterica* and *L. monocytogenes* are currently not available. Thus, the aim of this study was to develop growth-survival models for *S. enterica* and *L. monocytogenes* to predict the population changes of these pathogens in leafy greens during varying temperature conditions.

2. Materials and Methods

2.1 Data

Data for growth and survival of *S. enterica* and growth of *L. monocytogenes* were taken from different studies described in Tables 1, 2, and 3. Eight studies and seventeen studies were identified for growth data of *S. enterica* (Table 1) and *L. monocytogenes* (Table 3), respectively. Six studies were found for survival of *S. enterica* (Table 2). Growth and survival curves which included any active intervention step to affect the growth or survival rate of microorganism, such as application of chlorine washing (Li, Brackett, Chen, & Beuchat, 2002), and alkaline electrolyzed water (Ding et al., 2010) were excluded. Thirty five curves were identified for growth of *S. enterica*, 16 curves for survival of *S. enterica*, and 118 curves for growth of *L.*

monocytogenes (Tables 1-3). *L. monocytogenes* can grow at refrigerated temperatures down to 3°C, so few studies showing reduction of this pathogen at lower temperatures were identified, e.g. (Delaquis, Stewart, Cazaux, & Toivonen, 2002; Francis & O’Beirne, 1997), so only growth data were considered for model development of *L. monocytogenes*. Data were obtained from tables, graphs, text, or personal communication. Graphs were digitized using PlotDigitizer software (<http://plotdigitizer.sourceforge.net/>). Validation data for dynamic time-temperature profiles were taken from available studies (Koseki & Isobe, 2005b; Puerta-Gomez et al., 2013; Zeng et al., 2014). Table 4 shows different time-temperature profiles used for model validation.

2.2 Primary models

2.2.1 Growth

Three-phase linear model was used as a primary growth model. The three-phase model fits lag-phase, log-phase and stationary-phase as straight lines (Buchanan, Whiting, & Damert, 1997). A major advantage of using the three-phase linear model is that it does not require initial concentration of pathogens to predict the growth. The equations (Eq. 1) of the three-phase linear model were fitted on the growth data using the Integrated Pathogen Modeling Program (i-PMP), 2013 (<http://www.ars.usda.gov/Main/docs.htm?docid=23355>).

$$\log N_t = \log N_o \quad \text{for } t \leq t_{lag} \quad (1a)$$

$$\log N_t = \log N_o + \frac{\mu}{2.303}(t - t_{lag}) \quad \text{for } t_{lag} < t < t_{max} \quad (1b)$$

$$\log N_t = \log N_{max} \quad \text{for } t \geq t_{max} \quad (1c)$$

where N_t = cell concentration (CFU g⁻¹) at time t ; N_o = initial cell concentration (CFU g⁻¹); N_{max} = maximum cell concentration (CFU g⁻¹); t = time (h); t_{lag} = lag time (h); t_{max} = time required for maximum growth (h); μ = specific growth rate (ln CFU g⁻¹h⁻¹).

2.2.2 Survival Model

A log-linear survival model (McKellar & Delaquis, 2011) was used for survival of *S. enterica* at lower temperatures ($\leq 5^{\circ}\text{C}$). i-PMP software was used for linear regression of *S. enterica* survival. Equation 2 was used as the survival model for *S. enterica*.

$$\log(N_t) = \log(N_o) - \frac{k}{2.303} \times t \quad (2)$$

Where, k is death rate parameter in $\ln \text{CFU g}^{-1} \text{h}^{-1}$.

2.3 Secondary models

2.3.1 Growth model

The growth rate data were fitted to the square-root model (Ratkowsky, Lowry, McMeekin, Stokes, & Chandler, 1983) using MATLAB (The MathWorks Inc., Natwick, MA, ver. 2015b) software.

$$\sqrt{\mu} = b(T - T_{min}) \quad (3)$$

Where μ is specific growth rate ($\ln \text{CFU h}^{-1}$) mentioned in Eq. 1; b is the temperature coefficient, T is the temperature ($^{\circ}\text{C}$) and T_{min} is the notational minimum temperature ($^{\circ}\text{C}$) for growth of the bacterium. Regression line was calculated using least-square method and 95% confidence interval (CI) and 95% prediction interval (PI) for growth rate R were calculated using Equations 4-7 (Leininger, 2013).

$$CI = y^* \pm t_{n-2}^* \sigma \sqrt{\frac{1}{n} + \frac{(x^* - \bar{x})^2}{(n-1)s_x^2}} \quad (4)$$

$$PI = y^* \pm t_{n-2}^* \sigma \sqrt{1 + \frac{1}{n} + \frac{(x^* - \bar{x})^2}{(n-1)s_x^2}} \quad (5)$$

$$\sigma = \sqrt{\frac{SSE}{n-2}} \quad (6)$$

$$SSE = \sum (y_i - y^*)^2 \quad (7)$$

Where CI is confidence interval, y^* is the predicted value of dependent variable, n is the total number of samples, t^* is student's t-value, σ is standard deviation of residual of y , x^* is the value of independent variable, $\bar{x}$ is the mean of independent variable values in the data-set, s_x^2 is standard deviation of x , and SSE is the sum of square of errors.

2.3.2 Survival model

The values of death coefficient 'k' in Equation 2 were fitted for several distributions using @RISK 6.0 software (Palisade Corporation, Ithaca, NY). The survival data for *S. enterica* from different studies (Table 2) were used. Log-logistic distribution provided the best fits for survival of *S. enterica* in leafy greens. A log-logistic distribution is defined in @Risk as Riskloglogistic (γ, β, α), where γ is continuous location parameter, β is continuous scale parameter, and α is continuous shape parameter. Mean of this distribution is calculated using Equation 8.

$$\text{Mean} = \beta \theta \operatorname{cosec}(\theta) + \gamma \quad (8)$$

Where, $\theta = \pi / \alpha$

2.4 Tertiary model

Tertiary models were developed that could predict the growth and survival of *S. enterica* and *L. monocytogenes* with respect to the varying temperatures. These models were developed by integrating the three-phase linear (primary) and the square-root (secondary) models in MATLAB (The MathWorks Inc., Natwick, MA, ver. 2015b). Growth of *S. enterica* was modeled at temperatures above 7°C, decline was modeled at temperatures below 5°C (Equation 10), and *S. enterica* concentration was assumed to remain constant if the temperature was between 5 and 7°C. *L. monocytogenes* growth was modeled at temperatures at or above 3°C (Equation 12). The

188 models for *S. enterica* and *L. monocytogenes* growth and *S. enterica* survival were defined using
 189 Equation 9 (McKellar & Delaquis, 2011):

$$190 \quad \frac{dN_t}{dt} = Rate * N_t \quad (9)$$

191 For *S. enterica*,

$$Rate = Growth \text{ (if } T \geq 7)$$

$$192 \quad 0 \text{ (if } 7 > T > 5) \quad (10)$$

$$193 \quad Death \text{ (if } T \leq 5)$$

$$194 \quad Growth = b(T - T_{min})^2 \quad (11a)$$

$$195 \quad Death = -k \quad (11b)$$

196 For *L. monocytogenes*,

$$197 \quad Rate = (if \ T \geq 3, Growth, 0) \quad (12)$$

198 2.5 Model Validation

199 2.5.1 Profiles

200 Tertiary models developed to predict the changes in the levels of *S. enterica* and *L.*
 201 *monocytogenes* were used to simulate the changes in bacterial concentration with respect to the
 202 dynamic time-temperature profiles from available literature (Koseki & Isobe, 2005b; Puerta-
 203 Gomez et al., 2013; Zeng et al., 2014) shown in Table 4. The 95% confidence band of pathogen
 204 growth at different time intervals was also calculated using the 95% confidence interval
 205 (Equation 4) of the square-root growth model (Equation 3). Model validation was performed
 206 using MATLAB.

2.5.2 Goodness of fit

Goodness of fit of the tertiary models were evaluated using accuracy and bias factors (Ross, 1996), coefficient of determination (R^2), and root mean square error (RMSE) (Chai & Draxler, 2014) as represented by Equations 13-15.

$$\text{Accuracy factor } (A_f) = 10^{\frac{\sum |\log(\frac{\text{predicted}}{\text{observed}})|}{n}} \quad (13)$$

$$\text{Bias factor } (B_f) = 10^{\frac{\sum \log(\frac{\text{predicted}}{\text{observed}})}{n}} \quad (14)$$

If $A_f = B_f = 1$, there is an exact match between experimental observation and model predictions.

$$\text{Root mean square error (RMSE)} = \sqrt{\frac{1}{n} \sum (\text{predicted} - \text{observed})^2} \quad (15)$$

Acceptable prediction zone (APZ) approach was also used (Oscar, 2005). The difference between the observed and predicted values of bacteria concentration was prediction error (PE) with the units $\log \text{ cfu g}^{-1}$. PE values below 0 were considered fail-safe, while PE values above zero were considered fail-dangerous. The APZ was set between PE values of -1.0 and 0.5 (Argyri et al., 2013; McKellar & Delaquis, 2011).

3 Results

3.1 Growth models

3.1.1 *S. enterica*

Some *S. enterica* data sets had as few as three data points, and some had as high as 11 data points. It was difficult to judge whether there was any clear lag-phase or whether the maximum population density (MPD) was reached for curves with fewer than 5 data points. Eleven growth curves had a clear lag-phase, while 26 had a clearly defined MPD.

The Koseki & Isobe (2005b), Puerta-Gomez et al. (2013), and Sant'Ana, Franco, & Schaffner (2012) publications are examples where sufficient (up to 11) data points were available to distinguish lag-time and MPD. In contrast, the Brandl & Amundson (2008), Brandl & Mandrell (2002), and Ma, Zhang, Gerner-Smidt, Tauxe, & Doyle (2010) publications are examples of data sets where there was no lag phase, but a MPD was reached. In this case, the t_{lag} was set to zero. The Oliveira et al. (2010) publication is an example where data were not sufficient to identify MPD. In the cases of fitting where the MPD was not reached, the parameter t_{max} was fixed at the time corresponding to the highest cell number. In a few cases, both t_{lag} and t_{max} were fixed (no lag or MPD), and specific growth rate was obtained by linear regression. Specific growth rates corresponding to different temperatures are shown in Fig. 1(a). Fitted parameters for square-root model for *S. enterica* and *L. monocytogenes* are presented in Table 5.

3.1.2 *L. monocytogenes*

Some *L. monocytogenes* growth curves had as few as 4 data points (Farber, Wang, Cai, & Zhang, 1998; Li et al., 2002), whereas others had more than 10 points (Koseki & Isobe, 2005a; Sant'Ana, Franco, et al., 2012). The estimated exponential growth rates for *L. monocytogenes* are shown in Fig. 1(b).

3.2 Survival model

Sixteen survival curves showing the decline of *S. enterica* population in leafy greens corresponding to 4 and 5°C. Log-logistic distribution provided the best fits for survival of *S. enterica* in leafy greens, and distribution parameters are shown in Fig. 2.

The mean death rate was calculated as $0.0128 \ln \text{CFU g}^{-1} \text{h}^{-1}$, and the log-logistic fit of the *S. enterica* survival model and the values of γ , β , and α parameters are shown in Figure 2,

3.3 Tertiary model

The mean values of parameters b and T_{min} for *S. enterica* and *L. monocytogenes* are shown in Table 5. The notational minimum temperatures (T_{min}) were -0.57°C and 0.60°C for *S. enterica* and *L. monocytogenes*, respectively. The slope parameters (b) for *S. enterica* and *L. monocytogenes* were 0.020 and 0.023, respectively. The 95% confidence interval values used in the tertiary models are also shown in Table 5.

3.4 Validation of developed models using non-isothermal time-temperature profiles

Koseki & Isobe (2005) had two temperature profiles with the higher maximum temperature ($\sim 25^{\circ}\text{C}$) and lower maximum temperature ($\sim 15^{\circ}\text{C}$), respectively for both *S. enterica* and *L. monocytogenes*. Puerta-Gomez et al. (2013) had a cooling temperature profile for *S. enterica* with the temperature constantly dropping from 30°C to 2°C in about 5 hours. Zeng et al. (2014) recorded several dynamic time-temperature profiles during transportation to retail, retail storage and retail display, and one profile from each category was used. The predicted values from the developed tertiary model and observed values from published studies are presented in Fig. 3 and Fig. 4. Because the bacteria population data for different time-temperature profiles (Table 4) were reported in $\log_{10}$ scale, the validations presented in Fig. 3 and Fig. 4 were also calculated in $\log_{10}$ scale by dividing growth and survival rate parameters by 2.303, as shown in Equation 1b and Equation 2. Simulations were done using the temperature profiles with parameters and the lower and upper 95% prediction limits for the models (Table 5), and the predicted change in the bacteria population was compared to the data reported by Koseki & Isobe (2005), Puerta-Gomez et al. (2013), and Zeng et al. (2014) for the appropriate temperature profile (Table 4). Bacteria survival was also simulated for lower temperatures (Equation 11b), although survival was not assumed in the models presented by Koseki & Isobe (2005), Puerta-

Gomez et al. (2013), and Zeng et al. (2014). The graphs for *S. enterica* (Fig. 3) show that for Profile 1 (Fig. 3a), all the points reported (except the second point) by Koseki & Isobe (2005) were below the predicted values in the presented model in this study. For profile 2 (Fig. 3b), and profile 3 (Fig. 3b), the model followed the data points very well, with points both above and below the mean line. Similarly for *L. monocytogenes* (Fig. 4), all the models predicted the population change of the bacteria around the reported data points by Koseki & Isobe (2005), Puerta-Gomez et al. (2013), and Zeng et al. (2014). For *L. monocytogenes*, the 95% prediction bands were very narrow because of the less variability in the growth data of this pathogen (Fig. 1b).

3.5 Goodness of fit

Prediction error (PE) values for different non-isothermal time-temperature profiles and the acceptable prediction zone (APZ) analysis are presented in Fig. 5. In time-temperature profiles, all of the 28 observed points for *S. enterica* and the 41 observed points of *L. monocytogenes* profiles were found to be within the APZ. Fifteen out of 28 *S. enterica* predictions were in the fail-safe zone ($-1 \leq PE < 0$) and 21 out of 41 data points of *L. monocytogenes* were fail-safe.

Table 6 shows the A_f , B_f , R^2 , and RMSE values for *S. enterica* and *L. monocytogenes* for different temperature profiles mentioned in Table 4. The A_f values ranged from 1.005-1.059, whereas B_f ranged from 0.985-1.057. A_f , B_f , R^2 , and RMSE values predictions given for the respective time-temperature in the original studies are also shown in Table 6. While Koseki & Isobe (2005) and Puerta-Gomez et al. (2013) had reported graphical predictions from their models, Zeng et al. (2014) only reported the root mean squared error (RMSE), biases, and APZ results. As such, A_f , B_f , R^2 , and RMSE values could not be calculated for Profiles 4-6 (Zeng et

al., 2014) because prediction curves were not available. Also, the equation used by Zeng et al. (2014) to calculate the bias was different from the bias factor calculated in this study (Equation 14). The values of A_f and B_f for *S. enterica* in Profile 1 were quite high (1.059 and 1.057, respectively) because of systematic over-predictions in this profile. It has been suggested that models with B_f in the range of 0.7 to 1.15 can be regarded as “acceptable” for describing a pathogen growth rate (Oscar, 2005; Ross, 1996). The B_f values reported in Table 6 were consistently in the acceptable range of 0.7 to 1.15.

4 Discussion

Predictive models have significant importance in estimating the microbial behavior during the farm-to-fork food chain and have tremendous applications in risk assessments efforts to manage and control public health risk arising from microbial pathogens. *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes* are three major pathogenic bacteria of concern for leafy greens, as suggested by the high number of outbreaks and recalls of leafy greens contaminated with these pathogens (Herman et al., 2015; Zeng et al., 2014). McKellar and Delaquis (2011) developed a growth-death model for *E. coli* O157:H7 in leafy greens by compiling growth and survival data from 17 studies. Danyluk and Schaffner (2011) collected growth data of *E. coli* O157:H7 in leafy greens from 7 studies, and fitted those data using the three-phase linear and square-root models for model development and used that developed model for a quantitative microbial risk assessment (QMRA) for *E. coli* O157:H7 in leafy greens. We recognize the importance of developing such models for *S. enterica* and *L. monocytogenes*, as key to applications in predictive microbiology and future quantitative microbial risk assessment studies. We focused on growth and survival data of *S. enterica* and *L. monocytogenes* in leafy greens and used those data to develop growth-survival models for these two pathogens.

There is a great deal of variability in the growth data of *S. enterica*, evident from the coefficient of determination of 0.41 (Table 5). It was clear on further analysis that at any particular temperature growth rates in cut leafy greens (Brandl & Amundson, 2008; Chang & Fang, 2007; Koseki & Isobe, 2005b; Oliveira et al., 2010; Sant'Ana, Barbosa, et al., 2012; Sant'Ana, Franco, et al., 2012) were higher than the growth rates in uncut leafy greens (Brandl & Mandrell, 2002; Ma, Zhang, Gerner-Smidt, Tauxe, & Doyle, 2010; Puerta-gomez et al., 2013). Pathogens are not easily internalized in uncut leafy greens, and are exposed to the relative humidity of the incubation environment. Once leafy greens are cut however, moisture and sugar are made readily available to support bacterial growth. Lettuce is one of few edible crops that produce latex (Sessa, Bennett, Lewis, Mansfield, & Beale, 2000). Upon cutting of lettuce stems, a large quantity of latex is released onto the cut surfaces (Brandl, 2008). As early as 2 h and 4 h after its inoculation onto lettuce stem discs, populations of *E. coli* O157:H7 can increase by 0.7 or 1 log CFU and by 22 h, the *E. coli* O157:H7 populations increased by more than 4 log CFU, suggesting that the surface of cut lettuce stems holds large quantities of substrates that allow for the multiplication of bacteria (Brandl, 2008). There was relatively little variability in *L. monocytogenes* growth, shown by coefficient of determination value 0.83 (Table 5). Since all 17 *L. monocytogenes* studies analyzed uncut leafy greens, the growth of *L. monocytogenes* may be greater than the values predicted by the growth-survival model presented in this study.

For the death rate (k) of *S. enterica*, there was considerable variability within individual studies and between different studies, which could possibly be attributed to differences in vegetable types and experimental conditions. Death rate of <0.01 ln CFU/h was reported by Chang & Fang (2007), Kakiomenou, Tassou, & Nychas (1998), Oliveira et al. (2010), whereas Ma et al. (2010) reported a death rate of 0.07 ln CFU/h. This variability was addressed by fitting

the survival data to different distributions and on the basis of Akaike information criterion (AIC), the log-logistic distribution was found to best represent the survival data used in this study.

The minimum temperature supporting the growth of *S. enterica* and *L. monocytogenes* are 7°C and 3°C shown in Table 1 and Table 3, respectively. The notational minimum temperature (T_{min}) is often lower than the minimum temperature supporting the growth of bacteria (McKellar & Delaquis, 2011). Our study supports this finding as T_{min} for *S. enterica* and *L. monocytogenes* were calculated as -0.57°C and 0.60°C, respectively (Table 5).

We present the 95% confidence interval and 95% prediction interval for the square-root models for *S. enterica* and *L. monocytogenes* in Fig. 1. Although both are centered at the mean value of growth rate (μ) for a given temperature, the PI takes into account the tendency of any data point of growth rate to fluctuate from its mean value whereas the CI accounts for the uncertainty in estimating the mean value (Leininger, 2013). Prediction intervals must account for both the uncertainty in knowing the value of the population mean, plus data scatter, thus a PI is always wider than a CI (Fig. 1).

5. Conclusions

The models developed in this study can predict the growth-survival of *S. enterica* and *L. monocytogenes* during varying temperature conditions likely to be encountered by leafy greens along supply chain. We observed more variability in the growth data of *S. enterica* as compared to the variability in the growth data for *L. monocytogenes*. This may be because experiments using cut/shredded and uncut leafy greens were used to develop the growth models for *S. enterica*. The data we analyzed show higher growth rates of *S. enterica* on cut leafy greens compared to uncut leafy greens. All the studies for *L. monocytogenes* included in this study had data of growth of pathogen in uncut leafy greens. More studies need to be conducted to

differentiate the growth of pathogens in cut and uncut leafy greens. This will greatly impact the results of future quantitative microbial risk assessments (QMRAs) of leafy greens.

Acknowledgement:

This work was supported through a grant (award 2011-51181-30767) from the U.S. Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA). Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the USDA-NIFA. We thank Dr. Anderson Sant'Ana for providing us with data of their research.

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513

Fig. 1. Square-root model for growth of (a) *S. enterica*, and (b) *Listeria monocytogenes*. Symbols represent experimental data from available literature, solid line represents mean square-root model; inner and outer bands of dotted lines represent 95% confidence intervals and 95% prediction intervals, respectively.

Fig. 2. Distribution fitting for inactivation data for *S. enterica*.

Fig. 3. Validation for (a) *S. enterica* during Profile 1; symbols represent Koseki and Isobe (2005) data points; (b) *S. enterica* during Profile 2, symbols represent Koseki and Isobe (2005) data points; (c) *S. enterica* during Profile 3, symbols represent Puerta-Gomez et al. (2013) data points.

Fig. 4. Validation for (a) *L. monocytogenes* during Profile 1; symbols represent Koseki and Isobe (2005) data points; (b) *L. monocytogenes* during Profile 2, symbols represent Koseki and Isobe (2005) data points; (c) *L. monocytogenes* during Profile 4 (transportation from processing to retail), symbols represent Zeng et al. (2014) data points; (d) *L. monocytogenes* during Profile 5 (retail storage), symbols represent Zeng et al. (2014) data points; (e) *L. monocytogenes* during Profile 6 (retail display), symbols represent Zeng et al. (2014) data points.

Fig. 5. Acceptable Prediction Zone (APZ) analysis: (a) Data for time-temperature profiles for *S. enterica*, and (b) *L. monocytogenes*.

530 Fig. 1

531 (a)

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533 (b)

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535 Fig. 2

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538 Fig 3

542 Fig. 4

543

544

548 Fig. 5

549 (a)

551 (b)

553 **Table 1**

554 Growth data for *S. enterica*.

Authors	Temperature (°C)	Product	Data Source	No of curves
Brandl and Amundson (2008)	28	Romaine lettuce	Digitized	3
Brandl and Mandrell (2002)	22, 24, 26 and 30	Cilantro	Digitized	8
Chang and Fang (2007)	22	Iceberg lettuce	Table	1
Koseki and Isobe (2005)	10, 15, 20, and 25	Iceberg lettuce	Digitized	4
Ma et al. (2010)	12 and 21	Cilantro	Digitized	2
Oliveria et al. (2010)	25	Shredded romaine lettuce	Digitized	3
Puerta-Gomez et al. (2013)	10, 20, 30, 37	Baby spinach	Digitized	8
Sant’Ana et al. (2012)	7, 10, 15, 20, 25, 30	Lettuce	Personal communication	6

555 **Table 2**

556 Survival data for *S. enterica*.

Authors	Temperature (°C)	Product	Data Source	No of curves
Ma et al. (2010)	4	Cilantro	Digitized	1
Kakiomenou et al. (1998)	4	Lettuce	Digitized	2
Vandamm et al. (2013)	4	Fresh-cut celery	Table	8
Chang and Fang (2007)	4	Iceberg lettuce	Table	1
Weissinger et al. (2000)	4	Shredded lettuce	Table	1
Oliveria et al. (2010)	5	Lettuce	Digitized	3

557 **Table 3**

558 Growth data for *Listeria monocytogenes*.

Authors	Temperature (°C)	Product	Data Source	No of curves
Carlin et al. (1995)	10	Endives	Table	6
Carlin et al. (1996)	3 and 10	Endives	Table	16
Carrasco et al. (2008)	5 and 13	Shredded iceberg lettuce	Digitized	2
Ding et al. (2010)	4, 10, 15, 20, 25, 30 and 35	Iceberg lettuce	Digitized	7
Farber et al. (1998)	4 and 10	Caesar salad, coleslaw	Digitized	6
Francis and O’Beirne (1997)	8	Shredded iceberg lettuce	Digitized	6
Francis and O’Beirne (2001a)	8	Lettuce	Digitized	6
Francis and O’Beirne (2001b)	8	Lettuce	Digitized	8
Francis and O’Beirne (2005)	8	Lettuce and coleslaw mix	Digitized	15
García-Gimeno et al. (1996)	4	Mixed salad	Digitized	1
Gleeson and O’Beirne (2005)	8	Butterhead and iceberg lettuce	Digitized	5
Jacxsens et al. (1999)	7	Shredded chicory endives and iceberg lettuce	Digitized	2
Kaminski et al. (2014)	4, 7 and 10	Celery	Digitized	3

Koseki and Isobe (2005)	5, 10, 15, 20, and 25	Shredded iceberg lettuce	Digitized	5
Li et al. (2002)	5 and 15	Shredded iceberg lettuce	Digitized	12
Oliveria et al. (2010)	5 and 25	Shredded romaine lettuce	Digitized	12
Sant'Ana et al. (2012)	7, 10, 15, 20, 25, 30	Shredded lettuce	Personal communication	6

559 **Table 4**

560 Non-isothermal time temperature profiles for leafy greens supply chain.

Profile #	Pathogen in consideration	Description	Reference
Profile 1	<i>S. enterica</i> , <i>L. monocytogenes</i>	Farm to retail (initial temperature ~15°C)	(Koseki & Isobe, 2005b)
Profile 2	<i>S. enterica</i> , <i>L. monocytogenes</i>	Farm to retail (initial temperature ~25°C)	(Koseki & Isobe, 2005b)
Profile 3	<i>S. enterica</i>	Slow cooling	(Puerta-Gomez et al., 2013)
Profile 4	<i>L. monocytogenes</i>	Transportation to retail	(Zeng et al., 2014)
Profile 5	<i>L. monocytogenes</i>	Retail storage	(Zeng et al., 2014)
Profile 6	<i>L. monocytogenes</i>	Retail display	(Zeng et al., 2014)

561 **Table 5**

562 Fitted parameters for square-root model.

	<i>S. enterica</i>			<i>L. monocytogenes</i>		
	Model	2.5 percentile	97.5 percentile	Model	2.5 percentile	97.5 percentile
b	0.020	0.011	0.028	0.023	0.021	0.025
T_{min}	-0.571	-1.668	0.747	0.599	-1.691	0.338
R²	0.41			0.83		

563 **Table 6**

564 A_f , B_f , R^2 , and RMSE values for predicted growth of *S. enterica* and *Listeria monocytogenes* in leafy greens for different time-
565 temperature profiles given in Table 4.

Pathogen	Profile	A_f (This study)	A_f (Original study)	B_f (This study)	B_f (Original study)	R^2 (This study)	R^2 (Original study)	RMSE (This study)	RMSE (Original study)
<i>S. enterica</i>	Profile 1	1.059	1.031	1.057	0.994	0.597	0.721	0.211	0.151
	Profile 2	1.028	1.033	1.002	1.032	0.847	0.783	0.123	0.178
	Profile 3	1.017	1.015	0.985	0.981	0.596	0.731	0.080	0.066
<i>Listeria monocytogenes</i>	Profile 1	1.021	1.020	1.004	0.993	0.889	0.910	0.131	0.112
	Profile 2	1.005	1.018	1.004	1.013	0.998	0.970	0.029	0.104
	Profile 4	1.019	NA	1.007	NA	0.832	NA	0.196	NA
	Profile 5	1.058	NA	1.045	NA	0.836	NA	0.203	NA
	Profile 6	1.032	NA	1.028	NA	0.865	NA	0.167	NA

566 NA: Value not provided in the original study