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UNCERTAINTY ASSESMENT AND VALIDATION OF PREDICTIVE MICROBIAL GROWTH MODELS

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KARINA G. MARTINO

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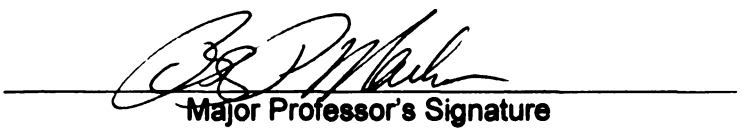

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UNCERTAINTY ASSESSMENT AND VALIDATION OF PREDICTIVE MICROBIAL GROWTH MODELS

By

Karina G. Martino

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ABSTRACT

UNCERTAINTY ASSESSMENT AND VALIDATION OF PREDICTIVE MICROBIAL GROWTH MODELS

By

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Microbial models enable a proactive approach, conveniently used by the food industry and risk assessors, to predict microbial food safety. However, the validity, reliability, and uncertainty of these models in application to real food products are rarely well known. Therefore, the overall goals of this study encompassed validation of predictive microbial growth models, assessment of the uncertainty related to those models, and deconstruction of the different errors that contribute to the total uncertainty of a microbial growth model.

For illustration purposes, *Listeria monocytogenes* growth data from laboratory broth and meat and poultry products were used throughout. The primary and secondary models used in the U.S. Department of Agriculture – Agricultural Research Service (USDA-ARS) Pathogen Modeling Program (PMP), a widely used tool by the food industry to estimate pathogen growth/survival/inactivation in food, were the principal models analyzed throughout this study.

Robustness of the broth-based growth models was evaluated using a Robustness Index (RI). Inside the calibration domain of the PMP, the best RI for application to meat products was 0.37; the worst was 3.96. Outside the domain, the best RI was 0.40, and the worst was 1.22. Meat product type influenced the RI values ($P<0.01$).

Two different microbial modeling procedures, using the broth-based data, were compared and validated against independent data for microbial growth in meat and

poultry products. A global regression method yielded a lower root mean squared error, 0.95 log(CFU/ml) for aerobic and 1.21 log(CFU/ml) for anaerobic conditions, than did a two-step procedure, which yielded errors of 1.35 log(CFU/ml) for aerobic and 1.62 log(CFU/ml) for anaerobic conditions. Validating with data from meat and poultry, the global regression was more robust than the two-step procedure for 65% of the cases studied. However, the predictions were overestimated (fail-safe) in more cases for the two-step than for the global regression.

In deconstructing the overall model error, the total uncertainty was assumed to be an aggregated contribution of errors due to organism, substrate, laboratory methodologies, replications, and primary and secondary regressions. The total uncertainties for aerobic and anaerobic conditions, with the PMP *L. monocytogenes* growth models, were 1.35 and 1.62 log(CFU/ml), respectively. The errors from the primary regression were 1.02 and 1.22 log(CFU/ml), for aerobic and anaerobic conditions, respectively. The errors from the secondary regression were 1.48 and 1.42 log(CFU/ml), for aerobic and anaerobic conditions respectively. The variability due to replications was 0.26 log(CFU/ml) for aerobic and 0.21 log(CFU/ml) for anaerobic conditions.

Following the methodologies described here could lead to better informed and more reliable decisions, for ensuring food safety and for evaluating consumer risk.

To my family

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CHAPTER 1

INTRODUCTION

1.1 STRUCTURE AND SCOPE OF THE DISSERTATION

The overall subject of this study was the influence of predictive microbiology on the food safety system. Chapter 1 is comprised of an introduction to this subject, followed by a description of the need and specific research objectives of this dissertation. In order to conduct a quantitative analysis, the growth of *Listeria monocytogenes* in broth was used as the case study for the dissertation.

A general literature review, with respect to the basic topics that are covered in this study, is presented in Chapter 2, including an overview of predictive microbiology, *L. monocytogenes*, and growth models. This overview encompasses the basic concepts that were used throughout this study.

Chapters 3, 4, and 5 are "stand-alone" manuscripts. Chapter 3 is a growth model validation study, which was published in the *Journal of Food Protection* (Martino *et al.*, 2005). An introduction of the currently used validation methods is presented. The Robustness Index (RI) was applied in order to validate broth-based models against data collected from actual food products. The results quantified model robustness inside and outside their original domain.

Chapter 4 is a paper that addresses model fitting procedures (submitted for publication in August 2006). This chapter analyzes how different fitting procedures affect the overall uncertainty of a growth model and its robustness when applied to a food system. Knowing the influence of the fitting methods on overall uncertainty of the model can help improve the accuracy of model predictions by choosing the methodology that

gives lower prediction errors.

Chapter 5 presents the deconstruction of model uncertainty by identifying and quantifying the different sources of error that contributed to it. These insights give a better understanding of how these errors can be quantified and segregated, so efforts to reduce them can be prioritized.

Chapter 6 presents overall conclusions and recommendations for future work.

1.2 IMPACT OF PREDICTIVE MICROBIOLOGY IN FOOD SAFETY

Current food safety tools used by food processors and risk assessors rely on predictive tools. These predictive tools (e.g., predictive microbial software programs) utilize models that describe the behavior of microorganisms under different physical or chemical conditions, such as temperature, pH, and water activity. An example of this kind of tool is the USDA-ARS Pathogen Modeling Program (PMP, (U.S. Department of Agriculture, 2003b)), which is a widely used tool in the food industry. Model predictions enable a proactive approach to avoid undesirable results or consequences (mainly human illness or death). They allow the prediction of microbial food safety or shelf life of products, the detection of critical parts of the production and distribution process, and the optimization of production and distribution chains (Zwietering *et al.*, 1990). In general, in the food area, prediction comes from mathematical models that were developed from broth-based data; however, some tools are being updated with models developed using data from actual food systems. The food safety and food microbiology community is working to account for variables that influence growth or death of foodborne pathogens of concern. Unfortunately, the accuracy, or uncertainty of the predictions is often not well

known.

The predictive tools that are currently used in the food industry are still empirically managed. There is no systematic or standardized approach to develop a model, conduct a specific laboratory analysis for a particular microorganism, conduct statistical analysis, or determine the uncertainty of the outcomes of a particular model.

Furthermore, regulatory agencies are putting increasing pressure on industries. For example, the USDA Food Safety and Inspection Service (FSIS, 2003), following several outbreaks, confirmed that it will maintain its "zero tolerance" policy with respect to *L. monocytogenes* in ready-to-eat meat and poultry products; no minimum lethality or maximum growth will be allowed, on a 25 gram sample, in any of these products (FSIS, 2003). Predictive tools represent a vital element in food processing, in order to enable rapid determination of potential pathogen growth.

Therefore, validation of these models is a critical component of predictive microbiology; however, validation is usually done in terms of parameters (growth rates, lag phase duration, etc.), forgetting that the true measure of product safety is actual microbial counts, not model parameters. Once predicted values are calculated, their uncertainty must also be established in order to determine limits, and to ensure that these limits do not present unacceptable risk to consumers.

In order to perform an accurate quantitative analysis of microbial growth, predictive microbiology needs to become a reliable tool, that is, a valid and systematic approach that can be directly used by the food industry, regulators, and/or risk assessors. Moreover, an increase in demand for predictive microbial software programs with application to food systems is expected (Tamplin *et al.*, 2004), which includes the use of predictive models

for research, HACCP development, product formulation, and risk assessment.

1.3 JUSTIFICATION

From the farm to the table, there are unavoidable risks to consumers; a very significant one is microbial contamination, either intentional or not, of the food system.

Pathogens are present across the entire harvesting-processing-distribution chain; therefore, it is necessary to take a proactive approach to minimize/prevent their survival. Predictive tools enable this type of approach, and are especially useful for processors who must comply with government regulations. However, problems can arise if processors rely on these tools without questioning their validity.

For example, food processors calculating microbial growth have no reliable estimate of uncertainty in those calculations; therefore, given normal process variability over time, and an unknown uncertainty in the calculations, processors may under- or over-process the product. Even though their prediction outcome is above the regulatory target, their uncertainty limits could extend below the target, which could represent a risk to the consumer (Fig. 1.1). However, if that processor had a tool that produced a reliable estimate of the uncertainty in the process survival or growth calculations, then the degree of over or under-processing could be based on real statistical information (prediction error + normal process variability), and the safety of the product could be better ensured (Fig. 1.1).

Therefore, there is a need to improve microbial prediction tools in order to help food processors, academia, and regulatory agencies to better validate processes, conduct microbial risk assessments, and better assess the uncertainty behind these predictions.

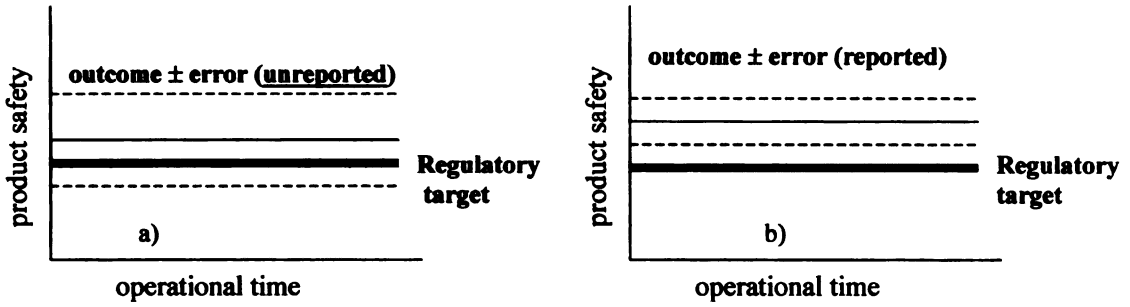

FIGURE.1.1. Illustration of the impact that uncertainty in process calculations can have on product safety.

1.4 OBJECTIVES

A quantitative assessment of errors in predictive microbiology has not been conducted previously. Furthermore, acceptable limits of prediction have not been established, meaning that confidence of the actual prediction values have not been defined or calculated. Therefore, the overall goal of this study was to provide background information with respect to overall model uncertainty, in order to improve current tools used in predictive microbiology.

To advance toward that goal, the specific objectives of this study were:

1. To validate *L. monocytogenes* broth-based growth models in terms of microbial counts.
2. To assess the performance of different fitting procedures in predictive microbiology.
3. To identify and quantify sources of error that contribute to microbial model uncertainty.
4. To demonstrate that microbial food safety limits should be represented by prediction intervals instead of confidence intervals.

CHAPTER 2

LITERATURE REVIEW

2.1 PREDICTIVE MICROBIOLOGY

Predictive food microbiology was defined by Schaffner and Labuza (Schaffner and Labuza, 1997) as the gathering of "the disciplines of food microbiology, engineering, and statistics to provide useful predictions about microbial behavior in food systems." Currently, predictive microbiology is considered an essential element of modern food microbiology; furthermore, in the future, it could be accepted as a mature subdiscipline of microbiology (McMeekin and Ross, 2002).

Microorganisms are primarily characterized by their adaptation to and exploitation of change. They can colonize almost every habitat on earth, such as brine ponds in the frozen wastes of polar regions, boiling water of hot springs, thermal volcanic vents, and the bottom of the deep ocean (Adams and Moss, 1995). A rich microflora of bacteria, yeasts and fungi can be found in structures such as leaves, fruits and roots, which could be used as raw ingredients for food processing. Therefore, assurance of food safety requires proactive and adaptive strategies. Generally, challenge tests are used to describe the relationship between pathogens and the influence of environmental conditions in their growth or decline. However, this traditional approach is typically expensive and slow, and requires specialized facilities and microbiological skills (Baranyi and Roberts, 1995). Therefore, with predictive microbiology, time and effort can be minimized by quickly giving the ranges of concern for a factor and thereby guiding the design of challenge tests, storage trials, and other conventional techniques to assess the probability of pathogen growth (Whiting, 1995). However, specific

interactions between the microorganisms and their environment have to be known, the predictive models have to be validated, and their related uncertainty should be carefully assessed.

Predictive microbiology mathematically describes, using microbial models, the growth or decline of foodborne microbes under specific environmental conditions, allowing the prediction of microbial food safety or shelf life of products, the detection of critical parts of the production and distribution process, and the optimization of production and distribution chains (Zwietering *et al.*, 1990).

Microbial models can be classified as primary, secondary, or tertiary (Whiting, 1995). Primary models describe how the number of microorganisms in a population changes with time under specific conditions. Secondary models relate the primary model parameters to environmental or intrinsic variables. Tertiary models combine primary and secondary models with a computer interface, providing a complete prediction tool.

2.1.1 Primary growth models

Primary growth models can be classified as follows.

van Gerwen and Zwietering (1998) stated that assuming first-order kinetics was the simplest way to describe microbial growth, such that:

$$\ln N = \ln N_0 + \mu t \qquad \text{Eq. 2.1}$$

where:

$\ln N$ = microbial counts at time t

$\ln N_0$ = initial microbial counts

μ = growth rate

t = time

In contrast, the lag-exponential function includes the lag time (van Gerwen and Zwietering, 1998):

$$\ln N = \ln N_0, \text{ for } t < \lambda \qquad \text{Eq. 2.2}$$

$$\ln N = \ln N_0 + \mu(t - \lambda), \text{ for } t \geq \lambda \qquad \text{Eq. 2.3}$$

λ = lag time

The growth curve also can be empirically described by several sigmoidal functions, such as logistic (eq. 2.4), Gompertz (eq. 2.5), or Richards, Schnute, and Stannard (van Gerwen and Zwietering, 1998).

$$\ln N = \ln N_0 + \frac{a}{[1 + \exp(b - cx)]} \qquad \text{Eq. 2.4}$$

where a, b, and c are fit parameters.

Or,

$$\ln N = \ln N_0 + A \exp\left\{-\exp\left[\frac{\mu_{max} \times e}{A}(\lambda - t) + 1\right]\right\} \qquad \text{Eq. 2.5}$$

where:

μ_{max}= maximum specific growth rate (h^{-1})

A = maximum level of increase: $\ln(N_\infty/N_0)$

A typical bacterial growth curve is shown in Figure 2.1.

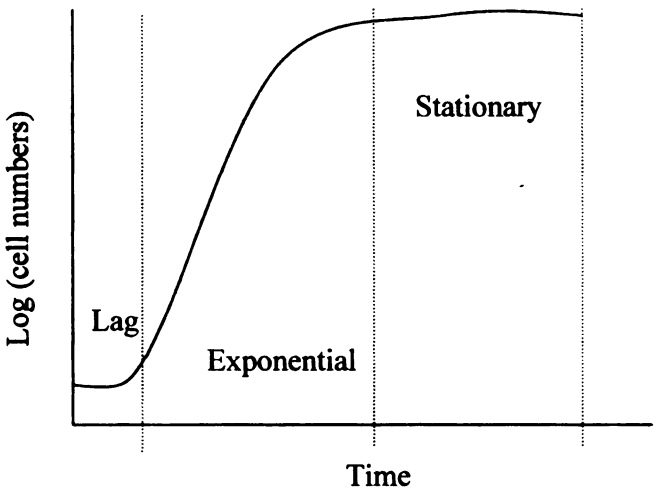

FIGURE 2.1. Microbial growth curve.

In the lag-phase, there is no apparent growth, while the inoculum adjusts to the new environment, synthesizes the enzymes required for its exploitation, and repairs any lesions resulting from earlier injury (e.g., freezing, drying, heating) (Adams and Moss, 1995). The next phase, the exponential or logarithmic, is characterized by an increase in cell numbers; the slope corresponds to the organism's specific growth rate, μ, which depends on the experimental conditions. When key nutrients become depleted (after the exponential growth), or inhibitory metabolites accumulate, the culture moves to the stationary phase (Adams and Moss, 1995).

There are two levels of detail that can be used, depending on the necessity, for microbial growth estimation (van Gerwen and Zwietering, 1998). For rough risk assessments (level 1), orders of magnitude for growth can be estimated easily using equation 1. Neglecting λ results in fail-safe predictions, and stationary growth is generally not relevant in risk assessments. However, if growth is one of the main determinants of risk (level 2), the description of the entire growth curve (equation 5, for

example) would be useful.

2.1.2 Secondary growth models

The three approaches most frequently used with secondary growth models are the response surface model (regression equation that is fitted using standard regression techniques, which may contain linear, quadratic, cubic, or reciprocal terms and includes interaction or cross product terms, (Whiting, 1995)), the Arrhenius relationship (logarithm of the rate versus the reciprocal of the temperature (K)), and the square root model.

The square root model (eq. 2.6) is a simple empirical model suggested by Ratkowsky *et al.* (1982), in which the data (experimental growth rate) were transformed, by taking their square root, to stabilize their variance (making the variance of the distribution independent of its mean, and close to normal distribution, (Ratkowsky, 2004)). This model and its numerous expansions are called square-root-type, Ratkowsky-type, or Bêlerádek-type models (Ross and Dalgaard, 2004).

$$\sqrt{\mu_{\max}} = b \times \left(T - T_{\min}\right) \qquad \text{Eq. 2.6}$$

where:

b = constant

T = temperature

T_{min} = parameter, a theoretical minimum temperature for growth.

Growth rates of microorganisms are described less appropriately by the Arrhenius-type equations than by square-root-type models (Ross and Dalgaard, 2004).

However, the Arrhenius-type models remain useful as secondary kinetic models for less extensive ranges of storage temperatures. The empirical Arrhenius-van't Hoff relationship is (Ross and Dalgaard, 2004):

$$rate = A\exp\left(-\frac{E_a}{RT}\right) \qquad \text{Eq. 2.7}$$

where:

A = constant

E_a = activation energy

R = the gas constant (8.314 J/K/mol)

T = temperature in Kelvin

The most common secondary models applied within predictive microbiology are the polynomial models. They are relatively easy to fit to experimental data by multiple linear regression, and they allow virtually any of the environmental variables and their interactions to be taken into account (Ross and Dalgaard, 2004); however, higher order polynomial models (e.g., cubic or quadratic) have been criticized for being too flexible and for attempting to model, rather than eliminate, experimental error; furthermore, these models also include coefficients with no biological interpretation (Baranyi *et al.*, 1996b; Ross and Dalgaard, 2004). Furthermore, because of their flexibility, they should only be used to provide predictions by interpolation(Baranyi *et al.*, 1996a; Baranyi *et al.*, 1996b). A quadratic equation is an illustration of such polynomial models:

$$\begin{aligned}\ln y = {} & \beta_1 + \beta_2 x_1 + \beta_3 x_2 + \beta_4 x_3 + \beta_5 x_1 x_2 + \beta_6 x_1 x_3 \\ & + \beta_7 x_2 x_3 + \beta_8 x_1^2 + \beta_9 x_2^2 + \beta_{10} x_3^2 + e\end{aligned} \qquad \text{Eq. 2.8}$$

where:

ln y = natural logarithm of the modeled growth responses (y = μ_{max}, lag time, or maximum population density [MPD]).

β_i $(i = 1, ..., 10)$ = coefficients to be estimated.

x_1, x_2, x_3 = environmental variables (e.g., temperature, pH, salt)

e = random error

2.1.3 Tertiary growth models

In tertiary models, environmental values of interest are entered into secondary models to obtain specific parameter values for the primary model. The primary model is then solved for increasing periods of time to obtain the growth or inactivation curve expected from that combination of environmental values. The primary and secondary models are used in conjunction with spreadsheets or other software programs, which avoids the reentering of equations, takes advantage of graphics capabilities of the software, and allows performance of other calculations. Tertiary systems vary in complexity from an equation on a spreadsheet to expert systems or risk assessment simulations (Doyle *et al.*, 2001).

An example of the tertiary model is the USDA Pathogen Modeling Program (PMP, (U.S. Department of Agriculture, 2003b)). The PMP (Fig. A.2) is a free software package of microbial models that describes growth, survival, inactivation, or toxin production of several pathogens, under various conditions defined by the user. Depending on the specific model, environmental inputs include atmosphere (aerobic or anaerobic), temperature, pH, water activity, ionizing radiation, varying concentrations of lactic acid,

sodium chloride, nitrite, and sodium pyrophosphate, or all of these. Lag phase duration, generation time, and time are displayed either in hours or days. Growth/inactivation curves are displayed in both graphical and tabular formats, with their respective confidence intervals. In addition, the PMP contains dynamic temperature models for the growth of *Clostridium perfringens* and *Clostridium botulinum* (McKellar and Lu, 2004; Tamplin *et al.*, 2004; Whiting, 1995).

Once these predictive tools are validated and tested, they can be used in a variety of ways. For example, quantitative risk assessment for the fate of pathogens in food products relies on predictive models for growth, survival, and inactivation, in order to determine strategies to improve food safety. In the same way, predictive microbiology assists in identifying hazards and critical control points, and in specifying limits for corrective actions in the formulation of hazard analysis critical control point (HACCP) plans (McMeekin and Ross, 2002). Furthermore, the consequences of reformulating a food product can be evaluated. Alternative formulations can be evaluated using models, the influence of different factors can be obtained, and formulations with similar or enhanced resistance to growth can be identified (Whiting, 1995).

Computer technology and advances in computational power make predictive microbiology readily available for the ultimate user. However, this user (i.e., a food processor) generally operates these tools "as is", confident that the elements provided were already validated and tested, because he/she does not have the time, facilities, or knowledge to corroborate the validity of that tool. Therefore, it is necessary to improve currently available tools, to avoid unknown predictions that could lead to dangerous outcomes, causing an increasing risk for consumers.

2.2 LISTERIA MONOCYTOGENES

Listeria is a small, regular Gram-positive rod with round ends. The genus *Listeria* contains six species: *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, and *L. grayi* (Ryser and Marth, 1999).

L. monocytogenes, in particular, represents a threat to the food industry, due to its resistance (it can survive adverse environmental conditions), its ability to colonize, multiply, and persist on processing equipment, and its ability to multiply at refrigeration temperatures. It can be found in soil, water, and in plant material. *L. monocytogenes* also has been recognized as a human pathogen since 1929, and in 2000 the Centers for Disease Control and Prevention (CDC) reported that of all the foodborne illnesses tracked by CDC, *L. monocytogenes* had the second highest case fatality rate (21%) and the highest hospitalization rate (90.5%) (U. S. Department of Health and Human Service, 2003). This disease usually occurs in high-risk groups, including pregnant women, neonates, and immunocompromised adults (Ryser and Marth, 1999; U. S. Department of Health and Human Service, 2003).

Foodborne listeriosis outbreaks could be dated back to 1914, even before Murray isolated it in 1926 (Ryser and Marth, 1999). Vehicles of infection, reported since 1949, can range from raw milk, sour milk, cream, pork, raw vegetables, shellfish, raw eggs, processed meats or pâté, sweet corn, cheese, to rice salad (Ryser and Marth, 1999). These foods are usually preserved by refrigeration and offer an appropriate environment for the multiplication of *L. monocytogenes* during manufacturing, aging, transportation, and storage.

Accurate prediction via mathematical models is becoming more important, not

only for risk assessors, but also for food processors, to evaluate the production-processing-consumption chain for food products that can be suitable substrates for *L. monocytogenes* growth. There are several models currently used to predict *L. monocytogenes* growth, which were developed either from broth-based data or actual food systems. A description of some of the more widely used models follows.

2.3 GROWTH MODELS

The most frequently used primary growth models are the Gompertz (modified model, eq. 2.7, (Gibson *et al.*, 1987)) and Baranyi equations (eq. 2.8, (Doyle *et al.*, 2001)) the first being a sigmoidal relationship, and the second being based in part on the concept that the rate of bacterial growth is controlled by the rate of a "bottleneck" biochemical reaction.

$$L(t) = A + Ce^{-e^{\left[-B\left(t-M\right)\right]}} \qquad \text{Eq. 2.9}$$

where:

$L(t)$ = log counts of bacteria at time t (log(CFU/ml))

A = Asymptotic log count of bacteria as t decreases indefinitely (log(CFU/ml))

C = Asymptotic, incremental increase in log count of bacteria as t increases indefinitely (log(CFU/ml))

M = Time at which the absolute growth rate is maximum (h)

B = Relative growth rate at M (1/h)

t = time (h)

and,

$$Y_t = Y_0 + \mu_{max} A(t) - \ln\{1 + [\exp(\mu_{max}\alpha(t) - 1)\exp(Y_{max} - Y_0)]\} \quad \text{Eq. 2.10}$$

where:

Y_t, Y_0, Y_{max} = log cell concentration (log(CFU/ml)) at time t, at inoculation, and at maximum cell density (stationary phase), respectively.

μ_{max} = maximum specific growth rate (1/h)

$A(t)$ = integral of the adjustment function $\alpha(t)$.

Several authors compared the performance of these two models, plus some other models, applied to different microorganisms (Baty and Delignette-Muller, 2004; Buchanan *et al.*, 1997; Juneja *et al.*, 1999). For example, Baty *et al.* (2004) found that the Gompertz model seems to be influenced more by the quality of the data set than is the Baranyi model. They also concluded that the Baranyi model provided the best fit for the majority of their data and gave reasonably precise estimates of the lag time. Another study also found that the Gompertz equation can overestimate the model parameters, which could bias the comparison with a different model (Membre *et al.*, 2004).

There are a numerous studies on secondary models (Augustin *et al.*, 1999; Augustin *et al.*, 2000; Buchanan and Phillips, 1990; Buchanan *et al.*, 1989; Cheroutre-Vialette *et al.*, 1998; Dalgaard and Jorgensen, 1998; Delignette-Muller *et al.*, 1995; Farber *et al.*, 1996; Fernandez *et al.*, 1997; George *et al.*, 1996; Whiting and Bagi, 2002; Wijtzes *et al.*, 1993), which include square-root, polynomial, Arrhenius, etc., applied to *L. monocytogenes*. These studies focused mainly on how the lag phase and/or growth rate is influenced by pH, water activity, carbon dioxide, lactic and acetic acids, salt, nitrite, and/or temperature. These parameters are important mainly because they give an estimate

of the shelf life (how long a product will last under its storage conditions without *L. monocytogenes* growing) of food products.

L. monocytogenes is a pathogen that has one of the highest rates of mortality; fortunately, predictive microbiology is becoming a very significant tool to limit risks associated to this pathogen. Research done so far has extensively focused on the influence of experimental conditions on growth parameters, most of them in broth (Augustin *et al.*, 1999; Augustin *et al.*, 2000; Breand *et al.*, 1999; Buchanan and Phillips, 1990; Buchanan *et al.*, 1989; Cornu *et al.*, 2002; Delignette-Muller *et al.*, 1995; Farber *et al.*, 1996; Fernandez *et al.*, 1997; George *et al.*, 1996; Whiting and Bagi, 2002; Wijtzes *et al.*, 1993), and some in food systems (Bovill *et al.*, 2000; Cheroutre-Vialette *et al.*, 1998; Dalgaard and Jorgensen, 1998). However, to be confident about the results that users are currently getting from models, more research is needed on validation of these models, in terms of actual microbial counts; the previous cited literature only focused on validation of models for growth parameters. Moreover, an overall assessment of the accuracy and uncertainty of these predictions will further contribute to the true estimates of either growth or inactivation of this foodborne pathogen.

2.4 MODEL LIMITATIONS

Models in general have inevitable limitations, either statistical or biological, that need to be addressed before they are applied. For instance, these limitations could be the inherent uncertainty of the model itself, the lack of fit of the model to a specific data, the type of fitting, and the domain in which the model was developed.

Before analyzing a model, it is important to understand that the growth process is

both variable and uncertain. Growth is variable, because the growth curve of one population will never be exactly the same as that of another population, not even for the same strain under identical circumstances; this variability is reflected by a spread of values for any particular property of the individuals that make up the population (Barker *et al.*, 2005; Nauta, 2000). Growth is uncertain, because growth progress will never be exactly known; microbiological measurements used to construct the growth curve will always be somehow imperfect (Nauta, 2000). Both uncertainty and variability greatly influence the performance of a model.

The goal is to use the simplest model that describes the response of an organism to a specific food system. Ideally, the model should give parameters that help to understand the system and design new experiments. Linear and nonlinear regressions can be used to fit a mathematical model to the data in order to determine the best-fit values of the model parameters (Motulsky and Christopoulus, 2004). Reliable prediction of risk can be made by incorporating uncertainty in the predictions; in order to do that, the accountability of its limits is necessary. Various methods can be used to characterize uncertainty in statistics, such as the mean or standard deviation, including analytical solutions and numerical solutions. Some transformations are used to homogenize the variances for fitting the models, so normal distribution can be attained. Although the process of reparameterization can be controversial, it makes variances more uniform and normally distributed and makes the parameters more interpretable (Ross and McMeekin, 2003; Whiting, 1995; Zheng and Frey, 2004).

When using nonlinear regression to fit a model to raw data, resulting confidence and prediction intervals are only approximations. When a model is nonlinear in the

parameters, no explicit analytical solutions are available for the parameters or the confidence intervals, and a solution must be found by linear approximation; meaning, iteratively, starting from initial values supplied by the analyst, or estimated by the computer program. Determination of the prediction limits is difficult; however the easiest method is to use asymptotic standard errors (SE), which are found from the matrix of variances and covariances of the parameters (Van Boekel, 1996). Once the SE is calculated, the confidence intervals can be computed using the *t*-parameter for a confidence level (1-0.5α) and degrees of freedom $\nu = n-p$, where n is the number of data points and p is the number of parameters. The Monte Carlo method is considered the best method (Motulsky and Christopoulus, 2004; Van Boekel, 1996), in which synthetic data are produced based on the model function and the obtained parameters, but with addition of random errors. The synthetic data set is then analyzed again with the same model function to obtain new parameter estimates. Doing this many times yields a distribution of parameter values, from which a confidence interval of the parameter and/or the dependent variable can be found.

Besides the uncertainty, lack of fit, and type of fitting of a model, the domain of the model has to be clearly specified, meaning, what microorganisms, what factors, the ranges of each factor, and what combination of factors give valid answers (biological limitations); otherwise, cautious interpretation of the predictions is required (Whiting, 1995). The overall uncertainty of a model accounts for both the statistical and biological limitations. Therefore, its assessment and understanding, either inside or outside the model domain, would contribute to the improvement of risk and food safety predictions.

CHAPTER 3

ROBUSTNESS OF MICROBIAL GROWTH MODELS

3.1 SUMMARY

Given the importance of *Listeria monocytogenes* as a risk factor in meat and poultry products, there is a need to evaluate the relative robustness of predictive growth models applied to meat products. The U.S. Department of Agriculture – Agricultural Research Service (USDA-ARS) Pathogen Modeling Program (PMP) is a tool widely used by the food industry to estimate pathogen growth/survival/inactivation in food. However, the robustness of the PMP broth-based *L. monocytogenes* growth model in meat and poultry application has not been specifically evaluated. In the present study, this model was evaluated against independent data in terms of predicted microbial counts covering a range of conditions inside and outside the original model domain. The Robustness Index (RI) was calculated as the ratio of the standard error of prediction, SEP (root mean square error [RMSE] of the model against an independent data set not used to create the model), to the standard error of calibration, SEC (RMSE of the model against the data set used to create the model). Inside the calibration domain of the PMP, the best RI for application to meat products was 0.37; the worst was 3.96. Outside the domain, the best RI was 0.40, and the worst was 1.22. Product type influenced the RI values ($P<0.01$). In general, the results indicated that broth-based predictive models should be validated against independent data in the domain of interest; otherwise, significant predictive errors can occur.

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3.2 INTRODUCTION

Quantitative risk assessments for the fate of pathogens in food products depend heavily on the validity of predictive models for pathogen growth, survival, and inactivation. An accurate prediction may have to consider whether a model is easy to use (the simplest one for a given purpose and data quality), whether it is robust and accurate (it must reflect reality), and whether it is validated against independent data sets (Ratkowsky, 2004). The validation or performance evaluation of a model can also be referred to as the robustness of the model (Campos *et al.*, 2004). The robustness indicates how well a model predicts future independent results across a wide domain of conditions. However, experimental data and associated models are rarely available to account for all of the relevant variables and range of conditions for a specific pathogen, product, and process being analyzed. Therefore, a risk assessment might extrapolate the predictive models, either in terms of the process parameters (e.g., temperature) or product parameters (e.g., fat content). Even though this practice is fundamentally undesirable, it might be the only means to completing a risk assessment for a given product/system; therefore, it is desirable and necessary to fully understand the implications of this practice.

In particular, given the importance of *Listeria monocytogenes* as a risk factor in meat and poultry products, there is a need to evaluate the relative robustness of predictive microbial growth models for this specific pathogen. Previous research has shown that product/process variables (e.g., pH, water activity) significantly affect *L. monocytogenes* response (Cheroutre-Vialette *et al.*, 1998). However, knowing that an effect exists is not sufficient to account for that effect quantitatively in predictive models. Some previous

studies only reported descriptive models (meaning that experimental data are generated and a model is fit to those data), which described the combined effect of temperature, pH, water activity, and CO_2 concentrations (Farber *et al.*, 1996; Wijtzes *et al.*, 1993). Other investigators compared their mathematical models (of the effect of CO_2, pH, temperature, NaCl, organic acids and modified atmospheres) against independent data sets (Buchanan and Phillips, 1990; Fernandez *et al.*, 1997; George *et al.*, 1996). Unfortunately, they made only qualitative comparisons between observed and predicted values and did not present a quantitative validation for their models.

On the other hand, Ross (Ross, 1996) presented the bias and accuracy factors as indices to evaluate the performance of predictive models in food microbiology, in terms of growth parameters (i.e., growth rates, lag phase duration). The bias factor is an overall average of the ratio of discrete model predictions to observations and assesses whether or not the model is "fail-safe", "fail-dangerous", or perfect. The accuracy factor is similar to the bias factor, except that it is the absolute value of the ratio of predictions to observation, thus providing an accumulated measure of overall model accuracy. However, these factors have only been used to evaluate the performance of secondary models in predicting growth parameters; actual log counts (from primary + secondary models) were not considered.

The true measure of product safety is actual microbial counts, not model parameters. Campos et al. (Campos *et al.*, 2004) introduced a new methodology to evaluate the robustness of a microbial growth model in terms of microbial counts. The robustness index (RI) was defined as the ratio of the standard error of prediction (SEP) to the standard error of calibration (SEC). The SEC and SEP are the root mean squared

errors (RMSE) calculated from the original and independent data sets, respectively. The RMSE is one of the most useful and informative measures of the goodness-of-fit against the model prediction for linear and nonlinear regressions. Moreover, it is a way to estimate the discrepancy between the observed and predicted data, which reflects whether a model truly fits the data well (Lammerding and McKellar, 2004). A robust model will have an RI value near or less than 1, meaning that the overall performance of a microbial model tested against an independent data set is within the expected error (SEC) of the model. Campos et al. (2004) also stated that the RI value alone does not tell whether observed values are above or below the predicted values; the mean relative error (RE) is used with the RI in order to provide this information.

The U.S. Department of Agriculture (USDA) – Agricultural Research Service (ARS) Pathogen Modeling Program (PMP, (2003b) and the U.K. Food MicroModel are tools used by the food industry to estimate pathogen growth/survival/inactivation in food. The majority of these models were developed from pure-culture, broth-based data. Because these models are based on pure-culture systems containing high level of nutrients and no competitive microbial flora, they are generally assumed to provide conservative estimates of pathogen growth.

Several authors have considered the performance of the PMP and other microbial growth models. For instance, te Giffel and Zwietering (1999) evaluated the prediction of *L. monocytogenes* growth rates in foods, including meat, by general models (e.g., Gamma-concept, PMP, Food MicroModel) and by specific models (e.g., modified Arrhenius equation, third-order polynomial model, quadratic equation, etc.). They tested these models against independent data sets and validated the models by graphical

comparison and mathematical/statistical comparison (mean squared error, regression coefficient, bias and accuracy factors). They recommended the use of a set of criteria to evaluate the performance of models, because the use of one criterion may fail to reveal some forms of systematic deviation between observed and predicted behavior. Again, the prior study evaluated the performance of only a secondary model; actual log count predictions were not evaluated.

Additionally, the evaluation of the models mentioned here did not include data outside their original domain, which is critically important if they are to be applied to broader risk analyses for foodborne pathogens in ready-to-eat food products. Furthermore, the prior studies only evaluated secondary models for growth parameters (i.e., growth rate, generation time, lag phase duration). They did not evaluate the robustness of the complete model (primary + secondary), which predicts the actual growth values, and gives the complete behavior (lag phase, exponential growth, and stationary phase) of the pathogen of interest.

Therefore, the objective of the present study was to evaluate the robustness, against independent data, of the PMP broth-based growth model for *L. monocytogenes* in meat and poultry products, in terms of predicted microbial counts covering a range of conditions inside and outside the original model domain.

3.3 MATERIALS AND METHODS

3.3.1 Data sources

ComBase ((Baranyi and Tamplin, 2004; U.S. Department of Agriculture, 2003a), Fig. A.3) was used as the main source of independent data sets. ComBase pre-distribution

version 2002, was searched for all records that included microbial counts with organism: "*L. monocytogenes/innocua*", and broth or food category: meat or meat products. In total, 65 data sets were found; 41 were within the domain of the PMP *L. monocytogenes* growth model, and 24 were outside the domain of the model (Table 3.1).

The original data sets used to develop the PMP broth-based[1] *L. monocytogenes* growth model were also obtained from ComBase ((U.S. Department of Agriculture, 2003a), source: "Buchanan_90", organism: "*L. monocytogenes/innocua*", environment: "culture medium", pH: "0.1 to 14", temperature: "-25 to 120", and a_w: "0.01 to 1"). These data sets were assumed to be the original ones used in the PMP, because they were in the same range of experimental conditions (pH: 4.5-7.5, nitrite: 50-1000 ppm, salt: 15-50 g/l, and temperature: 5-37 °C), and had a similar number of data sets (385 for anaerobic, and 553 for aerobic). The no-growth data were eliminated (Buchanan and Phillips, 1990; Buchanan *et al.*, 1989). The remaining data sets (n_{sets}= 291, n_{points}= 2,302 for anaerobic, and n_{sets}= 476, n_{points}= 3,680 for aerobic) were used to calculate the SEC of the PMP growth model.

[1] "Inoculum: Strains were maintained in BHI broth at 4° C and transferred monthly. Procedure: The test medium was 50 ml TPB in a 250 ml Erlenmeyer flask (aerobic) or a 250 trypsinizing flask fitted with a side arm (anaerobic, nitrogen flushed). Flasks were inoculated with 0.5 ml of a 24 h culture, shaken at 150 rpm. Enumeration: aliquots were plated on Tryptose Phosphate agar, and incubated for 24 h at 37 C", from Buchanan and Phillips (1990).

TABLE 3.1. References and keys in ComBase for meat and poultry data used in this study.

Data set No.	Key (ComBase)	Product type	Reference
1-6	J206_Lm to J211_Lm	Ground Beef	*(Nissen et al., 2000)*
7-12	J232_Lm to J237_Lm	Cooked Chicken	*(Barakat and Harris, 1999)*
13	M007	Pate	*(Bovill et al., 2000)*
14-18	M200_LM to M204_LM	Cooked Pork	*(Fang and Lin, 1994)*
19	M263_LM	Precooked Beef	*(Cooksey et al., 1993)*
20-24	M263_Lma to M263_Lme	Precooked Beef	*(Cooksey et al., 1993)*
25-26	M656_LM to M657_Lm	Cooked Beef w/ Gravy	*(Grant et al., 1993)*
27-28	M660_Lm to M661_Lm	Cooked Beef w/ Gravy	*(Grant et al., 1993)*
29	M921_LM	Home-style salad (chicken with no mayonnaise added)	*(Erickson et al., 1993)*
30	M921_LMa	Home-style chicken salad	*(Erickson et al., 1993)*
31	M921_LMb	Home-style salad (real mayonnaise + chicken)	*(Erickson et al., 1993)*
32	M921_LMd	Home-style salad (reduced calorie mayonnaise + chicken)	*(Erickson et al., 1993)*
33	M921_LMg	Home-style salad (real mayonnaise + chicken)	*(Erickson et al., 1993)*
34	M921_Lmi	Home-style salad (reduced calorie mayonnaise + chicken)	*(Erickson et al., 1993)*
35	SL113	Turkey	*(Mano et al., 1995)*
36	SL118	Turkey	*(Mano et al., 1995)*
37	SL123	Turkey	*(Mano et al., 1995)*
38-41	SL59 to SL62	Pork	*(Mano et al., 1995)*
42-52	M122_133 to M122_144	Pate or ham	*(Mano et al., 1995)*
54-65	M122_37 to M122_48	Pate or ham	*(Mano et al., 1995)*

3.3.2 Predictive models.

The robustness of the broth-based *L. monocytogenes* growth models (aerobic and anaerobic) in PMP version 7.0 ((U.S. Department of Agriculture, 2003b) was determined by testing the model predictions against the independent data sets. Because the PMP, as it is distributed, cannot run outside the calibration domain, the secondary models used to

calculate generation time (*GT*) and lag phase duration (*LPD*) within the PMP domain (Table 3.2) were implemented in a spreadsheet (source: A. Pickard, USDA-ARS Eastern Regional Center), to generate predictions for the data sets that were outside the original domain.

TABLE 3.2. Coefficient values for secondary models

	Aerobic		Anaerobic	
Variable	*Ln GT*	*Ln LPD*	*Ln GT*	*Ln LPD*
Int	21.45832	26.86796	13.51036	19.82645
T	-0.26798	-0.21535	-0.10334	-0.20281
pH	-5.29657	-6.5596	-3.34632	-4.34946
NaCl	0.012824	0.051605	0.042326	0.031356
NO_2	0.020202	0.019974	0.021956	0.024464
T×pH	0.00757	0.003684	-0.01424	-0.0032
T×NaCl	7.94E-06	0.000223	-3.5E-05	7.06E-05
T×NO_2	-5.1E-07	1.93E-05	4.83E-06	1.62E-05
pH×NaCl	-0.00137	-0.00686	-0.0036	-0.00181
pH×NO_2	-0.00278	-0.0028	-0.00282	-0.00321
NaCl×NO_2	5.28E-06	-3.7E-06	4.14E-06	-2.7E-06
T^2	0.002666	0.001918	0.002725	0.003123
pH^2	0.384181	0.487334	0.262941	0.310527
$NaCl^2$	0.000122	0.000102	-0.00027	-2.6E-05
NO_2^2	5.91E-07	7.36E-07	-8.6E-07	-4.8E-07

The primary model was the Gompertz equation:

$$L(t)=A+Ce^{-e^{\left[-B(t-M)\right]}} \qquad \text{Eq. 3.1}$$

where:

$L(t)$ = log counts of bacteria at time t (log (CFU/ml))

A = Asymptotic log count of bacteria as t decreases indefinitely (log (CFU/ml))

C = Asymptotic log count of bacteria as t increases indefinitely (log (CFU/ml))

M = Time at which the absolute growth rate is maximum (h)

B = Relative growth rate at M (1/h)

t = time (h),

and where (Buchanan and Phillips, 1990):

$$B = \frac{\log 2 \cdot e}{GT \cdot C} \qquad \text{Eq. 3.2}$$

$$M = LPD + \frac{1}{B} \qquad \text{Eq. 3.3}$$

Using the Gompertz primary model and the response surface secondary model from the PMP, log counts were predicted for conditions and times matching every experimental data point from the described data sources, given the initial log counts for the respective experimental growth curve.

Confidence intervals[2] (95%) were generated based on the following equation (adapted from (Neter *et al.*, 1992)):

$$CI = \hat{y}_j \pm (z)(SEC) \qquad \text{Eq. 3.4}$$

where:

CI = confidence interval

$\hat{y}_j$ = Predicted value of j^{th} data point (log (CFU/ml))

$z = z(1-\alpha/2)$, $\alpha=0.05$

SEC = Standard error of calibration (formula below)

[2] For the subsequent chapters (4 and 5) these confidence intervals were more accurately described as "prediction intervals", given that SEC is the root mean squared error between the observed values and the actual predicted values, not the mean responses.

3.3.4 Robustness Index (RI)

The RI for the PMP broth-based *L. monocytogenes* growth model was calculated based on the following equation (Campos *et al.*, 2004):

$$RI = \frac{SEP}{SEC} \qquad \text{Eq. 3.5}$$

where:

SEC = Standard error of calibration[3]

$$= \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n}} \qquad \text{Eq. 3.6}$$

where:

$\hat{y}_i$ = Predicted value of i^{th} data point (log(CFU/ml))

y_i = Observed value of the i^{th} data point from the original data sets used to develop the model (log(CFU/ml)), assuming 1 CFU/ml = 1 CFU/g

n = number of observed data points from the original data set

and

SEP = Standard error of prediction

$$= \sqrt{\frac{\sum_{j=1}^{n}(y_j - \hat{y}_j)^2}{n}} \qquad \text{Eq. 3.7}$$

where:

[3] In the following chapters, the denominator for SEC is more accurately represented as $n-p$, where p = number of parameters of the model. For this section p=2 and n~3000, so the final outcome was not significantly affected.

$\hat{y}_j$ = Predicted value of j^{th} data point (log(CFU/g))

y_j = Observed value of the j^{th} data point from an independent data set (log(CFU/g)), assuming 1 CFU/ml = 1 CFU/g

n = number of observed data points from an independent data set

The overall RI for each product type was calculated using the combined observed data from all independent sources:

$$RI = \frac{\sqrt{\frac{\sum_{k=1}^{n}(y_k - \hat{y}_k)^2}{n}}}{SEC} \qquad \text{Eq. 3.8}$$

where:

$\hat{y}_k$ = Predicted value of k^{th} data point (log(CFU/g))

y_k = Observed value of the k^{th} data point from all independent data sets corresponding to each product type (log(CFU/g)), assuming 1 CFU/ml = 1 CFU/g,

n = total number of observed data points from all independent data sets corresponding to each meat product type

Additionally, the mean relative error (RE) was calculated based on the following formula (Campos *et al.*, 2004):

$$RE = \frac{\sum_{j=1}^{n}\left(\frac{y_j - \hat{y}_j}{\hat{y}_j}\right)}{n} \qquad \text{Eq. 3.9}$$

where,

$\hat{y}_j$ = Predicted value of j^{th} data point (log(CFU/g))

y_j = Observed value of the j^{th} data point from an independent data set (log(CFU/g)), assuming 1 CFU/ml = 1 CFU/g

n = number of data points

To evaluate whether any of the product/process variables affected the RI, an analysis of variance (ANOVA) was conducted using JMP (SAS Institute Inc., Cary, N.C. Version 4.0.4).

3.4 RESULTS AND DISCUSSION

The SEC values for the PMP growth models for anaerobic and aerobic conditions were 1.49 log(CFU/ml) and 1.15 log(CFU/ml), respectively. This means that the model accuracy was ±1.32 log(CFU/ml) accuracy, on average, for the broth-based data for both atmospheric conditions.

The RI values for all the meat and poultry products that were inside the PMP domain were between 0.37 and 3.96 (Table 3.3). The mean relative error shows that the PMP growth model over-predicted (i.e., fail-safe) the log counts for 85% of the cases.

For the data set yielding the best RI value (Fig. 3.1), predicted and actual log counts were within the confidence levels, which implies that the model performed better than expected. On the other hand, for the data set yielding the worst RI value (Fig. 3.2), the actual log counts were outside the confidence bands predicted. This particular data set presented no growth in the total period that was studied.

TABLE 3.3. RI values for conditions inside the PMP model domain

Data set No.	Product type	Temp (°C)	pH [a]	a_w [b]	Atmosphere	RI	Mean Relative Error (RE)
1	Ground Beef	4	5.8	0.997	Anaerobic	2.44	-0.33
2	Ground Beef	4	5.8	0.997	Aerobic	2.21	-0.27
3	Ground Beef	4	5.8	0.997	Aerobic	2.18	-0.25
4	Ground Beef	10	5.8	0.997	Anaerobic	2.57	-0.39
5	Ground Beef	10	5.8	0.997	Aerobic	2.65	-0.25
6	Ground Beef	10	5.8	0.997	Aerobic	2.52	-0.28
7	Cooked Chicken	3.5	6	0.997	Anaerobic	1.15	-0.16
8	Cooked Chicken	3.5	6	0.997	Anaerobic	2.10	-0.32
9	Cooked Chicken	6.5	6	0.997	Anaerobic	0.45	0.02
10	Cooked Chicken	6.5	6	0.997	Anaerobic	0.78	-0.07
11	Cooked Chicken	10	6	0.997	Anaerobic	0.98	-0.08
12	Cooked Chicken	10	6	0.997	Anaerobic	1.52	-0.18
13	Pate	6.8	5.6	0.997	Aerobic	0.37	-0.03
14	Cooked Pork	4	6.3	0.997	Anaerobic	1.40	-0.21
15	Cooked Pork	4	6.2	0.997	Aerobic	1.20	-0.15
16	Cooked Pork	20	6.3	0.997	Anaerobic	0.95	-0.12
17	Cooked Pork	20	6.2	0.997	Aerobic	1.41	-0.14
18	Cooked Pork	20	6.3	0.997	Aerobic	0.66	-0.07
19	Precooked Beef	4	6	0.997	Vacuum [c]	1.40	-0.16
20	Precooked Beef	4	6	0.997	Vacuum [c]	0.41	-0.04
21	Precooked Beef	4	6	0.997	Vacuum [c]	2.76	-0.40
22	Precooked Beef	4	6	0.997	Vacuum [c]	1.94	-0.27
23	Precooked Beef	4	6	0.997	Vacuum [c]	3.96	-0.58
24	Precooked Beef	4	6	0.997	Vacuum [c]	2.36	-0.34
25	Cooked Beef w/ Gravy	5	6	0.997	Aerobic	0.48	0.06
26	Cooked Beef w/ Gravy	10	6	0.997	Aerobic	0.93	-0.08
27	Cooked Beef w/ Gravy	5	6	0.997	Aerobic	1.41	-0.26
28	Cooked Beef w/ Gravy	10	6	0.997	Aerobic	1.95	-0.24
29	Home-style salad (chicken with no mayonnaise added)	4	6	0.997	Aerobic	0.53	0.11

TABLE 3.3. Continuation.

Data set No.	Product type	Temp (°C)	pH [a]	a_w [b]	Atmosphere	RI	Mean Relative Error (RE)
30	Home-style chicken salad	4	6	0.997	Aerobic	0.56	0.08
31	Home-style salad (real mayonnaise + chicken)	4	6	0.997	Aerobic	1.29	-0.23
32	Home-style salad (reduced calorie mayonnaise + chicken)	4	6	0.997	Aerobic	1.47	-0.27
33	Home-style salad (real mayonnaise + chicken)	12.8	5	0.997	Aerobic	2.08	0.52
34	Home-style salad (reduced calorie mayonnaise + chicken)	12.8	5	0.997	Aerobic	1.28	0.31
35	Turkey	7	6	0.99	Anaerobic	1.16	-0.19
36	Turkey	7	6	0.99	Aerobic	1.52	-0.19
37	Turkey	7	6	0.99	Aerobic	1.78	-0.21
38	Pork	7	6	0.99	Aerobic	2.45	-0.30
39	Pork	7	6	0.99	Anaerobic	2.87	-0.45
40	Pork	7	6	0.99	Aerobic	3.45	-0.41
41	Pork	7	6	0.99	Aerobic	3.44	-0.40

[a] Assumed values for data sets 7-12.

[b] Assumed values for data sets 1-34.

[c] Assumed anaerobic for calculations

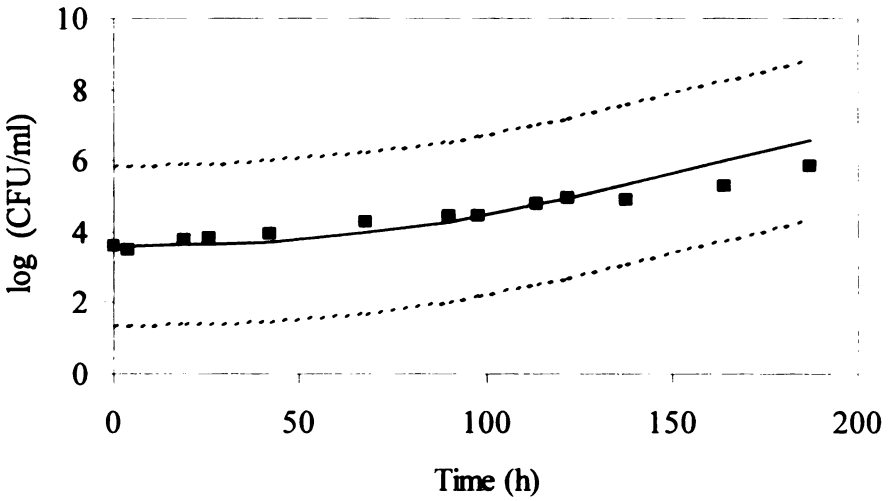

FIGURE 3.1. Comparison of the predicted (solid line) and actual (full squares) growth log counts from the data set (No. 13) resulting in the best RI value (0.37) inside the PMP model domain (95% confidence intervals, broken lines).

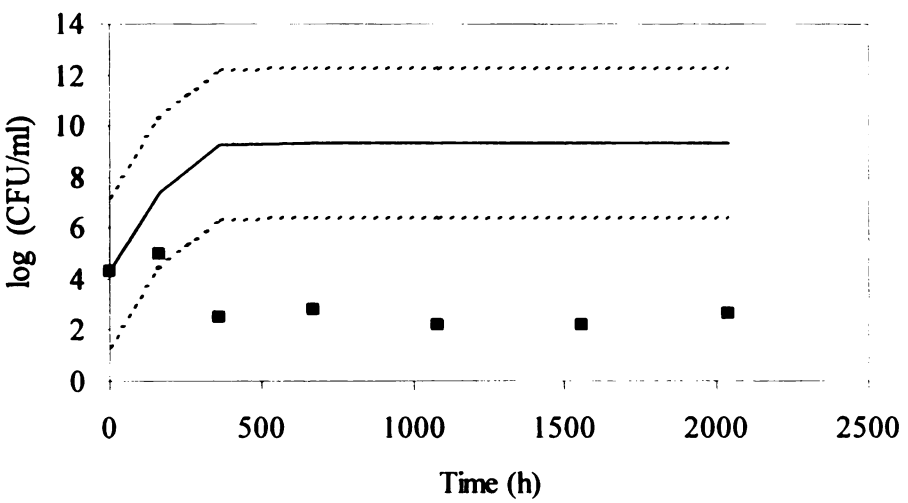

FIGURE 3.2. Comparison of the predicted (solid line) and actual (full squares) growth log counts from the data set (No. 23) resulting in the worst RI value (3.96) inside the PMP model domain (95% confidence intervals, broken lines).

Among the variables tested, only product type affected the RI value (Table 3.4)[4]. Therefore, the data were grouped into classes of similar product type, and an overall performance (RI) was calculated for each group (Table 3.5). The RI values between 0 and 2 (i.e., pate, cooked chicken, cooked pork and turkey) indicated satisfactory robustness for the PMP in the application. RI=2 is an arbitrary criterion; it means that the actual log counts were generally within the range described by standard error of calibration (SEC) of the model. The RI values above 2 (i.e., pork, ground beef, and precooked beef) suggest that actual log counts are more likely to fall outside the confidence limits of the model for this particular type of meat, and under these specific conditions, the model did not perform as expected. The differences in RI values might be due to the variability of the laboratory methods. For each product type, and even within the same product type, it is possible to find different inoculation and enumeration methods; different treatment or sample preparation; and different *L. monocytogenes* strains. All of these variables affected the error in the prediction. Controlling or coordinating the challenge tests could decrease the effects of those variables on the uncertainty of the model; however, these data sets represent the current state-of-data in this domain.

[4] In order to make a fair comparison (based on meat data) the chicken salad data were not included.

TABLE 3.4. ANOVA results for RI vs. product/process variables

Variable	*P* value
Product type	0.0004
pH	0.1484
Temp	0.0975
Atmosphere[a]	0.2856

[a] Atmosphere: aerobic or anaerobic

TABLE 3.5. Overall RI values for species inside PMP domain.

Product type	*Atmosphere*	*RI*
Ground beef	Anaerobic	2.20
	Aerobic	3.10
Cooked Chicken	Anaerobic	1.25
Pate	Aerobic	0.30
Cooked Pork	Anaerobic	1.10
	Aerobic	1.08
Precooked Beef	Vacuum	2.40
Turkey	Anaerobic	1.07
	Aerobic	1.63
Pork	Anaerobic	2.80
	Aerobic	3.40

For data sets under experimental conditions outside the PMP domain (i.e., low temperature), RI values were between 0.40 and 1.22 (Table 3.6). Again, for these data sets, the PMP growth model over-predicted (i.e., fail-safe) the log counts for the majority of the cases (83%). The ANOVA of these data showed no significant influence of the experimental conditions on the RI values, probably due to lack of variation in those

variables. As was the case for data within the model domain, for the best RI value, actual and predicted log counts fell within the confidence intervals (Fig. 3.3). For the data set yielding the worst RI value, the PMP growth model still performed as expected; most of the actual log counts fell within its confidence bands (Fig. 3.4), because the RI was still ~1.20. It should be noted that this evaluation of the model performance in an extrapolated domain was very limited, both in terms of the number and domain of the data. Extrapolation of predictive microbial models is always undesirable and not recommended; however, the RI is one possible method for evaluating the performance of models both within and outside the original calibration domain.

TABLE 3.6. RI values for conditions outside the PMP model domain.

Data set No.	Produt type	pH	Temp (°C)	A_W	Nitrite (ppm)	Salt (%)	RI	Mean Relative Error (RE)
42	Pate or ham	6.2	2	0.991	81.2	1.6	0.78	-0.03
43	Pate or ham	6.2	2	0.991	81.2	1.6	0.93	-0.05
44	Pate or ham	6.2	2	0.991	81.2	1.6	0.70	-0.07
45	Pate or ham	6.2	2	0.991	81.2	1.6	0.70	-0.06
46	Pate or ham	6.2	2	0.991	81.2	1.6	0.91	-0.11
47	Pate or ham	6.2	2	0.991	81.2	1.6	1.22	-0.10
48	Pate or ham	6.2	0	0.991	81.2	1.6	1.15	-0.03
49	Pate or ham	6.2	0	0.991	81.2	1.6	0.40	-0.06
50	Pate or ham	6.2	0	0.991	81.2	1.6	0.69	-0.09
51	Pate or ham	6.2	0	0.991	81.2	1.6	0.85	-0.10
52	Pate or ham	6.2	0	0.991	81.2	1.6	0.69	-0.10
53	Pate or ham	6.2	0	0.991	81.2	1.6	0.81	-0.14
54	Pate or ham	6.3	2	0.989	103	2.0	1.10	-0.03
55	Pate or ham	6.3	2	0.989	103	2.0	0.70	0.01
56	Pate or ham	6.3	2	0.989	103	2.0	0.78	-0.05
57	Pate or ham	6.3	2	0.989	103	2.0	0.57	-0.04
58	Pate or ham	6.3	2	0.989	103	2.0	0.87	-0.07
59	Pate or ham	6.3	2	0.989	103	2.0	0.93	-0.02
60	Pate or ham	6.3	0	0.989	103	2.0	0.47	-0.02
61	Pate or ham	6.3	0	0.989	103	2.0	0.62	0.00
62	Pate or ham	6.3	0	0.989	103	2.0	0.85	0.07
63	Pate or ham	6.3	0	0.989	103	2.0	0.70	0.07
64	Pate or ham	6.3	0	0.989	103	2.0	0.86	-0.11
65	Pate or ham	6.3	0	0.989	103	2.0	0.97	-0.07

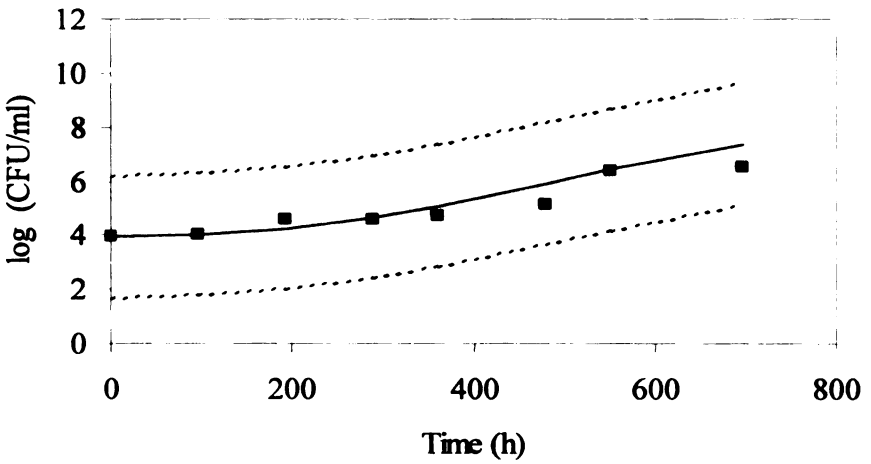

FIGURE 3.3. Comparison of the predicted (solid line) and actual (full squares) growth log counts from the data set (No. 48) resulting in the best RI value (0.40) outside the PMP model domain (95% confidence intervals, broken lines).

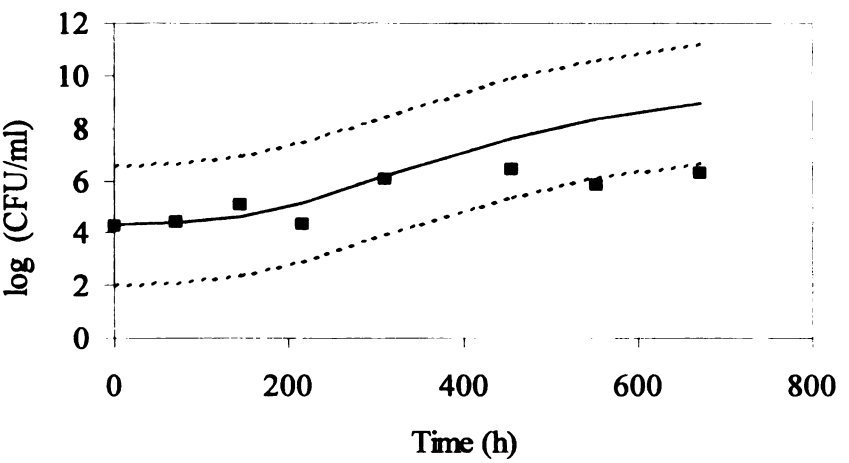

FIGURE 3.4. Comparison of the predicted (solid line) and actual (full squares) growth log counts from the data set (No. 46) resulting in the worst RI value (1.22) outside the PMP model domain (95% confidence intervals, broken lines).

To avoid dangerous errors when utilizing growth models for risk assessment (or other application), predictive models should be validated against independent data relevant to the application. In prior studies, the broth-based PMP growth model for *E. coli* O157:H7 under-predicted (i.e., fail-dangerous) microbial counts when compared to

data in ground beef (Campos *et al.*, 2004; Tamplin M., 2005; Tamplin, 2002; Tamplin *et al.*, 2005). Similar results were reported in the PMP for the *Clostridium perfringens* growth model against data from broth (Smith and Schaffner, 2004). In the present study, the broth-based PMP growth model for *L. monocytogenes* performed reasonably well overall for meat and poultry products, both inside and outside its original domain. In other words, it is a robust model for growth predictions that can be applied to meat and poultry products. Moreover, in some cases the model performed better than expected (RI~ 0-1). In general, microbial counts for *L. monocytogenes* were over-predicted by the PMP growth model. However, the data outside the model domain were limited to a very small range (i.e., low temperature, and just one product type); future work should further evaluate models in a broader domain of extrapolation and/or generate more experimental data to widen the validated domain of predictive models.

CHAPTER 4

EFFECT OF DIFFERENT MODELING PROCEDURES ON MICROBIAL GROWTH MODEL PERFORMANCE

4.1 SUMMARY

Two different microbial modeling procedures were compared and validated against independent data for microbial growth. The most generally used method is two consecutive regressions: growth parameters are estimated from a primary regression of microbial counts, and then a secondary regression relates the growth parameters to experimental conditions. A global regression is an alternative method, in which the primary and secondary models are combined, giving a direct relationship between experimental factors and microbial counts. The Gompertz equation was the primary model, and a response surface model was the secondary model. Independent data from meat and poultry products were used to validate the modeling procedures. The global regression yielded the lower standard errors of calibration (SEC), 0.95 log(CFU/ml) for aerobic and 1.21 log(CFU/ml) for anaerobic conditions. The two-step procedure yielded errors of 1.35 log(CFU/ml) for aerobic and 1.62 log(CFU/ml) for anaerobic conditions. For food products, the global regression was more robust than the two-step procedure for 65% of the cases studied. Robustness Index (RI) values for the global regression ranged from 0.27 (performed better than expected) to 2.60. For the two-step method, RI values ranged from 0.42 to 3.88. The predictions were overestimated (fail-safe) in more than 50% of the cases using the global regression, and in more than 70% of the cases using the two-step regression. Overall, the global regression performed better compared to the two-step procedure, for this specific application.

4.2 INTRODUCTION

In general, predictive microbiology models are fitted to observed data in a two-step regression process (Baranyi *et al.*, 1999; Baty and Delignette-Muller, 2004; Buchanan *et al.*, 1989; Dalgaard and Jorgensen, 1998; Delignette-Muller *et al.*, 1995; te Giffel and Zwietering, 1999): 1) The first step is to fit a primary growth model to observed experimental data, which yields estimated parameters, and 2) The second step is to independently fit a secondary model to each of these estimated parameters, as a function of experimental factors (e.g., temperature, pH, water activity, or nitrite). These two steps are usually not linked, and inherent uncertainty associated with the primary model is subsequently neglected. The lack of fit to an individual growth curve is not considered in the secondary model, and all parameters estimated from observed values are generally given the same weight in the second step, regardless of the goodness of fit of the primary model, potentially leading to poor estimates of the parameters (Pouillot *et al.*, 2003).

The Pathogen Modeling Program (U.S. Department of Agriculture, 2003b)) is a widely used tool in the food industry. This program (PMP) uses models developed via the two-step procedure to estimate, for example, the growth of *Listeria monocytogenes*. Its primary model is the Gompertz equation, and its secondary model is a response surface model. The *L. monocytogenes* growth models behind the PMP are based on the work of Buchanan et al. (1990), who evaluated the influences that several experimental conditions (pH, temperature, salt, and nitrite) have on *L. monocytogenes* growth.

In 2001, Claeys et al. (2001) studied the kinetics of hydroxymethylfurfural, lactulose, and furoxine formation using global and the two-step regression methods. They

agreed with Pouillot et al. (2003), regarding the two-step procedure; errors of the first regression influenced the exactness of the second regression, resulting in less accuracy and precision of the estimated parameters. By doing a global fit, the data set is considered as a whole, increasing the number of degrees of freedom and decreasing the confidence intervals for the model parameters. However, Claeys et al. (2001) concluded that when calculating kinetic parameters using both two-step linear (zeroth order reaction, and Arrhenius equation) and one-step nonlinear regression approaches, the results were comparable, superiority of either method was not clear, and the performance of a specific regression approach depends on the data set to which it is applied. However, these conclusions were based on zeroth order chemical kinetics, so the analysis may not necessarily be relevant to microbiological responses that follow first order or some other nonlinear behavior.

Few predictive microbiology studies have focused on the use of a one-step (global) method. Breand *et al.* (1999) used a primary model describing the lag time duration as a function of two different parameters related to stress temperature and duration. By replacing the two parameters with their corresponding secondary models, the lag time was directly related to stress temperature and duration. They found that the precision of the parameters improved with the global regression.

Membre *et al.* (2004) combined primary and secondary models that directly related the growth of *L. monocytogenes* to cooling temperature, simulating post-process contamination of packaged pork meats. Optimal growth rate was one of the parameters estimated from this regression, which was the main focus of the study. By using global regression, they lowered the bias factor of the growth rate compared to PMP results

(~40% lower).

In order to predict microbial inactivation under dynamic conditions (dynamic temperature profiles mimicking hot air treatments on a fully wetted and on a lean meat product), Valdramidis *et al.* (2005) used the global approach. They used the Bigelow model as their secondary model, which relates the specific inactivation rate to temperature. The model parameters (D_{ref}, z, and T_{ref}) were estimated by one-step regression. The authors mainly focused on the description of microbial inactivation under dynamic conditions. In a different study, Fernandez *et al.* (2002) reported the joint effect of pH and temperature on thermal resistance of *Bacillus cereus* in vegetable substrate, using the Weibull distribution. The parameters were described as a function of experimental conditions using Arrhenius-type relationships, which were replaced into the Weibull model. They concluded that the one-step analysis increased the precision of the estimated parameters, because it avoided estimation of intermediate parameters and used all the raw data. However, validation or comparison of this approach versus the two-step approach was not considered in either of these studies.

The previous literature (Breand *et al.*, 1999; Fernandez *et al.*, 2002; Membre *et al.*, 2004; Valdramidis *et al.*, 2005) that studied the global regression procedure focused on the estimation of model parameters, and evaluated different models that used the two-step modeling procedure. To our knowledge, no published studies have directly compared global versus two-step modeling procedures in terms of microbial counts. Therefore, the objective of this study was to compare different fitting procedures applied to broth-based growth models for *L. monocytogenes*, and validate them using independent data from meat and poultry products.

4.3 MATERIALS AND METHODS

4.3.1 Data sources

Broth-based *L. monocytogenes* growth data sets were obtained from ComBase (U.S. Department of Agriculture, 2003a), using source: "Buchanan_90", organism: "*L. monocytogenes/innocua*", environment: "culture medium", pH: "0.1 to 14", temperature: "-25 to 120", and a_w: "0.01 to 1". In total, 385 data sets for anaerobic conditions and 553 for aerobic conditions were found (Table B.1). The no-growth data were eliminated (Buchanan and Phillips, 1990; Buchanan *et al.*, 1989)(Table B.2). The remaining data sets (n_{sets}= 291, n_{points}= 2,302 for anaerobic, and n_{sets}= 476, n_{points}= 3,680 for aerobic) were considered for this study. The number of data points within each growth curve ranged from 4 to 19 for anaerobic conditions, and 4 to 21 for aerobic conditions.

To validate these fitting procedures, independent data (n_{sets}= 23, n_{points}= 174) from meat and poultry products were used (pH range: 5-6.3, and temperature range: 4-20 °C). The number of data points within each growth curve was between 3 and 15 points. These data sets were obtained from a pre-distribution version of ComBase 2002 (U.S. Department of Agriculture, 2003a), and all records found using the above search criteria were included; keywords and references are reported in Table 4.1.

TABLE 4.1. References and keys in ComBase for meat and poultry products used for model validation in this study.

Data set No.	Key (ComBase)	Product type	Reference
1-4	J207_Lm, J208_Lm, J210_Lm, and J211_Lm	Ground Beef	*(Nissen et al., 2000)*
5	M007	Pate	*(Bovill et al., 2000)*
6-8	M201_LM, M203_LM, and M204_LM	Cooked Pork	*(Fang and Lin, 1994)*
9-10	M656_LM to M657_Lm	Cooked Beef w/ Gravy	*(Grant et al., 1993)*
11	M660_LM	Cooked Beef w/ Gravy	*(Grant et al., 1993)*
12	M661_Lm	Cooked Beef w/ Gravy	*(Grant et al., 1993)*
13	M921_LM	Home-style salad (chicken with no mayonnaise added)	*(Erickson et al., 1993)*
14	M921_LMa	Home-style chicken salad	*(Erickson et al., 1993)*
15	M921_LMb	Home-style salad (real mayonnaise + chicken)	*(Erickson et al., 1993)*
16	M921_LMd	Home-style salad (reduced calorie mayonnaise + chicken)	*(Erickson et al., 1993)*
17	M921_LMg	Home-style salad (real mayonnaise + chicken)	*(Erickson et al., 1993)*
18	M921_LMi	Home-style salad (reduced calorie mayonnaise + chicken)	*(Erickson et al., 1993)*
19	SL118	Turkey	*(Mano et al., 1995)*
20	SL123	Turkey	*(Mano et al., 1995)*
21-23	SL59, SL61, and SL62	Pork	*(Mano et al., 1995)*

4.3.2 Predictive models

The total uncertainty was calculated based on the root mean squared error (RMSE) between the observed and predicted log counts, identified as the standard error of calibration, SEC (eq. 4.1).

SEC = Standard error of calibration

$$= \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n-p}} \qquad \text{Eq. 4.1}$$

where:

$\hat{y}_i$ = Predicted value of i^{th} data point (log(CFU/ml))

y_i = Observed value of the i^{th} data point from the original data sets used to fit the model (log(CFU/ml)), assuming 1 CFU/ml = 1 CFU/g

n = number of observed data points from the original data set

p = number of parameters

The two-step fitting procedure was as follows: first, the growth curves were fitted to the Gompertz equation (eq. 4.2), and growth parameters were obtained (B and M, (Martino *et al.*, 2005)).

$$L(t) = A + Ce^{-e^{\left[-B(t-M)\right]}} \qquad \text{Eq. 4.2}$$

where:

$L(t)$ = log counts of bacteria at time t (log(CFU/ml)), $\hat{y}_i$

A = Asymptotic log count of bacteria as t decreases indefinitely (log(CFU/ml)) = N_0

C = Asymptotic, incremental increase in log count of bacteria as t increases indefinitely (log(CFU/ml)) = $N_\infty - N_0$

M = Time at which the absolute growth rate is maximum (h)

B = Relative growth rate at M (1/h)

t = time (h)

N_0 = initial microbial concentration (log(CFU/ml)), at $t = 0$

Furthermore, N_∞ was not estimated as a parameter; instead, the N_∞ values were assumed to be the same fixed values as reported by Buchanan and Phillips (1990). For aerobic conditions, N_∞ was 9.57 log(CFU/ml), and for anaerobic conditions, N_∞ was 9.32 log(CFU/ml). The initial concentration (N_0) was a single fixed value for each data set (i.e., the reported count a $t = 0$).

Then, response surface models (eq. 4.3) were fitted to the natural logarithms (ln) of the B and M parameters, as functions of experimental factors (pH, temperature, salt, and nitrite) via multiple linear regression. The secondary models were then used to calculate B and M for each combination of experimental factors. These B and M parameters were then replaced in equation 2, and microbial counts were computed. The observed data (y_i, broth-based) and the predicted value ($\hat{y}_i$, from 2-step) were replaced in equation 1, and the total uncertainty was calculated.

$$\begin{aligned}\ln B \text{ or } \ln M = {} & \beta_1 + \beta_2 \mathrm{pH} + \beta_3 \mathrm{T} + \beta_4 \mathrm{NaCl} + \beta_5 \mathrm{N} + \beta_6 \mathrm{pH}^2 + \\ & \beta_7 (\mathrm{T} \times \mathrm{pH}) + \beta_8 \mathrm{T}^2 + \beta_9 (\mathrm{NaCl} \times \mathrm{pH}) + \\ & \beta_{10} (\mathrm{NaCl} \times \mathrm{T}) + \beta_{11} \mathrm{NaCl}^2 + \beta_{12} (\mathrm{N} \times \mathrm{pH}) + \\ & \beta_{13} (\mathrm{N} \times \mathrm{T}) + \beta_{14} (\mathrm{N} \times \mathrm{NaCl}) + \beta_{15} \mathrm{N}^2\end{aligned} \qquad \text{Eq. 4.3}$$

where:

β_n = coefficients

T = temperature (°C)

NaCl = salt (g/liter)

N = nitrite (ppm)

The global model was the combination of the Gompertz equation and the response surface models. Parameters B and M in equation 4.2 were replaced by the response surface model (eq. 4.3), and a global regression was performed (Fig. A.1). The observed data (y_i, broth-based) and the log predictions from the global regression ($\hat{y}_i$) were replaced in equation 4.1, and the total uncertainty was calculated.

To calculate the uncertainty resulting from the primary regression itself (calibration of the regression), first the Gompertz equation (eq. 4.2) was fitted to each growth curve, and the parameters B and M were obtained from each individual regression. The fitting procedure converged for most of the data sets using an optimization algorithm that included "second derivatives method" (Newton-Raphson method, it uses second derivatives as well as first derivatives in the iteration method to find a solution); for the cases where this procedure did not converge, a procedure without using "second derivatives method" always converged (Scripts used in JMP for the nonlinear regression can be found in appendix E). For aerobic conditions, 476 pairs of the parameters B and M were obtained (Table B.4), and for anaerobic conditions, 291 pairs were obtained (Table B.3). Second, each pair of parameters (obtained from the primary regression) was replaced in equation 4.2, and predicted microbial counts were computed for each individual growth curve. The difference from the previous procedures is that the obtained parameters were neither fitted to the response surface model (as a function of

the experimental conditions) nor replaced in the primary model by their respective secondary models. Rather, the values obtained from each individual regression were directly used in the primary model, and microbial counts were calculated independently for each growth curve. The observed data (y_i, broth-based) and the predicted values ($\hat{y}_i$) were replaced in equation 4.1, and the total uncertainty was calculated.

All the statistical analyses and nonlinear regressions were performed using JMP IN (SAS Institute Inc., Cary, N.C. Version 5.1.2).

4.3.3 Robustness Index (RI)

The predictions from the two-step and global procedures were validated using the RI (Martino *et al.*, 2005), based on:

$$RI = \frac{SEP}{SEC} \qquad \text{Eq. 4.4}$$

where,

SEC = Standard error of calibration. The SEC used in these calculations was the one obtained from either the two-step or global regression.

SEP = Standard error of prediction

$$= \sqrt{\frac{\sum_{j=1}^{n} \left(y_j - \hat{y}_j\right)^2}{n}} \qquad \text{Eq. 4.5}$$

where:

$\hat{y}_j$ = Predicted value of j^{th} data point (log(CFU/g)), from either two-step or global regression

y_j = Observed value of the j^{th} data point from an independent data set (log(CFU/g)), assuming 1 CFU/ml = 1 CFU/g

n = number of observed data points from an independent data set (from 3 to 15)

Additionally, the mean relative error (RE), which determines if the predictions are over/under estimated, was calculated based on the following formula (Martino *et al.*, 2005):

$$RE = \frac{\sum_{j=1}^{n}\left(\frac{y_j - \hat{y}_j}{\hat{y}_j}\right)}{n} \qquad \text{Eq. 4.6}$$

where,

$\hat{y}_j$ = Predicted value of j^{th} data point (log(CFU/g)), from either two-step or global regression.

y_j = Observed value of the j^{th} data point from an independent data set (log(CFU/g)), assuming 1 CFU/ml = 1 CFU/g

n = number of data points (from 3 to 15)

4.4 RESULTS AND DISCUSSION

Evaluation of the main effects and interactions among the experimental conditions, based on *P* values (Table 4.2), showed the following: temperature showed a significant influence ($P < 0.05$) in all cases (for both parameters and for both atmospheric conditions); salt and nitrite were only significant for the M parameter estimation, for both atmospheric conditions; pH was significant for all models, except aerobic ln B. In the

case of the B parameter (aerobic), the *P* values for the interactions ranged from <0.0001 to 0.9200, with T × pH, T^2, NaCl × pH, $NaCl^2$, and N × pH having a significant influence. For the M parameter (aerobic), the *P* value for the interactions ranged from <0.0001 to 0.6590, with pH^2, T × pH, T^2, N × pH, and N × T having a significant influence. For the anaerobic condition, *P* values for the interactions for the B parameter ranged from 0.0013 to 0.6630, with T × pH being the only second order term having significant influence; for the M parameter, *P* values ranged from <0.0001 to 0.8800, with pH^2, T^2, T × pH, and N^2 having a significant influence. The coefficient values for the secondary model are presented in Table 4.2 for the two-step regression, and Table 4.3 for the global regression. Even though some of the terms were not significant ($\alpha = 0.05$), all terms were included in the model, because they all were included in the PMP, which was the case study of this research Therefore, comparisons and analyses needed to use the same model as is used in the PMP.

However, when the non-significant terms from the secondary models (Table D.2) were eliminated, the SEP values decreased for 56% of the data sets. The SEP values increased in 44% of the cases, which directly affected the RI values. However, an overall SEP (including all the data for meat and poultry products, aerobic conditions) decreased from 1.78 (when including all terms) to 1.40 (when excluding the non-significant terms), indicating that the reduced model actually was slightly more robust than the complete model for these cases.

Overall, the global regression (Fig. 4.1) predicted the broth-based data better than the two-step (Fig. 4.2) procedure did, for both aerobic and anaerobic conditions.

TABLE 4.2. Coefficient values of secondary models for *L. monocytogenes* growth, fitted to broth-based data via two-step regression.

Variable		*ln B* ($ln\ h^{-1}$)	*P value*	*ln M* ($ln\ h$)	*P value*
		Aerobic			
Intercept	β_1	-5.01E+00	<.0001	6.42E+00	<.0001
pH	β_2	5.90E-02	0.3470	-2.26E-01	<.0001
T (°C)	β_3	1.44E-01	<.0001	-1.08E-01	<.0001
NaCl (g/l)	β_4	1.69E-03	0.5590	7.72E-03	<.0001
Nitrite (ppm)	β_5	-5.75E-04	0.4210	6.07E-04	0.0446
(pH-6.58876)×(pH-6.58876)	β_6	-1.95E-02	0.7510	2.63E-01	<.0001
(T-19.3929) × (pH-6.58876)	β_7	-1.68E-02	0.0048	8.96E-03	0.0004
(T-19.3929) × (T-19.3929)	β_8	-2.59E-03	<.0001	2.85E-03	<.0001
(NaCl-17.5525) × (pH-6.58876)	β_9	-1.55E-02	<.0001	-5.42E-04	0.6590
(NaCl-17.5525) × (T-19.3929)	β_{10}	-2.56E-04	0.1950	9.65E-05	0.2470
(NaCl-17.5525) × (NaCl-17.5525)	β_{11}	-6.53E-04	0.0025	1.15E-04	0.2070
(Nitrite-103.151) × (pH-6.58876)	β_{12}	1.40E-03	0.0016	-4.83E-04	0.0099
(Nitrite-103.151) × (T-19.3929)	β_{13}	-2.10E-05	0.1920	1.38E-05	0.0433
(Nitrite-103.151) × (NaCl-17.5525)	β_{14}	7.40E-06	0.4060	-5.00E-06	0.1600
(Nitrite-103.151) × (Nitrite-103.151)	β_{15}	-9.24E-08	0.9200	-2.54E-07	0.5120
		Anaerobic			
Intercept	β_1	-6.10E+00	<.0001	6.71E+00	<.0001
pH	β_2	3.49E-01	0.0027	-3.32E-01	<.0001
T (°C)	β_3	9.13E-02	<.0001	-7.88E-02	<.0001
NaCl (g/l)	β_4	6.33E-03	0.3450	9.92E-03	0.0003
Nitrite (ppm)	β_5	-1.70E-03	0.1670	1.15E-03	0.0215
(pH-6.66621) × (pH-6.66621)	β_6	-1.46E-01	0.2300	1.95E-01	<.0001
(T-21.269) × (pH-6.66621)	β_7	2.89E-02	0.0013	-1.62E-02	<.0001
(T -21.269) × (T -21.269)	β_8	-5.56E-04	0.4640	2.90E-03	<.0001
(NaCl-18.1724) × (pH-6.66621)	β_9	7.86E-03	0.1590	3.22E-03	0.1550
(NaCl -18.1724) × (T-21.269)	β_{10}	-1.88E-04	0.5890	-2.10E-05	0.8800
(NaCl -18.1724) × (NaCl-18.1724)	β_{11}	-2.69E-04	0.6630	-3.74E-04	0.1370
(Nitrite -142.759) × (pH-6.66621)	β_{12}	5.20E-04	0.2790	4.35E-05	0.8230
(Nitrite -142.759) × (T-21.269)	β_{13}	-1.50E-05	0.5960	2.03E-05	0.0753
(Nitrite-142.759) × (NaCl-18.1724)	β_{14}	-1.60E-05	0.2510	-2.00E-06	0.7100
(Nitrite-142.759) × (Nitrite-142.759)	β_{15}	2.50E-06	0.1300	-1.00E-06	0.0353

TABLE 4.3. Coefficient values computed after global regression for *L. monocytogenes* growth, fitted to broth-based data.

		Aerobic		Anaerobic	
	Variable	*ln B* ($ln\ h^{-1}$)	*ln M* (*ln h*)	*ln B* ($ln\ h^{-1}$)	*ln M* (*ln h*)
β_1	Int	-3.19E+01	2.92E+01	-1.26E+01	1.90E+01
β_2	pH	7.33E+00	-6.76E+00	1.84E+00	-4.13E+00
β_3	T	2.80E-01	-2.65E-01	1.32E-01	-7.58E-02
β_4	NaCl	-2.00E-02	3.93E-02	9.07E-04	5.16E-02
β_5	N	-2.00E-02	1.33E-02	-2.89E-02	1.36E-02
β_6	pH^2	-5.06E-01	4.93E-01	-1.23E-01	3.21E-01
β_7	TxpH	-1.10E-02	5.57E-03	2.18E-02	-2.53E-02
β_8	T^2	-2.18E-03	2.84E-03	-5.45E-03	4.13E-03
β_9	pHxNaCl	1.37E-03	-4.89E-03	-3.85E-03	-3.30E-03
β_{10}	TxNaCl	4.14E-04	-3.69E-05	7.98E-04	-3.28E-04
β_{11}	$NaCl^2$	-1.57E-04	1.06E-04	2.93E-04	-3.74E-04
β_{12}	pHxN	2.69E-03	-1.82E-03	3.72E-03	-1.56E-03
β_{13}	TxN	1.76E-05	3.56E-06	2.05E-04	-3.25E-05
β_{14}	NaClxN	-9.48E-06	-6.25E-07	-6.14E-05	-1.07E-06
β_{15}	N^2	-1.63E-06	3.15E-07	-6.97E-07	-7.42E-07

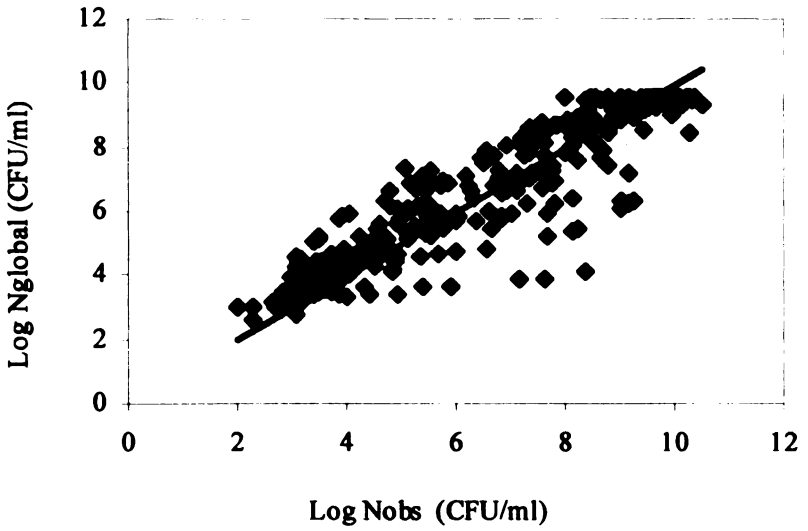

FIGURE 4.1. Observed versus predicted *L. monocytogenes* counts in broth from global regression, showing a randomly selected 10% of the total 3,680 data points and the 1:1 line (aerobic conditions).

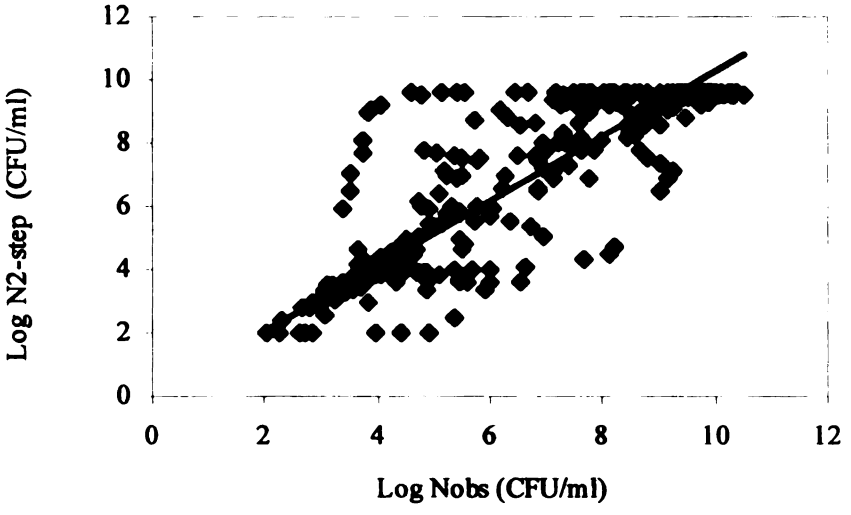

FIGURE 4.2. Observed versus predicted *L. monocytogenes* counts in broth from two-step regression, showing a randomly selected 10% of the total 3,680 data points and the 1:1 line (aerobic conditions).

Uncertainty of the model from the global regression was smaller than that from the two-step regression by approximately 30% for both atmospheric conditions (Table 4.4). Compared to the SEC of the primary regression itself (i.e., no secondary model), the two-step regression gave higher values, by ~25% for both atmospheric conditions. In the global regression case, the SEC values did not differ considerably from the primary regression itself, only 1% for anaerobic and 7% for aerobic; in this case, the global regression had lower uncertainty than the primary regression itself.

TABLE 4.4. SEC (uncertainty) of broth-based *L. monocytogenes* growth predictions resulting from different modeling regression procedures.

	Log(CFU/ml)	
	Aerobic	*Anaerobic*
Primary regression (no secondary model)	1.02	1.22
2-step	1.35	1.62
Global	0.95	1.21

These results reflect how the secondary regression in the two-step procedure can affect the overall performance of a model, in this case by increasing the uncertainty related to that model. Not only is uncertainty from the primary regression affecting the results, but the uncertainty related to the secondary regression also contributes to the overall error. On the other hand, by doing a global regression, the data set is considered as a whole, increasing the degrees of freedom, and making the confidence intervals of the parameters smaller (Claeys *et al.*, 2001); the estimation of intermediate parameters and giving the same weight to each data set used to estimate the parameters are avoided (Claeys *et al.*, 2001; Pouillot *et al.*, 2003).

Validation of these procedures was done using aerobic meat and poultry data. In 65% of the cases studied (Table 4.5), the global regression was more robust, compared to the two-step procedure, meaning that the global approach performed as expected, with respect to the model calibration, in the majority of the cases.

RI values for the global regression ranged from 0.27 (performed better than expected) to 2.60. In approximately 80% of the cases, RI values were lower than 2.0 (Fig. 4.3). For the two-step method, RI values ranged from 0.42 to 3.88, with almost 60% of the cases yielding RI values lower than 2 (Fig. 4.4). An RI less than 2.0 indicates that the model was reasonably robust, with errors less than double that expected, based on the

original model fitting (Martino *et al.*, 2005).

TABLE 4.5. RI and RE values with experimental conditions (assumed $a_w = 0.997$).

Data set No	RI global	RE global	RI 2-step	RE 2-step	pH	T °C
1	*1.03*	0.21	2.49	-0.24	4	5.8
2	*1*	0.19	2.46	-0.23	4	5.8
3	*0.27*	0.01	2.65	-0.2	10	5.8
4	*0.46*	0.07	2.85	-0.24	10	5.8
5	0.6	-0.07	*0.42*	0.03	6.8	5.6
6	1.62	-0.23	*1.35*	-0.07	4	6.2
7	*0.72*	0	1.59	-0.13	20	6.2
8	1.35	-0.11	*0.74*	-0.06	20	6.3
9	2.6	-0.61	*0.54*	0.16	5	6
10	2.54	-0.38	*1.05*	-0.04	10	6
11	1.91	-0.18	*1.59*	-0.2	5	6
12	*1.65*	-0.14	2.2	-0.23	10	6
13	1.71	-0.33	*0.6*	0.18	4	6
14	1.78	-0.33	*0.63*	0.17	4	6
15	*0.72*	0.12	1.45	-0.18	4	6
16	*0.92*	0.16	1.66	-0.22	4	6
17	*2.18*	-0.42	2.35	0.57	12.8	5
18	*1.28*	-0.23	1.44	0.35	12.8	5
19	*0.87*	0.11	1.71	-0.14	7	6
20	*1.19*	0.14	2.01	-0.17	7	6
21	*1.38*	0.2	2.76	-0.23	7	6
22	*2.51*	0.33	3.89	-0.35	7	6
23	*2.51*	0.33	3.88	-0.35	7	6

The predictions were overestimated (fail-safe) in more than 50% of the cases using the global regression (Fig. 4.5). On the other hand, for the two-step regression, predictions were overestimated in more than 70% of the cases (Fig. 4.6). Even though the global regression represents a more robust procedure, the two-step regression is on the fail-safe side of the curve in the majority of the cases, for these particular types of products.

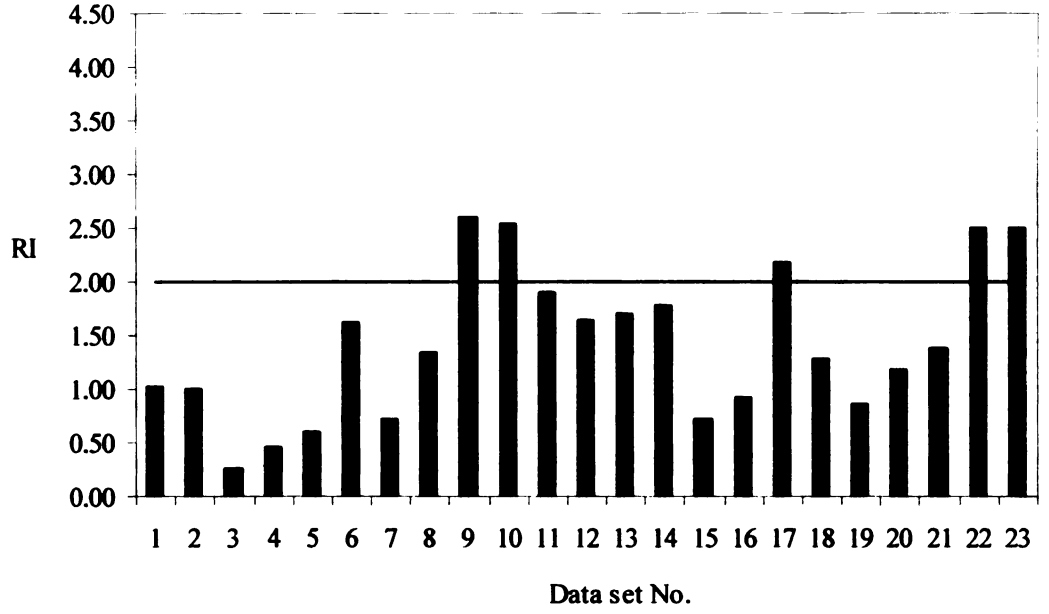

FIGURE 4.3. Robustness Index values of global regression applied to meat and poultry data.

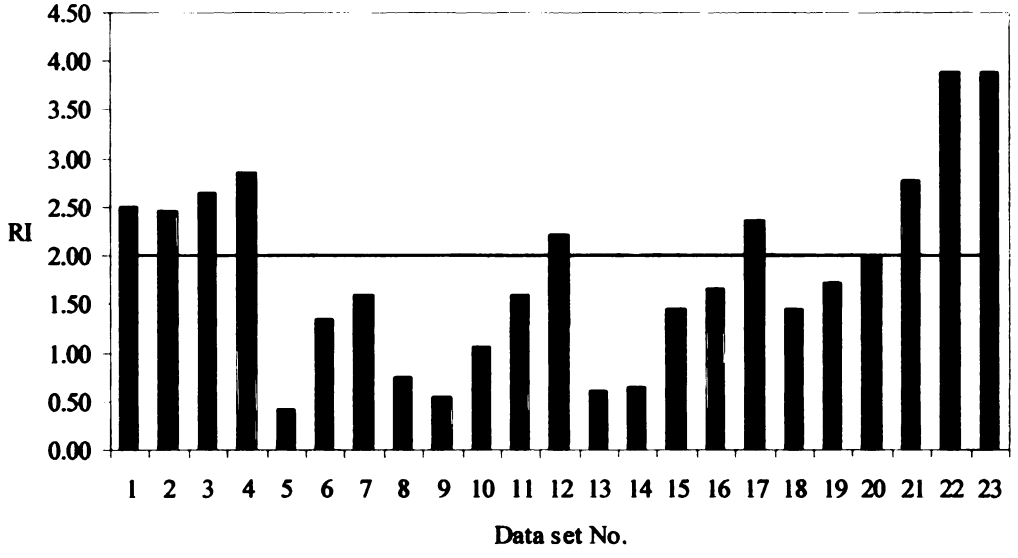

FIGURE 4.4. Robustness Index values of two-step regression applied to meat and poultry data.

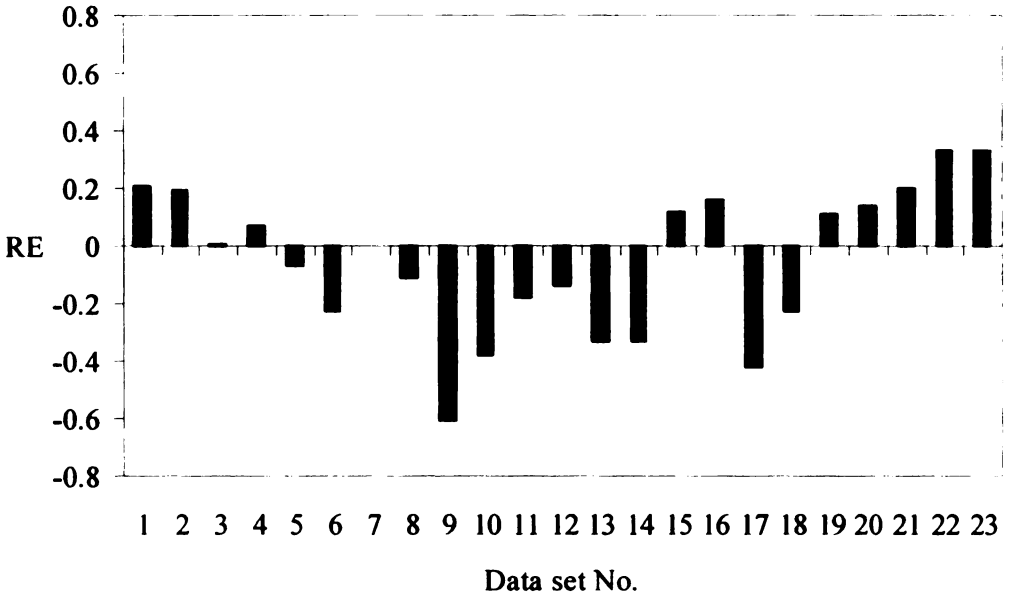

FIGURE 4.5. Relative error values of global regression applied to meat and poultry data.

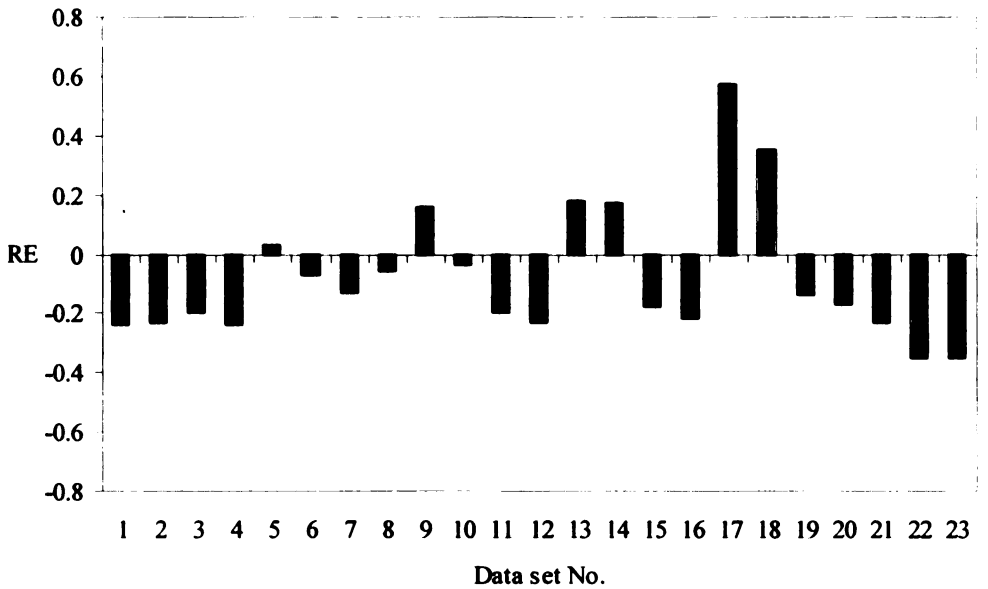

FIGURE 4.6. Relative error values of two-step regression applied to meat and poultry data.

When a model is considered, it is important not only to validate it against independent data (related to the specific application), but also to assess the modeling procedure used to obtain the predicted values. For *L. monocytogenes*, the broth-based growth model uncertainty is lowered by using a global regression, instead of applying the generally used method of two consecutive regressions, one to fit the raw data, and the second to fit the parameters to experimental conditions. By doing a one-step regression, the parameters can still be described as a function of the experimental factors (in this study: pH, temperature, salt, and nitrite).

In conclusion, in order to improve microbial growth (or inactivation) predictions, an overall knowledge of the model is needed, meaning its performance related to the application, and the best fitting procedure. In the present study, an approximation of the total uncertainty of different modeling procedures was estimated, and a global regression yielded a lower error than did the two-step regression. The Gompertz equation was used as a primary model and the response surface models as secondary models; however, this procedure can be applied to any combination of primary and secondary models.

CHAPTER 5

UNCERTAINTY ASSESSMENT IN BROTH-BASED MICROBIAL GROWTH MODELS

5.1 BACKGROUND

Uncertainty and variability of model parameters are increasingly important in several fields of risk analysis. Uncertainty is defined as the lack of perfect knowledge of the parameter value, and variability is the true population heterogeneity that is a consequence of the physical system. For example, some authors have quantified microbial growth variability among strains of a single species (Delignette-Muller, 2000). They found that biological variability has a great impact on the accuracy of the results and should not be systematically neglected.

Baranyi and Roberts (1995) described five different types of errors in predictive growth models: (a) the assumption that a bacterial population is homogeneous (*homogeneity error*), (b) the restriction of bacterial responses only to a few experimental factors, for instance, pH, salt, temperature, etc. (*completeness error*), (c) the error due to parameter estimations (*model function error*), and (d) the error due to laboratory replications (*measurement error*). The fifth error mentioned is the one due to fitting the model to the observed data (*numerical procedure error*). An example application was presented for growth parameters (maximum growth rate and generation time) based on broth-based data, where homogeneity, completeness, and numerical procedure errors were neglected, and only experimental and model function errors were considered. They stated that in real food systems, that are varied and complex structures, "the microbial

response is biochemically complex"; therefore, detailed information (cell distribution, heterogeneity, microbial population, etc.) is rarely available, too difficult, or too costly to acquire. Consequently, certain assumptions and simplifications must be made, that affect the uncertainty and variability of the predictions. For example, Pouillot *et al.* (2003) applied the Bayesian inference to quantify growth parameter uncertainty and growth variability between strains(Pouillot *et al.*, 2003); however, due to the nature of their data (collected from published literature), the physiological state of the strain, the exact nature of the isolate used and the counting method, were not considered, which can significantly influence the overall uncertainty of their results.

Users of predictive models should understand how various sources of uncertainty and variability contribute to potential predictive errors. These sources can ultimately underestimate and/or overestimate total uncertainty of a model, which can lead to significant problems in commercial food processing applications. If uncertainty is underestimated, processes might be designed or operated in a way that results in unacceptable risk to consumers. In contrast, if uncertainty is overestimated, processes will be over-designed, resulting in unnecessary expenditure of energy and/or a decrease in the quality and nutritional value of food products.

For example, the uncertainty of microbial growth models found in computer programs (e.g., Pathogen Modeling Program, PMP) is typically represented by 95% confidence intervals (CI, obtained from the 95% CI of the growth parameters (Juneja *et al.*, 1999; U.S. Department of Agriculture, 2003b)). However, these intervals reflect only the uncertainty of the mean response and are based only on the secondary regression, neglecting uncertainty of the actual predicted values, uncertainty from the primary

regression, and experimental variability.

In contrast, if prediction intervals are used, uncertainty of the curve itself, scatter of the data around the curve (Motulsky and Christopoulus, 2004), and total uncertainty related to that model and data are reported. Furthermore, using this simultaneous confidence intervals method (confidence intervals of the parameters used to calculate confidence intervals of the prediction), the actual 95% confidence intervals for the microbial counts end up to be ~ 90% confidence interval of the mean response (Neter *et al.*, 1992).

Determination of confidence and prediction intervals in nonlinear regression can be difficult, and these could be underestimated by a factor of 2-3 (Dolan *et al.*, 2006; Van Boekel, 1996). Dolan et al. (2006) proposed a technique to be used in nonlinear regression to estimate confidence and prediction intervals, which is among the first reported methodologies for the prediction intervals applied to actual survival of microorganisms, not just the model parameters.

Quantitative microbial risk assessors, food processors, and food regulators should utilize knowledge of the total uncertainty behind a model, in terms of prediction intervals of the actual predicted value, in order to improve ultimate estimates of risk. Moreover, once sources of errors are identified and quantified, they can be prioritized and targeted to reduce uncertainty.

Therefore, the objective this part of the study was to assess sources of uncertainty and variability in broth-based growth models, applied to *L. monocytogenes*, to better understand how the data, model, parameters, and methodologies contribute to the total uncertainty of a model.

5.2 METHODS

5.2.1 Data sources.

The same data sets described in Chapter 4, section 4.3.1 were used.

Additionally, the data sets were also classified based on the treatment (pH, temperature, salt, and nitrite). For aerobic conditions, 132 different treatments were identified (Tables B.6, B.7); 19 treatments that were not replicated were excluded from the analyses. There were 118 different anaerobic treatments (Tables B.5, B.8); 44 unreplicated treatments were excluded from the analyses.

5.2.2 Error sources

Total uncertainty was assumed to be an aggregated contribution of the uncertainties due to the primary and secondary regressions, and variability due to replications, substrate, organism, and laboratory methodologies, conceptually represented as:

$$\varepsilon_{Total} = f\left(\varepsilon_{organism}, \varepsilon_{substrate}, \varepsilon_{lab\ methods}, \varepsilon_{replic}, \varepsilon_{primary\ model}, \varepsilon_{secondary\ model}, \varepsilon_{random}\right)$$

where:

ε_{total}= Total uncertainty

$\varepsilon_{primary\ model}$= Error from the primary regression

$\varepsilon_{secondary\ model}$= Error from the secondary regression

$\varepsilon_{organism}$ = Error due to variability in microorganisms or strains

$\varepsilon_{substrate}$ = Error due to variability in substrate

$\varepsilon_{lab\ methods}$ = Error due to different laboratory methodologies

ε_{replic} = Error due to replications

ε_{random} = Random error

For this study, the errors due to organisms ($\varepsilon_{organism}$), substrate ($\varepsilon_{substrate}$), and laboratory methodologies ($\varepsilon_{lab\ methods}$) were neglected, because the same microorganism, substrate, and methodology were used for all of the data, and the data were all generated in a single laboratory.

5.2.3 Error calculations

All statistical analyses and nonlinear regressions were performed using JMP IN (SAS Institute Inc., Cary, N.C. Version 5.1.2).

Total uncertainty (ε_{total}) was calculated based on the root mean squared error (RMSE) between the observed and predicted log counts, identified as the standard error of calibration, SEC (reported in Chapter 4, equation 1). In order to obtain the predicted microbial counts, the two-step procedure described in Chapter 4, section 4.3.2 was followed.

To quantify the contribution from the secondary regression ($\varepsilon_{secondary\ model}$), the upper and lower limits of the primary models parameters ($UPI_{B,M}$, and $LPI_{B,M}$) were computed using equation 5.1 (adapted from (Neter *et al.*, 1992)). The RMSE for both parameters was obtained from the secondary regression (Table 5.1). Then, the upper and lower limits of the log predictions (UPI, and LPI) were calculated (with equation 4.2,

Chapter 4), using the upper and lower values of the parameters B and M (obtained from equations 5.1 and 5.2). Having the upper and lower limits of the log count predictions, the error from the secondary regression ($\varepsilon_{secondary\ model}$) in terms of microbial count was calculated with equation 5.3.

TABLE 5.1. RMSE of the parameters B (h^{-1}) and M (h) estimated from the secondary regression.

	RMSE from secondary regression	
	B	M
Aerobic	1.6	1.4
Anaerobic	4.1	1.8

$$UPI_{B,M} = \hat{y}_j + \left(z \times RMSE_{B.M}\right) \quad \text{Eq. 5.1}$$

$$LPI_{B.M} = \hat{y}_j - \left(z \times RMSE_{B,M}\right) \quad \text{Eq. 5.2}$$

where:

$\hat{y}_j$ = predicted B or M

$UPI_{B,M}$ = Upper prediction limit for B or M

$LPI_{B,M}$ = Lower prediction limit for B or M

$z = z(1-\alpha/2)$, $\alpha=0.05$

$RMSE_{B,M}$ = root mean squared error estimated from secondary regression, for B or M

Subsequently,

$$\varepsilon_{\text{secondary regression}} = \frac{UPI - LPI}{z \times 2} \qquad \text{Eq. 5.3}$$

where,

UPI = Upper prediction limit for log(CFU/ml)

LPI = Lower prediction limit for log(CFU/ml)

To quantify the error contribution from the primary regression ($\varepsilon_{\text{primary model}}$), the method described in Chapter 4, section 4.3.2, was followed (calibration of regression), in which the B and M parameters estimated from the primary regression were directly replaced in the Gompertz equation to calculate the log predictions (without using secondary models).

The error due to replications ($\varepsilon_{\text{replic}}$) was computed as follows. A two-way ANOVA (factors: time and treatment, general linear model, and 2-factor factorial design) was performed on the whole set of treatments, using JMP. Effects between factors were neglected, and the error from replications was then obtained (represented in JMP by "pure error").

5.2.4 Calculation of the prediction limits and parameter errors

The asymptotic (or approximate) standard error (SE) accounts for uncertainty in both parameter estimates and error in the model. It was calculated from the variance-covariance matrix associated with the parameter estimates, the composition of the partial derivatives of the model with respect to each parameter, and the estimate of error variance, represented in JMP by SQRT(VecQuadratic(*matrix1,vector1*)+mse (JMP,

2006). The diagonal elements of the variance-covariance matrix represent the asymptotic SE, and the off-diagonal elements represent covariance between parameters (Van Boekel, 1996).

This SE is used to calculate the prediction limit values for an individual predicted value, adding the variance of the error term to the variance of prediction involving the estimates, to form the interval. A specific SE corresponds to each data point, which is used to construct the prediction intervals (JMP, 2006).

To calculate the confidence intervals, the SE accounts for all of the uncertainty in the parameter estimates, but does not account for the uncertainty in predicting individual responses (Neter *et al.*, 1992). The SE for the confidence intervals is calculated from the covariance matrix associated with the parameter estimates, and the composition of the partial derivatives of the model with respect to each parameter. The command in JMP is Sqrt(VecQuadratic(*matrix1*,*vector1*, (JMP, 2006)).

An illustration of how the specific SE of each data point varies with time, and how SE of each data set varies with pH, temperature, nitrite, and salt is presented in appendix C.

The uncertainty of the parameters was also calculated using the RMSE between the B and M parameters estimated from the primary regression (observed values), and the predicted values, which were obtained from the response surface model (Chapter 4, equation 4.3, and the coefficients from Table 4.2) for the two-step procedure. For the global procedure, the coefficients from Table 4.3 were replaced in equation 4.3 (Chapter 4), the B and M parameters were computed.

5.3 RESULTS AND DISCUSSION

5.3.1 Deconstruction of the model uncertainty

Although the total uncertainty for anaerobic conditions was almost 20% higher than for aerobic conditions (Table 5.2), the relative contributions of the various components of the total uncertainty were fairly consistent across the two conditions. The largest contribution to the total uncertainty came from the secondary regression uncertainty (1.48 log(CFU/ml) for aerobic, and 1.42 log(CFU/ml) for anaerobic). Experimental variability was the smallest component (0.26 log(CFU/ml) for aerobic, and 0.21 log(CFU/ml) for anaerobic). The uncertainty due to the primary regression also had a high relative contribution to the total uncertainty (1.02 log(CFU/ml) for aerobic, and 1.22 log(CFU/ml) for anaerobic). As stated at the beginning of section 5.2.2, even though these errors represent the aggregated contribution to the total uncertainty, they cannot be directly added (Neter *et al.*, 1992), so the numbers calculated in this section represent how much each source is contributing to the total uncertainty, separately and independently.

TABLE 5.2. Relative contributions of the different sources of error to the total model uncertainty for aerobic and anaerobic conditions.

	Log (CFU/ml)	
Errors	*Aerobic*	*Anaerobic*
ε_{total}	1.35	1.62
$\varepsilon_{primary\ regression}$	1.02	1.22
$\varepsilon_{secondary\ regression}$	1.48	1.42
ε_{replic}	0.26	0.21

When experiments are conducted under controlled conditions (microorganisms, substrate, temperature, etc.), variability due to experiments is assumed to have less effect on the overall uncertainty of a model, as was found in this study. However, when experiments are run with food matrices, larger contribution of the experimental variability could be expected. A test of this is presented in appendix D.1.

Uncertainty due to the primary regression is assumed to contain not only the error of the regression itself, but also, the variability that comes with the data (in this study: ε_{replic}). For this particular study, error due to the primary regression was smaller than error due to the secondary regression. When food matrices are considered, the results could be the opposite, because of the natural variability of food matrices and how microorganisms react to these systems. However, given the experimental control for the data used here, this error did not have a great impact on the primary regression uncertainty.

The uncertainty due to the secondary regression had the largest contribution to the overall uncertainty, probably because the response surface model that was fitted to the parameters, as a function of the experimental conditions, was not the model with the best performance for this specific application, meaning that a more robust model could lead to a smaller uncertainty.

These results demonstrated that the impact that the various sources of error had on the total uncertainty of a model cannot be ignored. This assessment of the relative magnitude of the various sources of uncertainty can help prioritize efforts to minimize uncertainty in model development, by choosing a different model, redesigning the experimental methodology, and/or improving the statistical analyses.

For example, if two-step regression is used, different secondary models should be tested to determine which yields the smallest secondary model uncertainty. Alternatively, a global regression (combining primary and secondary models, giving a direct relationship between experimental factors and microbial counts) might be advised. By doing a global regression, only the error due to this regression contributes to total uncertainty, avoiding errors due to the addition of more parameters (Fernandez *et al.*, 2002), and avoiding giving the same weight to each data set used to estimate the parameters (Pouillot *et al.*, 2003). In Chapter 4, it was found that by performing a global regression instead of a two-step regression, the total uncertainty was reduced ~30%, with respect to predicted microbial counts. Another study showed that by fitting the response surface model to the natural logarithms (ln) of the parameter, uncertainty of the estimated parameter increased by 20%, compared to the error estimated from the primary fit (Baranyi and Roberts, 1995).

Experimental variability could greatly depend on personnel, which is controllable to some degree, through training and management. Furthermore, other factors can contribute to this error, including the materials and equipments used for the experiments, laboratory/ambient conditions, etc. All these factors, either together or separately, can be controlled to minimize the error due to experiments.

Uncertainty from the primary regression gave an idea of how well the primary model represented the growth pattern of the experimental data. This uncertainty would be affected by the particular choice of primary model, assuming that one model form would fit a given data set better than another.

5.3.2 Prediction intervals and errors of the parameters

Two examples of PI versus CI are shown in Figures 5.1 and 5.2. Even though the model fitted the data better on Figure 5.2, prediction intervals in both cases still contain most of the data. However, this was not the case for the confidence intervals; in both cases, the majority of the data were outside the limits.

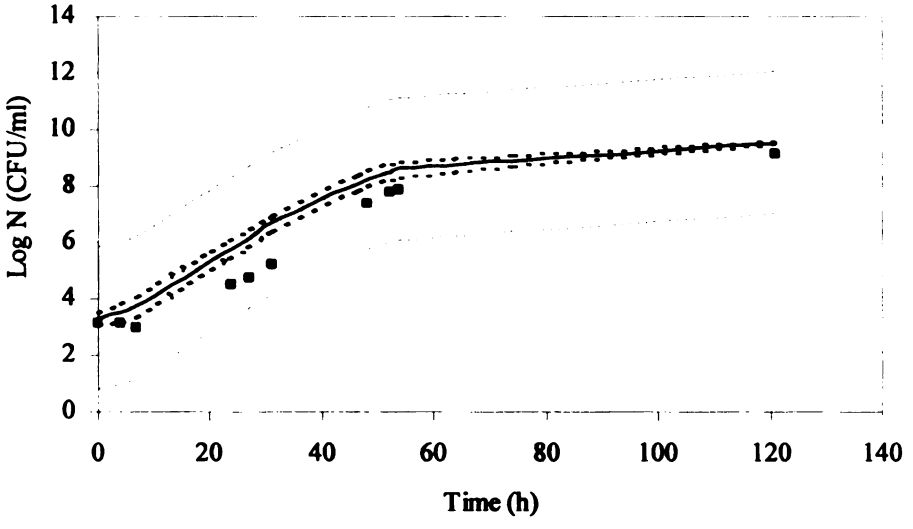

FIGURE 5.1. Confidence (small dashed lines) and prediction (wide dashed lines) intervals for global regression (data set No. 623, aerobic conditions, treatment No. 26: pH = 6, T =19°C, nitrite = 0 ppm, salt = 0 g/liter). Solid line: predicted curve; full squares: observed data.

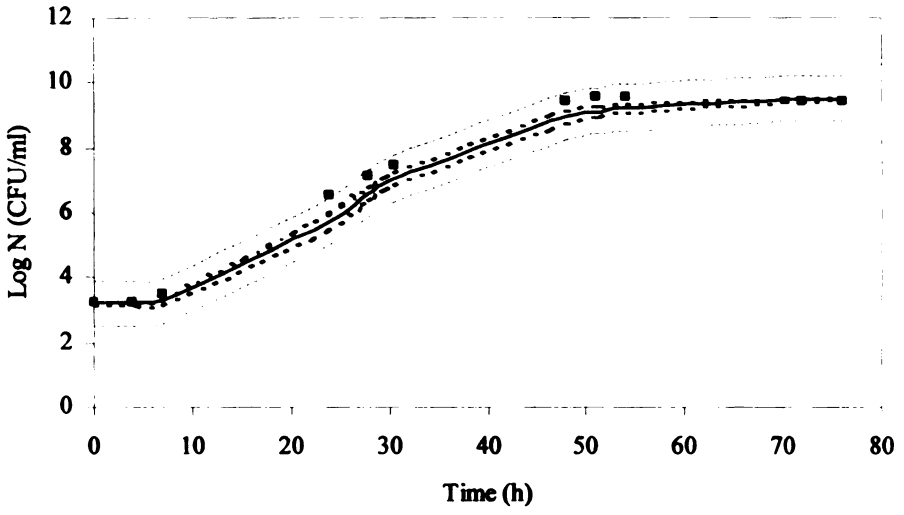

FIGURE 5.2. Confidence (small dashed lines) and prediction (wide dashed lines) intervals for global regression (data set No. 646, aerobic condition, treatment No. 80: pH = 7, T = 19°C, nitrate = 0 ppm, salt = 25 g/liter). Solid line: predicted curve; full squares: observed data.

The natural logarithm of B and M calculated with the coefficients obtained from the global regression gave higher RMSEs, for both atmospheric conditions, than did the values calculated from the response surface model on the two-step regression (Table 5.3).

TABLE 5.3. RMSE of the model parameters.

	RMSE			
	Aerobic		Anaerobic	
	ln B ($\ln h^{-1}$)	ln M ($\ln h$)	ln B ($\ln h^{-1}$)	ln M ($\ln h$)
Global	1.58	0.48	2.14	0.77
2-step	0.91	0.38	1.42	0.59

For ln B, aerobic conditions, the global regression values had ~42% higher error compared to the two-step regression. For anaerobic conditions, it was ~33% higher.

For ln M, aerobic and anaerobic conditions, the global regression had ~20% higher error for the global regression compared to the two-step regression.

These values showed that the methodologies used for model development do not always have the same impact on the results. For instance, in terms of microbial counts, the global regression performed better than the two-step regression; however, in terms of model parameters (e.g., B, and M), the two-step procedure gave a lower prediction error.

To conclude, by assessing the different sources of error that might contribute to the total uncertainty of a model, a better understanding of the model and the data used to parameterize it is also attained. Therefore, the techniques illustrated here can lead to improvement of a model, by reducing uncertainty, and/or improving the way that a model is utilized for process validation or risk analysis.

CHAPTER 6

CONCLUSIONS AND FUTURE WORK

6.1 CONCLUSIONS

1. The growth models used in the Pathogen Modeling Program (PMP) for *L. monocytogenes* (aerobic and anaerobic conditions) were validated, inside and outside their domain with growth data from meat and poultry products, and an acceptable robustness for this specific application was established. A Robustness Index (RI) between 0 and 2.0 indicated satisfactory robustness for the PMP growth models. RI values above 2.0 suggested that the model did not perform as expected. The type of food product (e.g., pâté, cooked chicken, turkey) significantly affected ($P < 0.05$) RI values. The standard errors of calibration for the growth models used in the PMP were ± 1.30 log(CFU/ml), on average, for the broth-based data. Inside the model domain, RI values for the meat and poultry products were between 0.37 and 3.96; the model overestimated the log counts for 85% of the cases. Outside the model domain, RI values were between 0.40 and 1.22. In most cases (83%), the log counts were overestimated. Overall, for meat and poultry products, both inside and outside the model domain, the PMP growth models for *L. monocytogenes* were robust models, although they were developed from broth-based data.

2. The uncertainty related to the global (one-step) regression, in terms of microbial counts, was almost 30% smaller than from two-step regression for both

atmospheric conditions. For the primary regression itself (using B and M estimated from the primary regression, instead of those calculated from the secondary regression), the standard error of calibration (SEC) was about 25% smaller than when using the two-step regression. By doing a global regression, instead of two consecutive regressions, the process avoids giving the same weight to each data set used to estimate the parameters. Moreover, with the global regression, the data set is considered as a whole, increasing the degrees of freedom, which makes the confidence intervals smaller. When validating against data from food products (meat and poultry), global regression gave more robust results, compared to two-step regression. RI values for global regression ranged from 0.27 to 2.60; in 80% of the cases, RI values were lower than 2.0. RI values for the two-step regression ranged from 0.42 to 3.88, with ~60% of the cases yielding RI values lower than 2.0. The predictions of microbial counts were overestimated in more than 50% of the cases using the global regression, and in more than 70% of the cases using the two-step regression. For this particular type of food product, global regression represented a more robust procedure; however, the two-step regression was on the fail-safe side of the curve in the majority of the cases. However, for the primary model parameters (B and M), global regression results gave higher errors compared to those generated from the two-step regression, for both atmospheric conditions. When assessing model performance, it is important to consider both the model form and the fitting procedure.

3. The sources of errors that contributed to the total uncertainty of a bacterial growth model were identified and quantified. The overall uncertainty of a model was assumed to be the result of the relative contributions of the error due to experimental variability, error due to primary regression, and error due to secondary regression. For the *L. monocytogenes* broth-based data, the secondary regression had the highest relative contribution, followed by the primary regression uncertainty, and the experimental variability. The lower relative contribution of the experimental variability might be due to controlled experimental conditions (microorganisms, laboratory procedures, and substrate did not change). On the other hand, the secondary regression uncertainty had the higher relative contribution, possibly due to lack of a good fit for the secondary model. Choosing a secondary model that better fits the data could possibly lower this contribution. The experimental variability was assumed to form part of the primary regression uncertainty. In this study, its contribution did not greatly affect the error due to the primary regression; different results might be expected for models developed from microbial data in food products.

4. When the true risk to the consumers, based on food safety, is assessed by the food industry, the uncertainty of the actual predicted value should be reported. It is common to find in the literature the uncertainty reported as confidence intervals of the mean prediction, not the individual prediction. However, by reporting the prediction intervals of the predicted values, the uncertainty of an individual outcome is known, and the risk related to the specific prediction can be assessed.

For example, for a processor of liquid eggs, it is important to know whether each individual package is safe (environmental conditions are controlled, so *Salmonella* growth is inhibited); only predicting that the mean package is safe is not sufficient control. Therefore, it is important that predictive microbial tools predict the risk limits for each specific package/serving that is processed.

6.2 SUGGESTIONS FOR FUTURE WORK

1. First, a simple summation of sources of error was assumed to be the total uncertainty of the model; however, later it was found that direct summation of errors is not statistically possible. The purpose at the beginning of the study was to quantify the direct contribution of each error to total uncertainty. The relative contribution of each error was assessed; however, assessment of the exact contribution of each error to total uncertainty of a model is still needed.

2. The magnitude of each source of error varies with time, which could be explained with the growth curve. For example, during the lag phase duration, only error due to experimental variability was assumed to affect overall uncertainty; during exponential growth, errors due to primary and secondary regression were assumed to affect total uncertainty. Then, during the stationary phase, once again the experimental variability was assumed to affect overall uncertainty. These assumptions could be explained with the variation of the asymptotic SE with time. This variation was small at the beginning and end of the curve, and slightly increased in the middle. However, this trend needs more analysis in order to

specifically quantify and confirm that the changes in magnitude of the different error sources vary with the different phases of the growth curves.

3. In the present study, the relative contribution of errors to the model uncertainty based on broth-based data was assessed. Further analysis of the relative contribution of errors to model uncertainty based on data from food products is still needed.

4. For the same data used in this study, evaluation of different secondary models and the impact on the secondary regression uncertainty, and later the total uncertainty, would be interesting.

5. Development of a "model diagnostic tool" would be interesting and very useful for academia and government. For example, assume ground beef data had been collected from a laboratory, and a model to describe the growth of *E. coli* is needed. The data sets could be downloaded to the software, including all the experimental variables; then the primary and secondary models could be chosen, the software runs the models, and calculates the relative contribution of each error and the total uncertainty. From the results, it would be possible to evaluate and compare model performance, so informed decisions could be made. Such decisions could involve model choice (primary or secondary), subsequent experimental design, data collection methodology, or number of replications. Once these changes are done, accuracy of the predictions could be improved, and

the uncertainty related to those predictions could be decreased.

APPENDIX A

COMPUTER PROGRAMS USED FOR DATA ANALYSIS AND DATA SOURCE

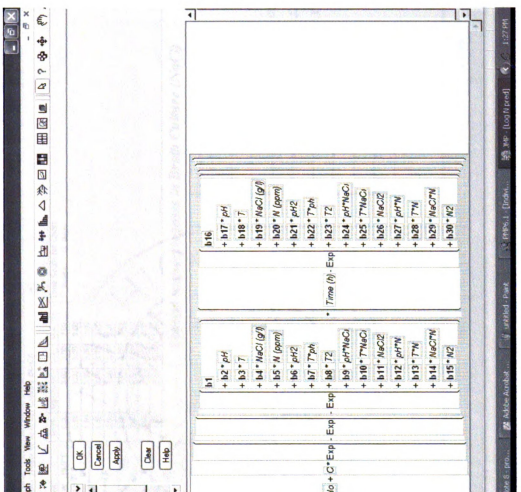

FIGURE A.1. Printed screen of JMP (SAS Institute Inc., Cary, N.C. Version 4.0.4) formula box that contains the global model.

FIGURE A.2. Screen picture of PMP (U.S. Department of Agriculture, 2003b), which shows the growth curve for *L. monocytogenes*, the growth parameters, and the experimental variables.

FIGURE A.3. Screen picture of ComBase (U.S. Department of Agriculture, 2003a). **Experimental conditions, microorganism, environment, and authors can be chosen, so related literature can be found.**

APPENDIX B

BROTH-BASED AND MEAT-BASED DATA DESCRIPTION AND ORGANIZATION

TABLE B.1. Keys to ComBase for aerobic (data set No. 386-938) and anaerobic (data set No. 1-385) conditions.

Key to ComBase	Data set No.	Key to ComBase	Data set No.	Key to ComBase	Data set No.	Key to ComBase	Data set No.
L1_A_1	1	L15_A_1	33	L17_A_3	65	L21_A_1	97
L1_A_2	2	L15_A_11	34	L17_A_4	66	L21_A_2	98
L1_A_3	3	L15_A_6	35	L17_A_5	67	L22_A_1	99
L1_A_4	4	L15_C_12	36	L17_A_6	68	L22_A_11	100
L1_A_5	5	L15_C_2	37	L18_A_1	69	L22_A_16	101
L1_A_6	6	L15_C_7	38	L18_A_2	70	L22_A_17	102
L1_A_7	7	L15_D_13	39	L18_A_3	71	L22_A_18	103
L1_A_8	8	L15_D_3	40	L19_A_1	72	L22_A_6	104
L1_A_9	9	L15_D_8	41	L19_A_11	73	L22_C_12	105
L10_A_1	10	L15_F_14	42	L19_A_16	74	L22_C_2	106
L10_A_11	11	L15_F_4	43	L19_A_18	75	L22_C_7	107
L10_A_6	12	L15_F_9	44	L19_A_19	76	L22_D_13	108
L10_C_12	13	L15_G_10	45	L19_A_6	77	L22_D_3	109
L10_C_2	14	L15_G_15	46	L19_C_12	78	L22_D_8	110
L10_C_7	15	L15_G_5	47	L19_C_2	79	L22_F_14	111
L10_D_13	16	L16_A_1	48	L19_C_7	80	L22_F_4	112
L10_D_3	17	L16_A_11	49	L19_D_13	81	L22_F_9	113
L10_D_8	18	L16_A_6	50	L19_D_17	82	L22_G_10	114
L10_F_14	19	L16_C_12	51	L19_D_3	83	L22_G_15	115
L10_F_4	20	L16_C_2	52	L19_D_8	84	L22_G_5	116
L10_F_9	21	L16_C_7	53	L19_F_14	85	L23_A_1	117
L10_G_10	22	L16_D_13	54	L19_F_4	86	L24_A_1	118
L10_G_15	23	L16_D_3	55	L19_F_9	87	L24_D_2	119
L10_G_5	24	L16_D_8	56	L19_G_10	88	L24_F_3	120
L11_C_1	25	L16_F_14	57	L19_G_15	89	L25_D_1	121
L11_E_2	26	L16_F_4	58	L19_G_5	90	L25_D_10	122
L12_C_1	27	L16_F_9	59	L2_A_1	91	L25_D_2	123
L12_E_2	28	L16_G_10	60	L2_A_2	92	L25_D_3	124
L13_C_1	29	L16_G_15	61	L2_A_3	93	L25_D_4	125
L13_E_2	30	L16_G_5	62	L20_A_1	94	L25_D_5	126
L14_C_1	31	L17_A_1	63	L20_A_2	95	L25_D_6	127
L14_E_2	32	L17_A_2	64	L20_A_3	96	L25_D_7	128

TABLE B.1. Continuation.

Key to ComBase	Data set No.	Key to ComBase	Data set No.	Key to ComBase	Data set No.	Key to ComBase	Data set No.
L25_D_8	129	L3_A_1	162	L4_A_6	195	L55_A_1	228
L25_D_9	130	L3_A_11	163	L4_C_12	196	L56_A_1	229
L26_D_1	131	L3_A_6	164	L4_C_2	197	L57_A_1	230
L27_A_1	132	L3_C_12	165	L4_C_7	198	L58_A_1	231
L27_A_11	133	L3_C_2	166	L4_D_13	199	L59_B_1	232
L27_A_6	134	L3_C_7	167	L4_D_3	200	L6_A_1	233
L27_C_12	135	L3_D_13	168	L4_D_8	201	L6_A_11	234
L27_C_2	136	L3_D_3	169	L4_F_14	202	L6_A_6	235
L27_C_7	137	L3_D_8	170	L4_F_4	203	L6_C_12	236
L27_D_13	138	L3_F_14	171	L4_F_9	204	L6_C_2	237
L27_D_16	139	L3_F_4	172	L4_G_10	205	L6_C_7	238
L27_D_3	140	L3_F_9	173	L4_G_15	206	L6_D_13	239
L27_D_8	141	L3_G_10	174	L4_G_5	207	L6_D_3	240
L27_F_14	142	L3_G_15	175	L40_A_1	208	L6_D_8	241
L27_G_5	143	L3_G_5	176	L41_A_1	209	L6_F_14	242
L28_A_1	144	L30_A_1	177	L42_A_1	210	L6_F_4	243
L28_A_11	145	L30_A_2	178	L43_A_1	211	L6_F_9	244
L28_A_6	146	L30_A_3	179	L44_A_1	212	L6_G_10	245
L28_C_12	147	L31_C_1	180	L45_A_1	213	L6_G_15	246
L28_C_2	148	L31_E_2	181	L46_A_1	214	L6_G_5	247
L28_C_7	149	L32_C_1	182	L47_A_1	215	L60_A_1	248
L28_D_13	150	L32_E_2	183	L48_A_1	216	L61_B_1	249
L28_D_3	151	L33_C_1	184	L49_A_1	217	L61_C_2	250
L28_D_8	152	L33_E_2	185	L5_D_1	218	L62_A_1	251
L28_F_14	153	L34_A_1	186	L5_D_2	219	L63_A_1	252
L28_F_4	154	L35_A_1	187	L5_D_3	220	L65_B_1	253
L28_F_9	155	L36_A_1	188	L50_A_1	221	L65_C_2	254
L28_G_10	156	L37_A_1	189	L51_A_1	222	L66_B_1	255
L28_G_15	157	L38_A_1	190	L52_A_1	223	L66_C_2	256
L28_G_5	158	L39_C_1	191	L52_A_2	224	L67_A_1	257
L29_A_1	159	L39_E_2	192	L52_A_3	225	L68_A_1	258
L29_A_2	160	L4_A_1	193	L53_A_1	226	L69_A_1	259
L29_A_3	161	L4_A_11	194	L54_B_1	227	L69_A_11	260

TABLE B.1. Continuation.

Key to ComBase	Data set No.	Key to ComBase	Data set No.	Key to ComBase	Data set No.	Key to ComBase	Data set No.
L69_A_16	261	L73_D_2	294	L85_D_8	327	L88_D_8	360
L69_A_6	262	L74_B_1	295	L85_F_14	328	L88_F_14	361
L69_C_12	263	L75_A_1	296	L85_F_4	329	L88_F_4	362
L69_C_2	264	L76_F_1	297	L85_F_9	330	L88_F_9	363
L69_C_7	265	L77_A_1	298	L85_G_10	331	L88_G_10	364
L69_D_13	266	L78_A_1	299	L85_G_15	332	L88_G_15	365
L69_D_3	267	L78_A_2	300	L85_G_5	333	L88_G_5	366
L69_D_8	268	L78_A_3	301	L86_D_1	334	L89_A_1	367
L69_F_14	269	L78_A_4	302	L86_D_2	335	L9_A_1	368
L69_F_4	270	L78_A_5	303	L86_D_3	336	L9_A_11	369
L69_F_9	271	L78_A_6	304	L87_A_1	337	L9_A_6	370
L69_G_10	272	L78_A_7	305	L87_A_11	338	L9_C_12	371
L69_G_15	273	L78_A_8	306	L87_A_6	339	L9_C_2	372
L69_G_5	274	L78_A_9	307	L87_C_12	340	L9_C_7	373
L7_A_1	275	L79_A_1	308	L87_C_2	341	L9_D_13	374
L7_A_11	276	L8_A_1	309	L87_C_7	342	L9_D_3	375
L7_A_6	277	L8_A_2	310	L87_D_13	343	L9_D_8	376
L7_C_12	278	L8_A_3	311	L87_D_3	344	L9_F_14	377
L7_C_2	279	L80_F_1	312	L87_D_8	345	L9_F_4	378
L7_C_7	280	L81_A_1	313	L87_F_14	346	L9_F_9	379
L7_D_13	281	L82_F_1	314	L87_F_4	347	L9_G_10	380
L7_D_3	282	L83_A_1	315	L87_F_9	348	L9_G_15	381
L7_D_8	283	L83_A_2	316	L87_G_10	349	L9_G_5	382
L7_F_14	284	L84_A_1	317	L87_G_15	350	L90_F_1	383
L7_F_4	285	L85_A_1	318	L87_G_5	351	L91_A_1	384
L7_F_9	286	L85_A_11	319	L88_A_1	352	L92_F_1	385
L7_G_10	287	L85_A_16	320	L88_A_11	353	LM002_1	386
L7_G_15	288	L85_A_6	321	L88_A_6	354	LM002_2	387
L7_G_5	289	L85_C_12	322	L88_C_12	355	LM002_3	388
L70_B_1	290	L85_C_2	323	L88_C_2	356	LM002_4	389
L71_C_1	291	L85_C_7	324	L88_C_7	357	LM002_5	390
L72_A_1	292	L85_D_13	325	L88_D_13	358	LM002_6	391
L73_C_1	293	L85_D_3	326	L88_D_3	359	LM003_1	392

TABLE B.1. Continuation.

Key to ComBase	Data set No.	Key to ComBase	Data set No.	Key to ComBase	Data set No.	Key to ComBase	Data set No.
LM003_10	393	LM006_2	426	LM009_10	459	LM012_10	492
LM003_11	394	LM006_3	427	LM009_11	460	LM012_11	493
LM003_12	395	LM007_1	428	LM009_12	461	LM012_12	494
LM003_13	396	LM007_10	429	LM009_13	462	LM012_13	495
LM003_14	397	LM007_11	430	LM009_14	463	LM012_14	496
LM003_15	398	LM007_12	431	LM009_15	464	LM012_15	497
LM003_2	399	LM007_13	432	LM009_2	465	LM012_16	498
LM003_3	400	LM007_14	433	LM009_3	466	LM012_17	499
LM003_4	401	LM007_15	434	LM009_4	467	LM012_18	500
LM003_5	402	LM007_2	435	LM009_5	468	LM012_19	501
LM003_6	403	LM007_3	436	LM009_6	469	LM012_2	502
LM003_7	404	LM007_4	437	LM009_7	470	LM012_20	503
LM003_8	405	LM007_5	438	LM009_8	471	LM012_21	504
LM003_9	406	LM007_6	439	LM009_9	472	LM012_22	505
LM004_1	407	LM007_7	440	LM010_1	473	LM012_23	506
LM004_2	408	LM007_8	441	LM010_2	474	LM012_24	507
LM004_3	409	LM007_9	442	LM010_3	475	LM012_25	508
LM005_1	410	LM008_1	443	LM011_1	476	LM012_26	509
LM005_10	411	LM008_10	444	LM011_10	477	LM012_27	510
LM005_11	412	LM008_11	445	LM011_11	478	LM012_3	511
LM005_12	413	LM008_12	446	LM011_12	479	LM012_4	512
LM005_13	414	LM008_13	447	LM011_13	480	LM012_5	513
LM005_14	415	LM008_14	448	LM011_14	481	LM012_6	514
LM005_15	416	LM008_15	449	LM011_15	482	LM012_7	515
LM005_2	417	LM008_2	450	LM011_2	483	LM012_8	516
LM005_3	418	LM008_3	451	LM011_3	484	LM012_9	517
LM005_4	419	LM008_4	452	LM011_4	485	LM013_1	518
LM005_5	420	LM008_5	453	LM011_5	486	LM013_2	519
LM005_6	421	LM008_6	454	LM011_6	487	LM013_3	520
LM005_7	422	LM008_7	455	LM011_7	488	LM013_4	521
LM005_8	423	LM008_8	456	LM011_8	489	LM014_1	522
LM005_9	424	LM008_9	457	LM011_9	490	LM014_2	523
LM006_1	425	LM009_1	458	LM012_1	491	LM014_3	524

TABLE B.1. Continuation.

Key to ComBase	Data set No.	Key to ComBase	Data set No.	Key to ComBase	Data set No.	Key to ComBase	Data set No.
LM014_4	525	LM018_4	558	LM024_1	591	LM030_2	624
LM015_1	526	LM018_5	559	LM024_2	592	LM031_1	625
LM015_2	527	LM018_6	560	LM025_1	593	LM031_2	626
LM015_3	528	LM018_7	561	LM025_2	594	LM032_1	627
LM015_4	529	LM018_8	562	LM026_1	595	LM032_2	628
LM016_1	530	LM018_9	563	LM026_2	596	LM033_1	629
LM016_2	531	LM019_1	564	LM026_3	597	LM033_2	630
LM016_3	532	LM019_10	565	LM026_4	598	LM034_1	631
LM016_4	533	LM019_11	566	LM027_1	599	LM034_2	632
LM017_1	534	LM019_12	567	LM027_10	600	LM035_1	633
LM017_10	535	LM019_13	568	LM027_11	601	LM035_2	634
LM017_11	536	LM019_14	569	LM027_12	602	LM036_1	635
LM017_12	537	LM019_15	570	LM027_13	603	LM036_2	636
LM017_13	538	LM019_16	571	LM027_14	604	LM036_3	637
LM017_14	539	LM019_17	572	LM027_15	605	LM036_4	638
LM017_15	540	LM019_18	573	LM027_16	606	LM037_1	639
LM017_2	541	LM019_19	574	LM027_17	607	LM037_2	640
LM017_3	542	LM019_2	575	LM027_18	608	LM037_3	641
LM017_4	543	LM019_3	576	LM027_19	609	LM038_1	642
LM017_5	544	LM019_4	577	LM027_2	610	LM039_1	643
LM017_6	545	LM019_5	578	LM027_20	611	LM039_2	644
LM017_7	546	LM019_6	579	LM027_3	612	LM039_3	645
LM017_8	547	LM019_7	580	LM027_4	613	LM040_1	646
LM017_9	548	LM019_8	581	LM027_5	614	LM041_1	647
LM018_1	549	LM019_9	582	LM027_6	615	LM041_2	648
LM018_10	550	LM020_1	583	LM027_7	616	LM042_1	649
LM018_11	551	LM020_2	584	LM027_8	617	LM043_1	650
LM018_12	552	LM021_1	585	LM027_9	618	LM043_2	651
LM018_13	553	LM021_2	586	LM028_1	619	LM044_1	652
LM018_14	554	LM022_1	587	LM028_2	620	LM044_2	653
LM018_15	555	LM022_2	588	LM029_1	621	LM045_1	654
LM018_2	556	LM023_1	589	LM029_2	622	LM045_2	655
LM018_3	557	LM023_2	590	LM030_1	623	LM046_1	656

TABLE B.1. Continuation.

Key to ComBase	Data set No.	Key to ComBase	Data set No.	Key to ComBase	Data set No.	Key to ComBase	Data set No.
LM046_2	657	LM050_2	690	LM055_2	723	LM065_2	756
LM047_1	658	LM051_1	691	LM056_1	724	LM066_1	757
LM047_10	659	LM051_10	692	LM056_10	725	LM066_2	758
LM047_11	660	LM051_11	693	LM056_11	726	LM067_1	759
LM047_12	661	LM051_12	694	LM056_12	727	LM067_2	760
LM047_13	662	LM051_2	695	LM056_13	728	LM068_1	761
LM047_14	663	LM051_3	696	LM056_14	729	LM068_2	762
LM047_15	664	LM051_4	697	LM056_15	730	LM069_1	763
LM047_16	665	LM051_5	698	LM056_2	731	LM069_2	764
LM047_17	666	LM051_6	699	LM056_3	732	LM070_1	765
LM047_18	667	LM051_7	700	LM056_4	733	LM070_2	766
LM047_19	668	LM051_8	701	LM056_5	734	LM071_1	767
LM047_2	669	LM051_9	702	LM056_6	735	LM071_2	768
LM047_20	670	LM052_1	703	LM056_7	736	LM072_1	769
LM047_21	671	LM053_1	704	LM056_8	737	LM072_2	770
LM047_22	672	LM053_10	705	LM056_9	738	LM073_1	771
LM047_23	673	LM053_11	706	LM057_1	739	LM073_2	772
LM047_24	674	LM053_12	707	LM057_2	740	LM074_1	773
LM047_25	675	LM053_13	708	LM058_1	741	LM074_2	774
LM047_26	676	LM053_14	709	LM058_2	742	LM075_1	775
LM047_27	677	LM053_15	710	LM059_1	743	LM075_2	776
LM047_3	678	LM053_16	711	LM059_2	744	LM076_1	777
LM047_4	679	LM053_2	712	LM060_1	745	LM076_2	778
LM047_5	680	LM053_3	713	LM060_2	746	LM077_1	779
LM047_6	681	LM053_4	714	LM061_1	747	LM077_2	780
LM047_7	682	LM053_5	715	LM061_2	748	LM078_1	781
LM047_8	683	LM053_6	716	LM062_1	749	LM078_2	782
LM047_9	684	LM053_7	717	LM062_2	750	LM080_1	783
LM048_1	685	LM053_8	718	LM063_1	751	LM081_1	784
LM049_1	686	LM053_9	719	LM063_2	752	LM082_1	785
LM049_2	687	LM054_1	720	LM064_1	753	LM083_1	786
LM049_3	688	LM054_2	721	LM064_2	754	LM084_1	787
LM050_1	689	LM055_1	722	LM065_1	755	LM084_2	788

TABLE B.1. Continuation.

Key to ComBase	Data set No.	Key to ComBase	Data set No.	Key to ComBase	Data set No.	Key to ComBase	Data set No.
LM085_1	789	LM099_4	823	LM113_5	857	LM127_14	891
LM085_2	790	LM099_5	824	LM113_6	858	LM127_15	892
LM086_1	791	LM099_6	825	LM113_7	859	LM127_16	893
LM087_1	792	LM099_7	826	LM113_8	860	LM127_2	894
LM088_1	793	LM099_8	827	LM113_9	861	LM127_3	895
LM089_1	794	LM099_9	828	LM114_1	862	LM127_4	896
LM089_2	795	LM100_1	829	LM115_1	863	LM127_5	897
LM089_3	796	LM100_2	830	LM115_2	864	LM127_6	898
LM090_1	797	LM101_1	831	LM116_1	865	LM127_7	899
LM090_2	798	LM101_2	832	LM116_2	866	LM127_8	900
LM091_1	799	LM102_1	833	LM117_1	867	LM127_9	901
LM091_2	800	LM102_2	834	LM117_2	868	LM128_1	902
LM092_1	801	LM103_1	835	LM118_1	869	LM128_2	903
LM093_1	802	LM103_2	836	LM118_2	870	LM129_1	904
LM093_2	803	LM104_1	837	LM119_1	871	LM129_2	905
LM094_1	804	LM104_2	838	LM119_2	872	LM129_3	906
LM094_2	805	LM105_1	839	LM120_1	873	LM130_1	907
LM095_1	806	LM105_2	840	LM120_2	874	LM130_10	908
LM095_2	807	LM106_1	841	LM121_1	875	LM130_11	909
LM096_1	808	LM107_1	842	LM121_2	876	LM130_12	910
LM096_2	809	LM108_1	843	LM122_1	877	LM130_13	911
LM097_1	810	LM108_2	844	LM122_2	878	LM130_14	912
LM097_2	811	LM109_1	845	LM123_1	879	LM130_15	913
LM098_1	812	LM109_2	846	LM123_2	880	LM130_2	914
LM099_1	813	LM110_1	847	LM124_1	881	LM130_3	915
LM099_10	814	LM110_2	848	LM124_2	882	LM130_4	916
LM099_11	815	LM111_1	849	LM125_1	883	LM130_5	917
LM099_12	816	LM111_2	850	LM125_2	884	LM130_6	918
LM099_13	817	LM112_1	851	LM126_1	885	LM130_7	919
LM099_14	818	LM112_2	852	LM127_1	886	LM130_8	920
LM099_15	819	LM113_1	853	LM127_10	887	LM130_9	921
LM099_16	820	LM113_2	854	LM127_11	888	LM131_1	922
LM099_2	821	LM113_3	855	LM127_12	889	LM131_2	923
LM099_3	822	LM113_4	856	LM127_13	890	LM132_1	924

TABLE B.1. Continuation.

Key to ComBase	Data set No.	Key to ComBase	Data set No.	Key to ComBase	Data set No.
LM132_10	925	LM132_15	930	LM132_6	935
LM132_11	926	LM132_2	931	LM132_7	936
LM132_12	927	LM132_3	932	LM132_8	937
LM132_13	928	LM132_4	933	LM132_9	938
LM132_14	929	LM132_5	934		

TABLE B.2. No-growth broth-based data sets (*L. monocytogenes*) eliminated for anaerobic and aerobic conditions.

Anaerobic			Aerobic		
Data set No					
1	211	293	386	741	912
4	212	294	387	742	913
9	213	295	388	745	916
26	223	331	389	746	917
29	224	332	390	747	921
30	225	333	391	748	
45	226	340	398	749	
46	227	341	402	750	
47	228	342	444	780	
63	229	343	449	781	
64	230	344	453	782	
82	231	345	482	794	
85	232	346	486	795	
86	233	347	527	796	
87	245	348	529	797	
88	248	349	535	798	
89	249	350	540	799	
90	250	351	544	800	
91	253	364	600	801	
117	254	365	605	802	
119	255	366	607	803	
120	256	380	614	804	
139	263	381	685	805	
142	264	382	686	806	
143	265		687	807	
160	266		688	808	
161	267		705	809	
174	268		709	814	
175	269		710	818	
178	270		711	819	
179	271		714	823	
180	272		715	824	
181	273		719	851	
184	274		739	852	
185	290		740	908	

TABLE B.3. B (h^{-1}) and M (h) estimated from the primary regression for anaerobic conditions.

Data set No.	B	M	Data set No.	B	M	Data set No.	B	M
2	0.016	182.517	41	0.046	43.205	80	0.042	32.080
3	0.956	10.666	42	0.950	10.670	81	0.036	36.910
5	1.710	23.193	43	0.031	51.691	83	0.039	35.301
6	0.205	80.177	44	0.045	39.534	84	0.028	47.227
7	0.106	302.736	48	3.060	23.094	92	0.016	183.557
8	0.484	201.306	49	0.889	10.654	93	0.280	98.601
10	0.209	6.622	50	2.858	22.809	94	0.184	19.073
11	0.203	6.932	51	0.195	10.324	95	0.190	19.118
12	0.195	7.086	52	0.162	11.838	96	0.107	13.118
13	0.203	6.774	53	0.684	19.824	97	0.088	16.009
14	0.871	10.996	54	0.228	9.961	98	0.090	16.605
15	0.861	10.915	55	3.209	23.183	99	0.097	14.935
16	0.870	11.034	56	3.965	7.179	100	0.128	11.787
17	0.867	10.808	57	0.761	20.187	101	0.101	13.946
18	1.136	8.871	58	1.151	21.657	102	0.104	14.103
19	0.169	8.090	59	0.561	21.222	103	0.105	14.102
20	0.176	7.831	60	0.582	22.527	104	0.115	12.831
21	0.164	8.120	61	0.125	14.657	105	0.108	15.200
22	0.109	11.606	62	0.471	19.653	106	0.084	16.792
23	0.147	9.115	65	0.421	94.777	107	0.176	11.394
24	0.122	10.194	66	0.020	80.855	108	0.103	13.586
25	1.327	57.243	67	0.019	80.491	109	0.104	15.160
27	1.062	6.968	68	0.020	76.174	110	0.074	19.583
28	1.093	7.052	69	0.921	45.057	111	0.097	14.779
31	1.990	7.127	70	0.117	29.015	112	0.103	14.170
32	1.500	7.350	71	0.097	29.061	113	1.643	6.394
33	0.260	17.849	72	0.109	13.803	114	1.855	8.884
34	0.230	10.649	73	0.244	19.947	115	0.952	10.703
35	0.922	10.170	74	0.156	19.483	116	4.164	23.700
36	0.962	10.512	75	0.114	15.284	118	0.822	29.874
37	0.058	26.198	76	0.182	19.079	121	0.041	47.743
38	0.078	29.784	77	0.176	19.963	122	0.035	48.385
39	0.090	25.531	78	0.074	24.201	123	0.034	51.268
40	0.234	20.497	79	0.064	29.694	124	0.046	44.100

TABLE B.3. Continuation.

Data set No.	B	M	Data set No.	B	M	Data set No.	B	M
125	0.027	59.629	164	1.115	9.545	206	0.912	8.184
126	0.041	47.975	165	0.177	11.823	207	3.904	16.487
127	0.040	46.321	166	7.804	7.105	208	0.024	57.146
128	0.036	48.095	167	0.514	20.642	209	0.023	56.600
129	0.042	48.345	168	0.933	10.175	210	0.022	61.343
130	0.039	41.167	169	0.096	13.451	214	0.975	20.753
131	1.929	47.204	170	0.148	13.157	215	0.978	10.863
132	0.085	21.158	171	0.986	9.934	216	0.014	129.406
133	0.087	19.229	172	2.501	23.648	217	0.017	85.001
134	0.094	21.453	173	0.970	9.981	218	0.119	13.246
135	0.053	43.004	176	0.982	9.946	219	0.100	16.191
136	0.024	85.145	177	0.003	494.122	220	0.105	15.305
137	0.082	44.055	182	0.033	41.969	221	0.011	155.611
138	0.064	36.615	183	0.033	44.217	222	0.052	149.320
140	0.049	38.124	186	0.020	70.745	234	1.585	7.330
141	0.031	57.037	187	0.025	56.194	235	0.185	10.074
144	0.138	20.848	188	0.027	54.055	236	0.050	30.102
145	4.599	26.821	189	0.023	59.368	237	0.076	26.661
146	0.093	21.540	190	0.026	57.652	238	0.070	28.490
147	0.087	22.303	191	0.023	65.533	239	0.080	26.354
148	0.093	21.884	192	0.065	72.028	240	0.067	27.105
149	0.088	21.224	193	0.240	6.650	241	0.067	26.575
150	0.952	10.076	194	0.271	6.103	242	0.065	37.214
151	0.919	10.140	195	0.256	6.446	243	0.957	10.446
152	0.062	24.221	196	2.089	8.945	244	0.955	10.563
153	0.106	23.297	197	0.281	6.122	246	0.063	55.242
154	0.141	25.824	198	1.744	8.570	247	1.013	11.762
155	0.641	29.639	199	0.274	6.066	251	0.011	183.021
156	0.549	23.887	200	0.300	6.304	252	0.005	296.848
157	0.605	24.053	201	0.264	6.114	257	0.010	232.661
158	0.181	23.577	202	1.833	8.829	258	0.005	477.574
159	0.003	494.198	203	0.249	6.174	259	0.353	48.701
162	0.291	7.092	204	0.243	6.287	260	25.923	120.120
163	0.783	7.270	205	1.372	7.744	261	0.989	10.697

TABLE B.3. Continuation.

Data set No.	B	M	Data set No.	B	M	Data set No.	B	M
262	0.975	10.945	307	0.010	143.720	339	0.954	200.734
275	0.426	16.715	308	0.045	239.062	352	0.011	142.644
276	1.345	7.479	309	0.054	41.014	353	0.014	135.248
277	0.929	10.825	310	0.036	49.552	354	0.009	161.137
278	0.998	10.304	311	0.039	46.982	355	0.016	137.446
279	0.927	10.831	312	0.012	138.531	356	0.011	144.186
280	1.148	8.899	313	0.007	236.116	357	0.012	136.504
281	1.343	7.498	314	0.993	154.908	358	0.010	157.094
282	0.577	15.293	315	0.239	164.455	359	0.010	157.447
283	1.332	7.583	316	0.276	164.132	360	0.006	217.585
284	1.144	8.924	317	0.009	169.041	361	0.004	413.335
285	1.361	7.350	318	0.010	142.339	362	0.012	248.843
286	1.147	8.904	319	0.009	146.859	363	0.266	284.116
287	0.951	10.620	320	0.009	192.921	367	0.012	139.988
288	1.143	8.937	321	0.015	172.845	368	2.937	22.552
289	1.145	8.928	322	0.009	156.028	369	0.193	8.077
291	0.020	271.282	323	0.009	159.316	370	1.011	9.921
292	0.010	171.758	324	0.009	165.278	371	0.070	17.900
296	0.012	166.289	325	0.009	145.480	372	0.056	22.260
297	0.164	179.709	326	0.010	144.412	373	0.096	16.181
298	0.009	168.174	327	0.011	141.319	374	0.077	21.589
299	0.010	169.396	328	0.009	171.077	375	0.066	22.340
300	0.017	123.855	329	0.007	178.235	376	0.069	22.020
301	0.014	120.746	330	0.009	184.649	377	0.071	22.860
302	0.018	102.843	334	0.012	391.181	378	0.064	24.001
303	0.016	111.003	335	0.010	380.690	379	0.067	23.108
304	0.015	110.348	336	0.968	382.834	383	0.009	191.736
305	0.015	114.322	337	0.957	200.690	384	0.009	205.806
306	0.010	152.000	338	0.952	200.764	385	0.004	427.784

TABLE B.4. B (h^{-1}) and M (h) estimated from the primary regression for aerobic conditions.

Data set No.	B	M	Data set No.	B	M	Data set No.	B	M
392	0.280	5.993	428	0.865	11.083	465	0.411	19.463
393	0.109	24.392	429	1.079	9.793	466	0.314	18.451
394	0.901	5.834	430	0.344	7.414	467	0.467	19.811
395	0.226	6.573	431	0.284	7.343	468	0.396	17.928
396	0.213	6.960	432	0.282	7.145	469	0.631	21.198
397	2.510	9.275	433	0.268	7.759	470	0.548	20.685
399	1.155	8.857	434	2.805	22.617	471	0.418	18.915
400	0.220	7.418	435	0.266	7.485	472	0.119	13.124
401	1.630	8.697	436	0.247	7.922	473	1.239	24.686
403	1.423	9.913	437	0.870	10.860	474	1.156	24.698
404	0.225	6.094	438	0.286	8.824	475	1.124	24.702
405	0.225	6.984	439	0.969	10.446	476	0.463	16.616
406	0.208	9.535	440	0.809	7.443	478	0.925	10.848
407	0.922	10.012	441	0.289	7.484	479	1.143	8.937
408	0.979	9.960	442	0.285	7.590	480	0.440	18.853
409	0.982	9.949	443	1.474	7.034	481	0.517	21.074
410	0.472	16.292	445	1.195	7.878	483	1.660	5.137
411	0.242	5.849	446	1.037	7.892	484	1.843	7.422
412	0.253	5.239	447	1.104	9.434	485	0.544	21.718
413	0.232	5.796	448	1.164	23.548	487	0.928	10.830
414	1.142	8.851	450	1.033	7.906	488	1.145	8.921
415	0.250	5.829	451	1.157	9.604	489	0.412	18.073
416	0.984	10.236	452	0.934	23.318	490	0.505	21.491
417	0.240	5.389	454	0.903	8.273	491	1.183	8.668
418	1.300	7.923	455	0.903	10.434	492	0.902	10.205
419	0.991	9.972	456	0.438	15.754	493	2.157	7.272
420	0.251	6.217	457	1.087	23.664	494	2.141	7.253
421	0.422	16.565	458	0.270	8.128	495	1.249	9.217
422	0.262	5.496	459	0.098	15.751	496	4.000	7.133
423	1.130	8.933	460	0.147	10.734	497	0.906	10.195
424	0.239	5.749	461	0.651	21.594	498	0.183	8.709
425	0.922	10.012	462	0.928	10.050	499	0.200	8.807
426	0.979	9.960	463	0.123	12.661	500	3.221	22.775
427	0.982	9.949	464	0.950	10.276	501	0.197	8.289

TABLE B.4. Continuation.

Data set No.	B	M	Data set No.	B	M	Data set No.	B	M
502	1.676	7.995	539	0.075	17.337	575	0.024	57.438
503	0.199	8.237	541	0.267	10.737	576	0.060	41.956
504	0.195	8.232	542	0.234	9.891	577	0.050	41.660
505	2.392	22.359	543	0.059	19.965	578	0.007	206.641
506	0.877	10.822	545	0.247	10.542	579	0.008	223.769
507	0.205	7.526	546	0.312	10.347	580	0.007	229.171
508	1.555	8.858	547	0.220	10.994	581	1.260	50.272
509	0.233	6.837	548	0.059	17.829	582	0.823	49.679
510	1.087	9.683	549	0.137	12.335	583	0.018	122.431
511	0.418	14.524	550	0.103	14.584	584	0.020	97.915
512	1.178	9.362	551	0.128	12.729	585	0.022	162.688
513	1.156	9.754	552	0.132	12.790	586	0.023	154.398
514	2.006	7.312	553	0.112	15.275	587	1.911	49.752
515	2.105	7.261	554	0.121	14.493	588	0.956	101.016
516	2.899	7.196	555	0.112	13.921	589	0.984	100.707
517	0.882	10.345	556	0.128	12.622	590	0.984	100.695
518	0.039	43.714	557	0.123	13.336	591	0.937	101.444
519	0.975	10.940	558	0.142	12.127	592	1.447	100.814
520	1.473	49.964	559	0.100	14.445	593	1.482	100.538
521	0.957	10.609	560	0.119	13.678	594	0.481	101.654
522	0.982	9.946	561	0.129	12.954	595	0.125	15.604
523	5.939	20.080	562	0.120	13.580	596	0.123	15.563
524	1.158	33.893	563	0.115	13.215	597	0.191	13.036
525	2.708	16.673	546	0.024	59.836	598	0.156	11.397
526	0.948	10.299	565	0.029	94.308	599	0.185	20.993
528	0.935	9.967	566	0.030	89.682	601	0.200	21.125
530	0.895	10.429	567	0.028	90.237	602	0.168	21.471
531	1.529	8.862	568	0.031	90.823	603	0.163	22.492
532	2.994	22.695	569	0.955	30.808	604	0.047	29.714
533	2.690	16.699	570	0.955	30.812	606	0.210	15.327
534	0.236	10.733	571	0.026	101.757	608	0.135	13.140
536	0.262	10.415	572	0.019	104.605	609	0.190	8.177
537	2.210	23.419	573	0.019	103.627	610	0.538	23.267
538	0.273	10.599	574	0.022	98.502	611	0.191	8.730

TABLE B.4. Continuation.

Data set No.	B	M	Data set No.	B	M	Data set No.	B	M
612	0.114	22.521	647	0.096	23.616	681	0.096	18.908
613	0.046	30.887	648	0.101	23.974	682	0.095	21.094
615	0.147	20.920	649	0.090	27.089	683	0.106	20.548
616	0.177	22.075	650	0.053	32.597	684	0.106	21.326
617	0.124	22.082	651	0.062	30.938	689	0.095	22.623
618	0.048	27.651	652	0.054	35.564	690	0.094	22.883
619	0.090	22.241	653	0.052	31.600	691	0.091	25.817
620	0.088	21.319	654	0.091	18.297	692	0.049	30.625
621	0.054	26.517	655	0.097	19.038	693	0.069	28.162
622	0.064	25.864	656	0.105	14.385	694	0.060	31.361
623	0.064	31.213	657	0.090	17.792	695	0.063	31.651
624	0.062	31.636	658	0.107	20.765	696	0.080	26.272
625	0.072	27.744	659	0.436	23.419	697	0.063	27.354
626	0.079	26.829	660	0.112	20.224	698	0.079	27.947
627	0.095	22.624	661	0.114	20.313	699	0.061	28.405
628	0.094	22.883	662	0.109	21.359	700	0.036	39.850
629	0.058	31.491	663	0.092	20.954	701	0.052	33.447
630	0.061	31.259	664	0.575	23.620	702	0.075	27.975
631	0.041	52.874	665	0.124	17.736	703	0.098	15.782
632	0.052	40.657	666	0.132	17.944	704	0.054	28.739
633	0.052	31.988	667	0.135	17.507	706	0.059	29.444
634	0.054	34.239	668	0.114	19.835	707	0.047	45.365
635	0.126	11.866	669	0.091	19.993	708	0.056	65.442
636	0.129	11.756	670	0.098	18.412	712	0.044	45.737
637	0.180	8.479	671	0.101	19.231	713	0.061	62.339
638	0.197	8.070	672	0.098	20.699	716	0.061	28.584
639	0.087	18.832	673	0.094	20.620	717	0.045	47.243
640	0.065	24.620	674	0.092	21.029	718	0.090	59.420
641	0.071	24.617	675	0.074	21.066	720	0.052	31.988
642	0.091	19.003	676	0.084	22.582	721	0.054	34.239
643	0.094	22.543	677	0.080	22.745	722	0.059	21.229
644	0.068	26.983	678	0.106	21.025	723	0.906	10.187
645	0.063	26.352	679	0.089	21.431	724	0.063	27.375
646	0.098	20.402	680	0.964	23.734	725	0.867	24.372

TABLE B.4. Continuation.

Data set No.	B	M	Data set No.	B	M	Data set No.	B	M
726	0.073	29.178	770	0.019	75.165	829	0.011	173.799
727	0.068	26.773	771	0.676	121.559	830	0.013	169.092
728	0.061	27.959	772	0.022	65.201	831	0.007	206.964
729	0.064	29.057	773	0.022	76.079	832	0.009	204.111
730	0.594	24.515	774	0.020	64.748	833	0.009	400.774
731	0.063	26.304	775	0.019	90.197	834	0.006	383.934
732	0.069	26.911	776	0.020	88.856	835	0.009	213.803
733	0.072	28.891	777	0.977	71.195	836	0.009	213.626
734	0.637	24.390	778	0.036	62.503	837	0.012	171.105
735	0.061	21.279	779	0.021	87.858	838	0.013	171.990
736	0.063	28.365	783	0.033	48.043	839	0.006	235.330
737	0.096	26.562	784	0.024	57.570	840	0.006	239.583
738	0.063	29.973	785	0.024	56.813	841	0.008	228.468
743	0.892	104.334	786	0.022	61.451	842	0.011	158.868
744	0.030	648.047	787	0.057	30.320	843	0.005	253.157
751	0.951	10.739	788	0.033	61.952	844	0.007	229.541
752	0.020	75.468	789	0.040	43.258	845	0.007	212.711
753	1.449	72.305	790	0.042	42.770	846	0.007	232.976
754	0.031	63.782	791	0.023	60.749	847	0.006	276.770
755	0.016	100.126	792	0.021	65.139	848	0.006	297.124
756	0.016	99.919	793	0.023	57.227	849	0.004	557.919
757	0.058	31.353	810	0.011	232.032	850	0.003	596.528
758	0.061	31.116	811	0.011	235.292	853	0.009	187.027
759	0.014	138.300	812	0.010	197.185	854	0.013	140.772
760	0.013	146.489	813	0.012	129.769	855	0.014	128.213
761	0.009	152.598	815	0.012	138.558	856	0.016	112.864
762	0.088	57.583	816	0.011	163.508	857	0.013	127.059
763	0.024	63.408	817	0.007	257.826	858	0.013	123.159
764	0.020	66.861	820	0.009	257.083	859	0.013	128.762
765	0.388	25.823	821	0.009	180.254	860	0.012	158.229
766	0.035	50.620	822	0.007	262.008	861	0.010	144.320
767	0.034	47.421	825	0.012	135.966	862	0.015	135.187
768	0.037	46.798	826	0.009	196.525	863	0.012	153.132
769	0.021	75.590	827	0.005	288.501	864	0.010	173.042

TABLE B.4. Continuation.

Data set No.	B	M	Data set No.	B	M
865	0.007	200.274	899	0.010	163.798
866	0.009	204.733	900	0.010	164.632
867	0.011	165.480	901	0.011	152.368
868	0.010	176.612	902	0.006	235.456
869	0.015	96.338	903	0.006	239.690
870	0.016	89.720	904	0.016	151.365
871	0.010	172.599	905	0.013	178.187
872	0.009	168.587	906	0.015	174.290
873	0.016	160.084	907	0.008	198.709
874	0.013	156.457	909	0.007	227.315
875	0.008	224.263	910	0.007	297.308
876	0.009	217.646	911	0.007	386.786
877	0.007	240.956	914	0.009	284.062
878	0.006	265.994	915	0.004	439.285
879	0.009	198.395	918	0.009	209.478
880	0.009	179.296	919	0.006	332.618
881	0.010	129.998	920	0.007	335.046
882	0.010	141.609	922	0.012	171.121
883	0.013	114.840	923	0.013	172.005
884	0.016	110.004	924	0.010	179.316
885	0.012	146.337	925	0.014	146.925
886	0.010	155.764	926	0.011	179.223
887	0.011	144.304	927	0.012	163.802
888	0.010	156.268	928	0.012	173.334
889	0.010	156.438	929	0.012	170.474
890	0.010	154.515	930	0.013	147.530
891	0.009	156.153	931	0.011	177.617
892	0.011	145.476	932	0.014	161.775
893	0.011	156.722	933	0.011	159.964
894	0.010	160.651	934	0.014	149.552
895	0.010	156.118	935	0.011	176.798
896	0.011	155.369	936	0.013	178.495
897	0.011	141.126	937	0.012	169.827
898	0.010	168.463	938	0.011	155.331

TABLE B.5. Treatment (treat.) and data set numbers for anaerobic conditions used to calculate error due to replication calculations.

Data set No.	Treat. No.	Data set No.	Treat. No.	Data set No.	Treat. No.	Data set No.	Treat. No.	Data set No.	Treat. No.
2	4	48	99	84	24	128	26	168	41
3	4	49	99	92	39	129	26	169	41
5	4	50	99	93	39	130	26	170	41
6	4	51	100	94	62	132	27	171	42
7	4	52	100	95	62	133	27	172	42
8	4	53	100	96	62	134	27	173	42
10	98	54	101	97	73	135	28	177	12
11	98	55	101	98	73	136	28	186	12
12	98	56	101	99	87	137	28	193	104
13	98	57	102	100	87	138	29	194	104
14	98	58	102	101	87	140	29	195	104
15	98	59	102	102	87	141	29	196	105
16	98	60	103	103	87	144	93	197	105
17	98	61	103	104	87	145	93	198	105
18	98	62	103	105	88	146	93	199	106
19	98	65	2	106	88	147	94	200	106
20	98	66	2	107	88	148	94	201	106
21	98	67	2	108	89	149	94	202	107
22	98	68	2	109	89	150	95	203	107
23	98	69	6	110	89	151	95	204	107
24	98	70	6	111	90	152	95	205	108
33	35	71	6	112	90	153	96	206	108
34	35	72	22	113	90	154	96	207	108
35	35	73	22	114	91	155	96	214	54
36	36	74	22	115	91	156	97	215	54
37	36	75	22	116	91	157	97	218	44
38	36	76	22	121	26	158	97	219	44
39	37	77	22	122	26	162	39	220	44
40	37	78	23	123	26	163	39	221	118
41	37	79	23	124	26	164	39	222	118
42	38	80	23	125	26	165	40	234	45
43	38	81	24	126	26	166	40	235	45
44	38	83	24	127	26	167	40	236	46

TABLE B.5. Continuation.

Data set No.	Treat. No.	Data set No.	Treat. No.	Data set No.	Treat. No.
237	46	304	57	359	83
238	46	305	57	360	83
239	47	306	57	361	83
240	47	307	57	362	84
241	47	308	57	363	84
242	48	310	3	369	30
243	48	311	3	370	30
244	48	312	3	371	30
246	49	316	72	372	31
247	49	317	72	373	31
251	8	319	76	374	31
252	8	320	76	375	32
260	13	321	76	376	32
261	13	322	76	377	32
262	13	323	77	378	33
276	109	324	77	379	33
277	109	325	77		
278	109	326	78		
279	110	327	78		
280	110	328	78		
281	110	329	79		
282	111	330	79		
283	111	335	17		
284	111	336	17		
285	112	337	17		
286	112	338	18		
287	112	339	18		
288	113	353	81		
289	113	354	81		
300	57	355	81		
301	57	356	82		
302	57	357	82		
303	57	358	82		

TABLE B.6. Treatment (treat.) and data set numbers for aerobic conditions used to calculate error due to replication calculations.

Data set No.	Treat. No.	Data set No.	Treat. No.	Data set No.	Treat. No.	Data set No.	Treat. No.	Data set No.	Treat. No.
392	44	428	123	464	132	500	108	536	40
394	44	429	127	465	129	501	108	537	41
395	45	430	123	466	130	502	109	538	42
396	46	431	124	467	131	503	108	539	43
397	47	432	125	468	132	504	108	541	41
399	45	433	126	469	128	505	108	542	42
400	46	434	127	470	129	506	108	543	43
401	47	435	124	471	130	507	108	545	40
403	44	436	125	472	131	508	108	546	41
404	45	437	126	473	2	509	108	547	42
405	46	438	127	474	2	510	108	548	43
406	47	439	123	475	2	511	110	549	113
407	49	440	124	476	36	512	111	550	117
408	49	441	125	478	36	513	112	551	113
409	49	442	126	479	37	514	108	552	114
410	118	443	50	480	38	515	109	553	115
411	122	445	50	481	39	516	110	554	116
412	118	446	51	483	37	517	111	555	117
413	119	447	52	484	38	518	9	556	114
414	120	448	53	485	39	519	10	557	115
415	121	450	51	487	36	520	9	558	116
416	122	451	52	488	37	521	10	559	117
417	119	452	53	489	38	522	68	560	113
418	120	454	50	490	39	523	69	561	114
419	121	455	51	491	108	524	68	562	115
420	122	456	52	492	112	525	69	563	116
421	118	457	53	493	108	526	11	564	1
422	119	458	128	494	109	528	11	565	1
423	120	459	132	495	110	530	70	566	1
424	121	460	128	496	111	531	71	567	1
425	125	461	129	497	112	532	70	568	1
426	125	462	130	498	108	533	71	569	1
427	125	463	131	499	108	534	40	570	1

TABLE B.6. Continuation.

Data set No.	Treat. No.	Data set No.	Treat. No.	Data set No.	Treat. No.	Data set No.	Treat. No.	Data set No.	Treat. No.
571	1	606	26	641	79	674	97	720	32
572	1	608	26	642	79	675	97	721	32
573	1	609	26	643	80	676	97	722	32
574	1	610	27	644	80	677	97	723	32
575	1	611	26	645	80	678	99	724	103
576	1	612	28	646	80	679	100	725	107
577	1	613	29	647	81	680	101	726	103
578	1	615	26	648	81	681	97	727	104
579	1	616	27	649	81	682	98	728	105
580	1	617	28	650	81	683	99	729	106
581	1	618	29	651	81	684	100	730	107
582	1	619	26	652	81	689	30	731	104
583	4	620	26	653	81	690	30	732	105
584	4	621	26	654	83	691	31	733	106
585	4	622	26	655	83	692	31	734	107
586	4	623	26	656	83	693	31	735	103
587	5	624	26	657	83	694	31	736	104
588	5	625	26	658	97	695	31	737	105
589	5	626	26	659	101	696	31	738	106
590	5	627	30	660	97	697	31	743	3
591	6	628	30	661	98	698	31	744	3
592	6	629	30	662	99	699	31	751	23
593	6	630	30	663	100	700	31	752	23
594	6	631	26	664	101	701	31	753	24
595	8	632	26	665	97	702	31	754	24
596	8	633	26	666	97	704	32	755	24
597	8	634	26	667	97	707	32	756	24
598	8	635	67	668	97	708	33	757	24
599	26	636	67	669	98	712	33	758	24
601	26	637	67	670	97	713	34	759	25
602	27	638	67	671	97	716	32	760	25
603	28	639	79	672	97	717	33	761	25
604	29	640	79	673	97	718	34	762	25

TABLE B.6. Continuation.

Data set No.	Treat. No.	Data set No.	Treat. No.	Data set No.	Treat. No.	Data set No.	Treat. No.
763	76	833	13	869	72	903	17
764	76	834	13	870	72	904	18
765	76	835	13	871	73	905	18
766	76	836	13	872	73	906	18
767	76	837	13	873	73	907	19
768	76	838	13	874	73	909	19
769	77	839	13	875	74	910	20
770	77	840	13	876	74	911	21
771	77	842	56	877	74	914	20
772	77	843	56	878	74	915	21
773	77	844	56	879	82	918	19
774	77	845	57	880	82	919	20
775	78	846	57	881	82	920	21
776	78	847	58	882	82	922	19
777	78	848	58	883	82	923	19
778	78	849	59	884	82	924	91
784	55	850	59	886	86	925	95
791	75	853	61	887	90	926	91
810	13	854	61	888	86	927	92
811	13	855	61	889	87	928	93
813	13	856	61	890	88	929	94
815	13	857	61	891	89	930	95
816	14	858	61	892	90	931	92
817	15	859	61	893	86	932	93
821	14	860	61	894	87	933	94
822	15	861	61	895	88	934	95
825	13	862	72	896	89	935	91
826	14	863	72	897	90	936	92
827	15	864	72	898	86	937	93
829	13	865	72	899	87	938	94
830	13	866	72	900	88		
831	13	867	72	901	89		
832	13	868	72	902	17		

TABLE B.7. Experimental variables each treatment (aerobic conditions, (U.S. Department of Agriculture, 2003a)).

pH	T °C	NaCl (g/l)	Nitrite (ppm)	No. of data points	No. of treat.	pH	T °C	NaCl (g/l)	Nitrite (ppm)	No. of data points	No. of treat.
4.5	19	0	0	200	1	6	19	25	0	64	30
4.5	28	0	0	30	2	6	19	25	100	92	31
5	12	25	0	28	3	6	19	45	0	60	32
5	19	0	0	66	4	6	19	45	50	24	33
5	19	25	0	38	5	6	19	45	100	16	34
5	19	45	0	50	6	6	19	45	1000	7	35
5.25	10	15	50	7	7	6	28	0	0	12	36
5.25	19	0	0	32	8	6	28	0	50	12	37
5.25	28	15	50	14	9	6	28	0	100	12	38
5.25	28	15	150	17	10	6	28	0	200	12	39
5.25	28	35	50	15	11	6	28	45	0	24	40
5.5	5	0	0	7	12	6	28	45	50	22	41
6	5	0	0	151	13	6	28	45	100	24	42
6	5	0	50	21	14	6	28	45	200	21	43
6	5	0	100	21	15	6	37	0	0	16	44
6	5	0	200	7	16	6	37	0	50	17	45
6	5	25	0	18	17	6	37	0	100	18	46
6	5	25	100	24	18	6	37	0	200	18	47
6	5	45	0	36	19	6	37	0	1000	6	48
6	5	45	50	21	20	6	37	25	100	15	49
6	5	45	100	21	21	6	37	45	0	15	50
6	10	0	0	14	22	6	37	45	50	15	51
6	12	0	0	27	23	6	37	45	100	15	52
6	12	25	0	66	24	6	37	45	200	15	53
6	12	45	0	42	25	6.25	5	0	0	7	54
6	19	0	0	177	26	6.25	10	0	0	13	55
6	19	0	50	18	27	6.5	5	0	0	25	56
6	19	0	100	18	28	6.5	5	20	0	18	57
6	19	0	200	18	29	6.5	5	40	0	20	58

TABLE B.7. Continuation.

pH	T °C	NaCl (g/l)	Nitrite (ppm)	No. of data points	No. of treat.	pH	T °C	NaCl (g/l)	Nitrite (ppm)	No. of data points	No. of treat.
6.5	5	60	0	26	59	7.5	5	0	100	24	88
6.5	10	0	0	13	60	7.5	5	0	200	24	89
6.75	5	0	0	75	61	7.5	5	0	1000	24	90
6.75	10	0	0	13	62	7.5	5	45	0	18	91
6.75	10	15	50	7	63	7.5	5	45	50	18	92
6.75	10	15	150	8	64	7.5	5	45	100	18	93
6.75	10	35	50	7	65	7.5	5	45	200	18	94
6.75	10	35	150	7	66	7.5	5	45	1000	15	95
6.75	19	0	0	32	67	7.5	10	0	0	13	96
6.75	28	15	50	13	68	7.5	19	0	0	114	97
6.75	28	15	150	16	69	7.5	19	0	50	15	98
6.75	28	35	50	14	70	7.5	19	0	100	15	99
6.75	28	35	150	15	71	7.5	19	0	200	15	100
7	5	0	0	84	72	7.5	19	0	1000	13	101
7	5	25	0	42	73	7.5	19	25	100	6	102
7	5	45	0	38	74	7.5	19	45	0	18	103
7	10	0	0	13	75	7.5	19	45	50	18	104
7	12	0	0	46	76	7.5	19	45	100	18	105
7	12	25	0	54	77	7.5	19	45	200	18	106
7	12	45	0	34	78	7.5	19	45	1000	18	107
7	19	0	0	38	79	7.5	28	0	0	90	108
7	19	25	0	39	80	7.5	28	0	50	18	109
7	19	45	0	68	81	7.5	28	0	100	18	110
7.2	5	0	0	46	82	7.5	28	0	200	18	111
7.2	19	0	0	34	83	7.5	28	0	1000	18	112
7.25	5	0	0	7	84	7.5	28	45	0	21	113
7.25	10	0	0	13	85	7.5	28	45	50	21	114
7.5	5	0	0	31	86	7.5	28	45	100	21	115
7.5	5	0	50	24	87	7.5	28	45	200	21	116

TABLE B.7. Continuation.

pH	T °C	NaCl (g/l)	Nitrite (ppm)	No. of data points	No. of treat.	pH	T °C	NaCl (g/l)	Nitrite (ppm)	No. of data points	No. of treat.
7.5	28	45	1000	21	117	7.5	37	25	100	33	125
7.5	37	0	0	17	118	7.5	37	25	200	18	126
7.5	37	0	50	17	119	7.5	37	25	1000	18	127
7.5	37	0	100	18	120	7.5	37	45	0	18	128
7.5	37	0	200	16	121	7.5	37	45	50	18	129
7.5	37	0	1000	17	122	7.5	37	45	100	17	130
7.5	37	25	0	18	123	7.5	37	45	200	18	131
7.5	37	25	50	18	124	7.5	37	45	1000	16	132

TABLE B.8. Experimental variables in each treatment (anaerobic conditions, (U.S. Department of Agriculture, 2003a)).

pH	T °C	NaCl (g/l)	Nitrite (ppm)	No. of data points	No. of treat.	pH	T °C	NaCl (g/l)	Nitrite (ppm)	No. of data points	No. of treat.
4.5	10	0	0	10	1	6	28	0	0	18	30
4.5	19	0	0	43	2	6	28	0	50	25	31
4.5	28	0	0	31	3	6	28	0	100	22	32
4.5	37	0	0	57	4	6	28	0	200	12	33
5.25	10	15	50	6	5	6	28	0	1000	9	34
5.25	19	0	0	30	6	6	28	45	0	16	35
5.3	28	15	50	8	7	6	28	45	50	17	36
5.5	5	0	0	26	8	6	28	45	100	22	37
5.5	5	0	50	15	9	6	28	45	200	23	38
5.5	5	5	0	5	10	6	37	0	0	42	39
5.5	5	25	0	14	11	6	37	0	50	16	40
5.5	10	0	0	23	12	6	37	0	100	17	41
6	5	0	0	19	13	6	37	0	200	14	42
6	5	0	25	10	14	6	37	0	1000	4	43
6	5	0	50	10	15	6	37	25	100	27	44
6	5	0	1000	5	16	6	37	45	0	16	45
6	5	25	100	26	17	6	37	45	50	26	46
6	5	45	0	16	18	6	37	45	100	28	47
6	5	45	25	7	19	6	37	45	200	24	48
6	5	45	1000	8	20	6	37	45	1000	20	49
6	10	0	0	13	21	6.25	5	0	0	6	50
6	19	0	0	53	22	6.25	10	0	0	13	51
6	19	0	50	29	23	6.5	5	0	0	7	52
6	19	0	100	33	24	6.5	5	0	200	7	53
6	19	25	0	8	25	6.5	8	0	0	19	54
6	19	25	100	100	26	6.5	8	50	0	9	55
6	19	45	0	21	27	6.5	10	0	0	13	56
6	19	45	50	24	28	6.75	5	0	0	75	57
6	19	45	100	24	29	6.75	10	0	0	13	58

TABLE B.8. Continuation.

pH	T °C	NaCl (g/l)	Nitrite (ppm)	No. of data points	No. of treat.	pH	T °C	NaCl (g/l)	Nitrite (ppm)	No. of data points	No. of treat.
6.75	10	15	150	6	59	7.5	19	0	100	24	89
6.75	10	35	50	6	60	7.5	19	0	200	27	90
6.75	10	35	150	7	61	7.5	19	0	1000	27	91
6.75	19	0	0	30	62	7.5	19	25	100	7	92
6.75	28	35	50	7	63	7.5	19	45	0	24	93
6.75	28	35	150	7	64	7.5	19	45	50	24	94
6.8	28	15	50	7	65	7.5	19	45	100	18	95
6.8	28	15	150	6	66	7.5	19	45	200	18	96
7	5	0	0	11	67	7.5	19	45	1000	16	97
7	5	0	200	10	68	7.5	28	0	0	105	98
7	5	5	0	7	69	7.5	28	45	0	18	99
7	5	5	200	6	70	7.5	28	45	50	18	100
7	10	0	0	13	71	7.5	28	45	100	18	101
7.2	5	0	0	13	72	7.5	28	45	200	18	102
7.2	19	0	0	16	73	7.5	28	45	1000	21	103
7.25	5	0	0	8	74	7.5	37	0	0	19	104
7.25	10	0	0	13	75	7.5	37	0	50	21	105
7.5	5	0	0	31	76	7.5	37	0	100	20	106
7.5	5	0	50	24	77	7.5	37	0	200	21	107
7.5	5	0	100	24	78	7.5	37	0	1000	20	108
7.5	5	0	200	16	79	7.5	37	45	0	14	109
7.5	5	0	1000	9	80	7.5	37	45	50	15	110
7.5	5	45	0	24	81	7.5	37	45	100	15	111
7.5	5	45	50	24	82	7.5	37	45	200	15	112
7.5	5	45	100	24	83	7.5	37	45	1000	10	113
7.5	5	45	200	15	84	8	5	0	0	6	114
7.5	5	45	1000	9	85	8	5	0	200	10	115
7.5	10	0	0	13	86	8	5	5	0	14	116
7.5	19	0	0	51	87	8	8	0	0	8	117
7.5	19	0	50	21	88	8	8	50	0	19	118

APPENDIX C

STANDARD ERROR ANALYSIS

After the nonlinear regression was performed to the broth-based data, the asymptotic standard error (SE) of each data set was obtained as described in chapter 5, section 5.2.4. A trend or significant relationship between SE and experimental conditions (pH, temperature, salt, or nitrite), time, microbial counts, and treatments (specific combination of the experimental variables) was assessed (aerobic conditions).

An analysis of variance for SE versus pH, temperature, salt, or nitrite, showed that, assuming there was no interaction between the variables, only pH had a significant influence on SE (Table C.1).

TABLE C.1. Effects tests for the experimental variables.

Variable	*P* value
pH	0.0017
T °C	0.1346
NaCl (g/L)	0.1952
Nitrite (ppm)	0.3796

An analysis of variances with the same variables, but including interactions showed that T^2 and pH^2 had a significant influence on SE (Table C.2).

TABLE C.2. Effects test for the experimental variables with interactions.

Source	*P* value
pH	0.1516
T °C	0.1066
NaCl (g/L)	0.0741
Nitrite (ppm)	0.1889
pH×pH	*0.0215*
pH× T °C	0.0677
T °C × T °C	*0.0005*
pH×NaCl (g/L)	0.3955
T °C ×NaCl (g/L)	0.3137
NaCl (g/L) ×NaCl (g/L)	0.5090
pH×Nitrite (ppm)	0.4696
T °C ×Nitrite (ppm)	0.7862
NaCl (g/L) ×Nitrite (ppm)	0.9335
Nitrite (ppm) ×Nitrite (ppm)	0.3758

No significant relationship was found between treatment and SE, meaning that specific combination of the variables did not affect SE.

The analysis to find the relationship between SE versus time and microbial counts was done separately because the experimental variables do not change within the same data set.

Both time and microbial counts had a significant influence ($P < 0.0001$) on the SE, for all treatments.

To illustrate the relationship of SE with time and microbial counts, treatment No. 1 was chosen as an example, because of its high number of replications (19 data sets, table C.3).

Additionally, it was found that SE had a unique trend as a function of time. SE slightly increased in value at the middle of the time period, decreasing at the beginning and end of the period (Fig. C.1). The same trend was found for all treatments.

TABLE C.3. Data sets included in treatment No. 1.

Data No.	Time (h)	Log (CFU/ml)	SE
564	0	1.97	1.1987
564	3	1.92	1.1989
564	20	2.76	1.1998
564	24	2.76	1.2
564	27	2.92	1.2001
564	44	3.98	1.2003
564	48	4.21	1.2003
564	51	4.39	1.2003
564	69	5.44	1.2002
564	75	5.97	1.2001
564	93	7.2	1.2002
564	99	7.61	1.2003
564	121	8.39	1.2012
565	0	3.59	1.1966
565	24	3.6	1.1974
565	48	3.35	1.1976
565	72	4.6	1.1975
565	96	5.08	1.1975
565	168	7.63	1.2006
565	216	8	1.2028
565	264	8.32	1.203
565	336	7.64	1.2005
566	0	3.61	1.1966
566	24	3.66	1.1974
566	48	3.74	1.1976
566	72	4.6	1.1975
566	96	5.38	1.1975
566	168	7.68	1.2005
566	216	8.29	1.2027
566	264	8.14	1.203
566	336	7.64	1.2005
567	0	3.63	1.1965
567	24	3.55	1.1973
567	48	3.65	1.1976
567	72	4.54	1.1974
567	96	5.31	1.1975
567	168	7.61	1.2005
567	216	6.96	1.2026
567	264	8.29	1.2029
567	336	7.72	1.2004
568	0	3.63	1.1965
568	24	3.65	1.1973
568	48	3.74	1.1976
568	72	4.48	1.1974
568	96	5.43	1.1975
568	168	7.69	1.2005
568	216	8.31	1.2026
568	264	8.26	1.2029
568	336	7.64	1.2004
569	0	3.56	1.1966
569	24	3.57	1.1974
569	48	3.56	1.1977
569	72	4.25	1.1975
569	96	5.24	1.1976
569	168	7.23	1.2006
569	216	8.21	1.2029
569	264	8.13	1.2031
569	336	7.7	1.2006
570	0	3.53	1.1967
570	24	3.55	1.1975
570	48	3.49	1.1977
570	72	4.4	1.1976
570	96	5.07	1.1976
570	168	7.15	1.2007
570	216	8.19	1.203
570	264	8.09	1.2032
570	336	7.84	1.2007
571	0	3.47	1.1967
571	24	3.49	1.1976
571	48	3.55	1.1978
571	72	4.06	1.1977
571	96	4.87	1.1977
571	168	7.18	1.2009
571	216	8.15	1.2032
571	264	8.1	1.2034
571	336	7.77	1.2008
572	0	3.26	1.197
572	24	3.35	1.1979
572	48	3.4	1.1981
572	72	4.19	1.198
572	96	4.76	1.198
572	168	6.73	1.2014
572	216	7.86	1.2039
572	264	8.27	1.2041
572	336	7.88	1.2014
573	0	3.26	1.197
573	24	3.31	1.1979
573	48	3.44	1.1981
573	72	4.13	1.198
573	96	4.81	1.198
573	168	6.67	1.2014
573	216	7.66	1.2039
573	264	8.13	1.2041
573	336	7.8	1.2014
574	0	3.2	1.197
574	24	3.26	1.198
574	48	3.4	1.1982
574	72	4.1	1.1981
574	96	4.92	1.1981
574	168	6.8	1.2016
574	216	8.23	1.2041
574	264	8.15	1.2043
574	336	7.7	1.2015
575	0	2.01	1.1986
575	3	2.27	1.1988
575	20	2.63	1.1998
575	24	2.69	1.1999
575	27	2.85	1.2
575	44	3.99	1.2003
575	48	4.93	1.2003
575	51	4.4	1.2003
575	69	5.39	1.2001
575	75	5.9	1.2001
575	93	7.15	1.2001
575	99	7.61	1.2002
575	121	8.35	1.2011
576	0	4.29	1.1958
576	3	4.06	1.1959
576	20	3.63	1.1964
576	24	4.93	1.1965
576	27	5.01	1.1965
576	44	5.96	1.1966
576	48	6.24	1.1966
576	69	8	1.1966
576	75	8.09	1.1965
576	93	8.54	1.1966
576	99	8.58	1.1966
576	121	8.25	1.1971

TABLE C.3. Continuation.

Data No.	Time (h)	Log (CFU/ml)	SE	Data No.	Time (h)	Log (CFU/ml)	SE
577	0	3.97	1.1962	580	0	3.05	1.1972
577	3	4.02	1.1963	580	6	2.99	1.1975
577	20	3.55	1.1968	580	24	3.08	1.1982
577	24	4.74	1.1969	580	48	3.09	1.1985
577	27	4.96	1.1969	580	54	3.26	1.1984
577	44	5.92	1.1971	580	72	3.55	1.1983
577	48	5.94	1.1971	580	78	3.65	1.1983
577	69	7.77	1.197	580	96	3.73	1.1984
577	75	7.96	1.197	580	100	3.74	1.1984
577	93	8.52	1.197	580	192	4.6	1.2035
577	99	8.71	1.197	580	240	5.16	1.205
577	121	8.3	1.1975	580	336	7.16	1.2019
578	0	3.01	1.1973	580	408	7.77	1.1982
578	6	3.12	1.1976	580	504	8.16	1.1951
578	24	3.14	1.1983	581	0	3.61	1.1966
578	48	3.25	1.1985	581	24	3.67	1.1974
578	54	3.33	1.1985	581	48	3.55	1.1976
578	72	3.53	1.1984	581	72	4.1	1.1975
578	78	3.63	1.1984	581	96	4.51	1.1975
578	96	3.75	1.1984	581	168	6.97	1.2005
578	100	3.83	1.1985	581	216	8.2	1.2027
578	192	4.74	1.2036	581	264	8.31	1.203
578	240	5.33	1.2052	581	336	7.97	1.2005
578	336	7.54	1.202	582	0	3.58	1.1966
578	408	7.93	1.1983	582	24	3.54	1.1974
578	504	8.04	1.1951	582	48	3.64	1.1976
579	0	3.05	1.1972	582	72	4.06	1.1975
579	6	2.98	1.1975	582	96	4.89	1.1976
579	24	2.85	1.1982	582	168	7.39	1.2006
579	48	3.13	1.1985	582	216	8.3	1.2028
579	54	2.98	1.1984	582	264	8.35	1.2031
579	72	3.42	1.1983	582	336	7.76	1.2005
579	78	3.55	1.1983				
579	96	3.64	1.1984				
579	100	3.69	1.1984				
579	192	4.63	1.2035				
579	240	4.99	1.205				
579	336	7.1	1.2019				
579	408	7.77	1.1982				
579	504	7.94	1.1951				

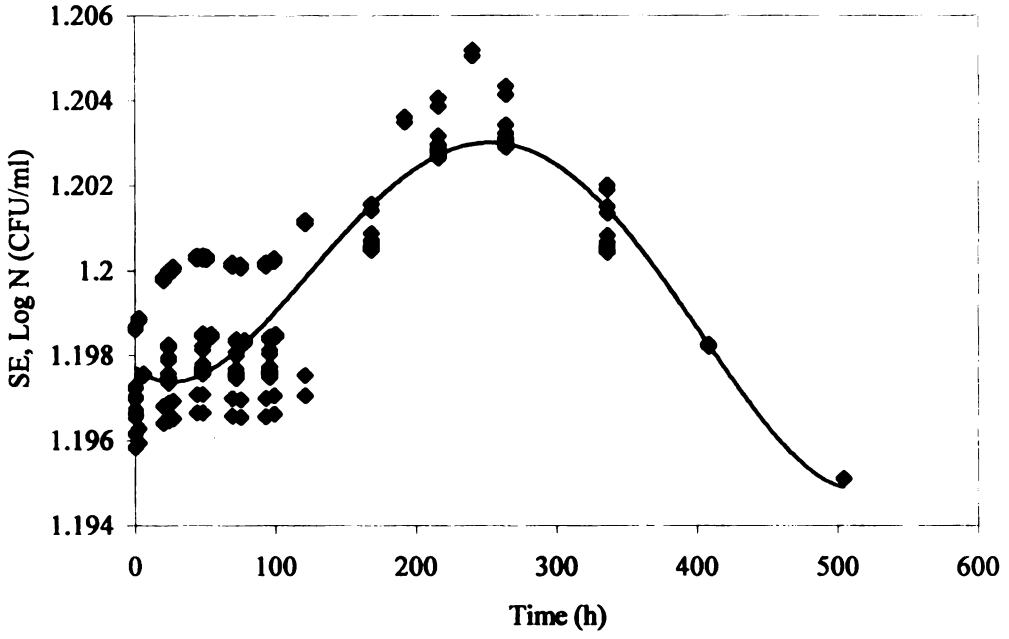

FIGURE C.1. Standard error (SE) versus time, treatment 1 for *L. monocytogenes* growth in broth (pH= 4.5, T= 19 °C, salt= 0 g/l, and nitrite= 0 ppm).

APPENDIX D

D.1 COMPARISON OF EXPERIMENTAL VARIABILITY BETWEEN BROTH AND MEAT-BASED DATA

In order to perform this analysis on the same basis, broth and meat-based data that had the same number of treatments and the same number of replications within those treatments, were randomly selected.

Three different treatments with two replications for each one were selected from the *L. monocytogenes* broth-based growth data, anaerobic conditions (Table D.1.1). The experimental variability was calculated as described in Chapter 5, section 5.2.3, and was found to be 0.05 log(CFU/ml).

L. monocytogenes growth data in cooked chicken were obtained from ComBase. Three different treatments with two replications for each one (anaerobic conditions) were selected (Table D.1.2). Again the experimental variability was calculated as described in Chapter 5, section 5.2.3, which was found to be 0.97 log(CFU/ml).

The experimental variability due to replications was approximately 95% higher for the food-based data than for the broth-based data.

TABLE D.1.1. Broth-based *L. monocytogenes* growth data.

Data set No.	Time (h)	Log (CFU/ml)	Treat. No.	pH	T °C	NaCl (g/l)	Nitrite (ppm)
378	0	2.94	33	6	28	0	200
378	3	3.14	33	6	28	0	200
378	7	3.35	33	6	28	0	200
378	24	5.23	33	6	28	0	200
378	48	8.33	33	6	28	0	200
378	54	8.33	33	6	28	0	200
379	0	3.01	33	6	28	0	200
379	3	3.15	33	6	28	0	200
379	7	3.46	33	6	28	0	200
379	24	5.38	33	6	28	0	200
379	48	8.48	33	6	28	0	200
379	54	8.37	33	6	28	0	200
234	0	3.72	45	6	37	45	0
234	3	3.72	45	6	37	45	0
234	7	4.69	45	6	37	45	0
234	24	9.15	45	6	37	45	0
234	27	9.11	45	6	37	45	0
234	31	9.23	45	6	37	45	0
234	48	9.07	45	6	37	45	0
234	54.5	8.26	45	6	37	45	0
235	0	3.67	45	6	37	45	0
235	3	3.66	45	6	37	45	0
235	7	4.59	45	6	37	45	0
235	24	8.42	45	6	37	45	0
235	27	8.26	45	6	37	45	0
235	31	8.92	45	6	37	45	0
235	48	8.69	45	6	37	45	0
235	54.5	8.76	45	6	37	45	0

TABLE D.1.1. Continuation

Data set No.	Time (h)	Log (CFU/ml)	Treat. No.	pH	T °C	NaCl (g/l)	Nitrite (ppm)
246	0	3.64	49	6	37	45	1000
246	3	3.52	49	6	37	45	1000
246	7	3.56	49	6	37	45	1000
246	24	3.21	49	6	37	45	1000
246	27	3.22	49	6	37	45	1000
246	31	3.66	49	6	37	45	1000
246	48	4	49	6	37	45	1000
246	54.5	4.13	49	6	37	45	1000
246	72	4.53	49	6	37	45	1000
246	79	4.85	49	6	37	45	1000
247	0	3.63	49	6	37	45	1000
247	3	3.57	49	6	37	45	1000
247	7	3.48	49	6	37	45	1000
247	24	3.21	49	6	37	45	1000
247	27	3.42	49	6	37	45	1000
247	31	3.44	49	6	37	45	1000
247	48	4.03	49	6	37	45	1000
247	54.5	4.29	49	6	37	45	1000
247	72	5.11	49	6	37	45	1000
247	79	5.21	49	6	37	45	1000

TABLE D.1.2. Meat-based *L. monocytogenes* growth data.

Key to ComBase	Time (h)	Log (CFU/ml)	Treat. No.	pH	T °C	NaCl (g/l)	Nitrite (ppm)
J232_Lm	0	2.5	1	3.5	6	5	0
J232_Lm	96	3.1	1	3.5	6	5	0
J232_Lm	168	3.4	1	3.5	6	5	0
J232_Lm	264	4.9	1	3.5	6	5	0
J232_Lm	360	5.9	1	3.5	6	5	0
J232_Lm	432	6.7	1	3.5	6	5	0
J232_Lm	552	8	1	3.5	6	5	0
J232_Lm	600	8.4	1	3.5	6	5	0
J232_Lm	696	9.2	1	3.5	6	5	0
J232_Lm	768	9.6	1	3.5	6	5	0
J232_Lm	840	9.4	1	3.5	6	5	0
J233_Lm	0	2.5	1	3.5	6	5	0
J233_Lm	96	2.7	1	3.5	6	5	0
J233_Lm	168	2.7	1	3.5	6	5	0
J233_Lm	264	2.5	1	3.5	6	5	0
J233_Lm	360	4	1	3.5	6	5	0
J233_Lm	432	4.9	1	3.5	6	5	0
J233_Lm	552	6.4	1	3.5	6	5	0
J233_Lm	600	6.9	1	3.5	6	5	0
J233_Lm	696	7.8	1	3.5	6	5	0
J233_Lm	840	9	1	3.5	6	5	0
J234_Lm	0	2.5	2	6.5	6	5	0
J234_Lm	96	5.5	2	6.5	6	5	0
J234_Lm	168	7.4	2	6.5	6	5	0
J234_Lm	264	10	2	6.5	6	5	0
J234_Lm	336	10.3	2	6.5	6	5	0
J235_Lm	0	2.7	2	6.5	6	5	0
J235_Lm	96	3.9	2	6.5	6	5	0
J235_Lm	168	6.5	2	6.5	6	5	0
J235_Lm	264	7.7	2	6.5	6	5	0
J235_Lm	336	9.8	2	6.5	6	5	0
J235_Lm	432	9.8	2	6.5	6	5	0
J235_Lm	504	10	2	6.5	6	5	0

TABLE D.1.2. Continuation.

Key to ComBase	Time (h)	Log (CFU/ml)	Treat. No.	pH	T °C	NaCl (g/l)	Nitrite (ppm)
J236_Lm	0	2.5	3	10	6	5	0
J236_Lm	72	4.2	3	10	6	5	0
J236_Lm	120	6.4	3	10	6	5	0
J236_Lm	240	9.2	3	10	6	5	0
J236_Lm	288	10.1	3	10	6	5	0
J236_Lm	360	10.2	3	10	6	5	0
J237_Lm	0	2.4	3	10	6	5	0
J237_Lm	72	2.8	3	10	6	5	0
J237_Lm	120	4.5	3	10	6	5	0
J237_Lm	240	7.3	3	10	6	5	0
J237_Lm	264	9.1	3	10	6	5	0
J237_Lm	360	9.6	3	10	6	5	0
J237_Lm	480	9.5	3	10	6	5	0

D.2 STANDARD ERROR OF PREDICTION AND ROBUSTNESS INDEX VALUES OBTAINED AFTER NON-SIGNIFICANT TERMS WERE ELIMINATED

TABLE D.2

Aerobic		Without non-significant terms		Without non-significant terms
Data No	SEP log(CFU/ml)	SEP log(CFU/ml)	RI	RI
1	1.8920	*1.1821*	1.4015	*0.8956*
2	1.8193	*1.2326*	1.3476	*0.9338*
3	2.1808	*0.9916*	1.6154	*0.7512*
4	2.4599	*1.3240*	1.8221	*1.0030*
5	*0.2097*	0.7492	*0.1553*	0.5676
6	*0.6434*	0.9811	*0.4766*	0.7433
7	1.4425	*0.6687*	1.0685	*0.5066*
8	*0.7040*	1.0798	*0.5215*	0.8180
9	*0.9086*	1.9229	*0.6731*	1.4568
10	*1.0226*	1.2218	*0.7575*	0.9256
11	*1.1478*	1.3936	*0.8502*	1.0558
12	1.9816	*0.8186*	1.4678	*0.6202*
13	*1.0640*	1.4165	*0.7882*	1.0731
14	*1.1691*	1.4804	*0.8660*	1.1215
15	0.9486	*0.8692*	0.7027	*0.6585*
16	1.1806	*1.0934*	0.8745	*0.8284*
17	*2.5245*	2.5351	*1.8700*	1.9205
18	*1.5938*	1.6042	*1.1806*	1.2153
19	1.1776	*0.7699*	0.8723	*0.5833*
20	1.5308	*1.0991*	1.1339	*0.8326*
21	1.8256	*1.0491*	1.3523	*0.7948*
22	3.0180	*2.2340*	2.2356	*1.6924*
23	3.0185	*2.2407*	2.2360	*1.6975*

APPENDIX E

SCRIPTS USED FOR NONLINEAR REGRESSION AND DATA ANALYSIS IN JMP

E.1. SCRIPT USED FOR NONLINEAR REGRESSION.

This script was applied to each data set in order to perform nonlinear regression, with second derivative, and the results were given in a separate table:

" column5 << set formula (Parameter({B = 1, M = 10, C = 5}, C * Exp(-Exp(-B * (:Time - M)))));nlin=Nonlinear(Y(:Name("Log(N/No)")), X(:Column 5), Second Deriv Method(1),finish, plot (1), save estimates);

```
nParameters = n row(report(nlin)["Solution"][table box(2)][1] << get as matrix);
    errorTable << add row(1);
    nRowsErrorTable = n row(errorTable);
    colSSE[nRowsErrorTable] = report(nlin)["Solution"][table box(1)][1][1];
    colDFE[nRowsErrorTable] = report(nlin)["Solution"][table box(1)][2][1];
    colMSE[nRowsErrorTable] = report(nlin)["Solution"][table box(1)][3][1];
    colRMSE[nRowsErrorTable] = report(nlin)["Solution"][table box(1)][4][1];
    for(j = 1, j <= nParameters, j++,
     parameterTable << add row(1);
     nRowsParamTable = n row(parameterTable);
     colParameter[nRowsParamTable] = report(nlin)["Solution"][table
box(2)][1][j];
     colEst[nRowsParamTable] = report(nlin)["Solution"][table box(2)][2][j];
     colApproxStdErr[nRowsParamTable] = report(nlin)["Solution"][table
box(2)][3][j];
     colLowerCL[nRowsParamTable] = report(nlin)["Solution"][table
box(2)][4][j];
     colUpperCL[nRowsParamTable] = report(nlin)["Solution"][table
box(2)][5][j]); ".
```

E.2. SCRIPT USED FOR NONLINEAR REGRESSION WITHOUT SECOND DERIVATIVE.

"col=new column ("Model"); col<<set formula (Parameter({B = 0.009, M = 80}, :Log C[1] + (9.57 - :Log C[1]) * Exp(-Exp(-B * (:Name("Time (H)") - M)))))".

E.3. SCRIPT USED FOR NONLINEAR REGRESSION WITH SECOND DERIVATIVE.

"col=new column ("Model"); col<<set formula (Parameter({B = 0.02, M = 114}, :Log C[1] + (9.57 - :Log C[1]) * Exp(-Exp(-B * (:Name("Time (H)") - M))))); Nonlinear(Y(:Log C), X(:Model), Second Deriv Method(1));".

E.4 SCRIPT USED FOR REITERATIVE ANALYSIS.

"for (i=1, i<=9, i++, column5 << set formula (Parameter({B = 0.02, M = 114, C = 5}, C * Exp(-Exp(-B * (:Time - M)))));nlin=Nonlinear(Y(:Name("Log(N/No)")), X(:Column 5), Second Deriv Method(1),finish, plot (1), save estimates;report (nlin) ["Solution"][table box (1)] << make into data table; report (nlin) ["Solution"][table box (2)] << make into data table));".

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