VALIDATION OF A PREDICTIVE MODEL FOR *SALMONELLA* GROWTH IN GROUND BEEF UNDER MULTIPLE CYCLES OF TEMPERATURE CHANGE

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ABSTRACT OF THE THESIS

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The advances of microbial predictive modeling techniques facilitate Quantitative Microbial Risk Assessment (QMRA), which can extensively contribute to the food safety in meat industry in the United States. However, the challenges of predictive modeling cannot be neglected, an example of which is the intermediately induced lag phase. This research sought to expose this problem by validation of a predictive model for *Salmonella* growth on ground beef. *Salmonella* is an important pathogen related to beef consumption. ComBase is a predictive database that can be used to predict microbial behaviors with a variety of environmental conditions in a dynamic manner. The literature review part of this thesis discusses general properties of *Salmonella*, the implication of Salmonella in beef and beef products, the applications of Hazard Analysis and Critical Control Points (HACCP) and QMRA in meat industry, methods of predictive modeling, challenges and validation of

predictive modeling. The laboratory work of this thesis exposed the fail-safe phenomenon under a fluctuating temperature profile. Ground beef (20% fat) was inoculated with a cocktail composed of five strains of *Salmonella*. The inoculated ground beef was subjected to programmable water bath with different temperature profiles. Enumeration of *Salmonella* was conducted with proper time intervals. The observed Salmonella concentrations were compared ComBase predictions, accuracy and bias factors, as well as final differences between the observed and the predicted were calculated. The results showed that the model was very fail-safe. According to literature review and previous study in our laboratory, we excluded background microflora from the major reasons causing the high deviation. And we speculated that the deviation was mainly resulted from temperature-induced intermediate lag phase.

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I. 1. Introduction to *Salmonella*

Salmonella is a Gram-negative rod-shaped bacterium that can be found in a wide variety of reservoirs (swine, cattle, poultry, etc.). *Salmonella* is a genus of the *Enterobacteriaceae* family and includes two species: *S. bongori* and *S. enterica*. It is reported that *S. enterica* species includes at least 2600 serotypes *(93)*. *Salmonella* can be classified into two general groups: typhoidal and non-typhoidal *Salmonella* based on the clinical syndromes. Typhoidal *Salmonella* includes four serotypes: *Typhi* and *Paratyphi* A-C, which cause systemic illness without diarrhea *(29)*. The other serotypes besides these four are non-typhoidal *Salmonella (29)*.

Salmonellosis is a result of the infection of *Salmonella* and is usually caused by ingestion of *Salmonella*-contaminated foods. *Salmonella* colonize the gastrointestinal tracts in a wide variety of animals, especially the livestock for human consumption *(96)*. *Salmonella* survive passage through the acidic human stomach to invade the mucosa of the small and large intestine, which subsequently triggers the release of proinflammatory cytokines and thereby induces inflammatory reactions *(26)*. The toxins produced by *Salmonella* result in cytoskeletal changes, and interfere with signaling pathways, resulting in disease manifestation *(84)*. *Salmonella* can adjust rapidly to stressful environments (e.g., high osmotic pressure, low pH) and utilize various virulence factors, such as flagella, fimbriae, adhesins/invasins, and effector proteins, to facilitate the invasion process *(24)*. The clinical symptoms of salmonellosis include diarrhea, fever, abdominal

cramps, etc. The dose of *Salmonella* needed to cause illness can be as low as a single cell, and the median dose varies from strain to strain, and can be 15 to 20 cells *(11)*.

Salmonella is a common foodborne pathogen in the United States. The Centers for Disease Control and Prevention (CDC) estimates that *Salmonella* causes 1.2 million of illnesses and 450 deaths every year *(16)*. *Salmonella* also imposes a great economic loss ($3.4 billion) in the U.S. annually *(30)*.Table I.1. summarizes the numbers of outbreaks, illnesses, hospitalizations, and deaths caused by *Salmonella* relative to all foodborne hazards (mostly microbial pathogens) from 1998 to 2014 in the US as recorded by CDC. Among the dozens of different pathogens, *Salmonella* accounts for 12.5%, 17.2%, 50.7%, and 24.8% of all outbreaks, illnesses, hospitalizations, and deaths, respectively. The serotypes of *Salmonella* that are most frequently implicated with outbreaks are Enteritidis, Typhimurium, Newport, and Javiana, which account for approximately 50% of all *Salmonella* illness cases *(16)*.

Table I.1. Numbers of outbreaks, illnesses, hospitalizations, and deaths caused by *Salmonella* or all foodborne hazards (1998-2014) *(16)*.

Pathogen	Outbreaks	Illnesses	Hospitalizations	Deaths
Salmonella	2,273	61,630	6,952	79
Overall	18,211	358,391	13,715	318

I. 2. *Salmonella* and Safety of Beef

Beef is one of the most important foods in the American diet. From 2002 to 2014, U.S. produced in average 25.9 billions pounds of beef every year, worth $75.3

billion *(90)*. Cattle are a natural reservoir for a variety of pathogens such as *Salmonella*, *Campylobacter*, *Listeria*, and *Escherichia coli* O157:H7. *Salmonella* is a major concern of food pathogen in meat industry *(87)*. Barham et al. (2002) reported that *Salmonella* prevalence level in cattle's hides and feces increased from 6% and 18% to 89% and 46%, respectively between feedlot and arrival at the packing facilty *(7)*. Similarly, Beach et al. (2002) reported that contamination of hides of cattle with *Salmonella* increased from about 19% to a 53% during transportation to abattoir *(8)*. The dehiding, evisceration, and rinsing processes in abattoirs may also increase the risk of contaminating the carcass. Once beef is contaminated, any subsequent packaging, transportation, or retail processes that result in temperature abuse increase the risk of illness to consumers.

Surveys have been conducted to assess the prevalence of *Salmonella* on beef in the U.S., some of which are summarized in Table I. 2. 1. One of the most representative studies *(11)* found that the overall prevalence of *Salmonella* in ground beef is 4.2%, and the most common serotypes include Montevideo (21.0%), Anatum (14.8%), and Muenster (8.5%). The other studies found 2.2-58.0% pre-evisceration carcass were positive for *Salmonella (7, 11, 13, 85).*

Another factor contributing to the beef-borne illness is that the cooking practices of the food handlers in households and restaurants are inconsistent and subject to personal preference. The Food Safety Inspection Service under the U.S. Department of Agriculture (USDA-FSIS) has made recommendations for freezing,

refrigeration, defrosting, and cooking ground beef products *(91)*. USDA FSIS recommends that a four-ounce-patty be grilled, boiled, or fried for 3-5 min per side, and the internal temperature needs to reach 160˚F (71.1˚C). However, a recent survey shows that, when cooking beef patties, 51% of consumers choose color as the criterion of doneness, which is not correct since interior color may be affected by many other factors *(50)*. Cooking to "well-done" may also not be the favorite choice for some consumers. The same survey shows that 21% consumers prefer pink interior *(50)*.

Table I. 2. 1. summarizes the outbreaks related to beef or beef products due to *Salmonella* or all pathogens separately from 1998 to 2014 based on the records of CDC. It shows that *Salmonella* is responsible for 10.7% and 16.7% beef-implicated outbreaks and illnesses, respectively.

Concerns about antibiotic-resistant strains of *Salmonella* have risen in recent years. Bosilevac et al. (2009) reported that 0.6% of the *Salmonella* tested in ground beef were multidrug-resistant *(11)*. Schmidt et al. (2015) reported that 7.6% and 0.5% *Salmonella* on hides at processing were resistant to third-generation cephalosporin and nalidxic acid, respectively *(85)*. The antibiotic-resistant property of *Salmonella* contributes to higher risk of infection during treatment, elevated severity of illness, as well as co-selection of higher virulence genes *(87)*.

Table I. 2. 1. Selected studies of *Salmonella* prevalence in beef in U.S.

Source	No. Samples	% positive	Comment
(2)	1,140	1.6%	Bovine lymph nodes used for ground beef
(7)		12.7%	Pre-evisceration carcass samples
(11)	4,136	4.2%	Nationwide ground beef samples in U.S.
(11)	1,995	58%	Pre-evisceration carcass samples
(13)	138	26.9-54.4% *	Pre-evisceration carcass
(85)	184	2.2%	Pre-evisceration carcass samples
(96)	210	4%	Beef samples from 59 stores in greater Washington, DC area

* Values represent four different plants.

Table I. 2. 2. Numbers of outbreaks, illnesses, hospitalizations, and deaths due to beef and beef products caused by *Salmonella* or all foodborne hazards (1998-2014) *(16)*.

Pathogen	Outbreaks	Illnesses	Hospitalizations	Deaths
Salmonella	104	3,242	373	4
Overall	969	19,349	1000	16

I. 3. HACCP and QMRA for Food Safety Assurance

Multiple US agencies collaborate to ensure the food safety of meat and meat products, including Food and Drug Administration (FDA), Center for Disease Control and Prevention (CDC), Environmental Protection Agency (EPA), and Department of Agriculture (USDA). Within USDA, several agencies participate in the regulation, including Animal and Plant Health Inspection Service (APHIS), Food and Nutrition Service (FNS), National Institute of Food and Agriculture (NIFA), and Food Safety Inspection Service (FSIS). Since 1960s, several laws and regulatory programs had been made to improve food safety in the meat industry, including Wholesome Meat

Act of 1967, Voluntary Quality Control Programs, Total Quality Control Programs, Partial Quality Control Programs, and the Federal Meat Inspection Act, etc. *(65)*. The FDA and USDA had made recommendations for the control of salmonellosis due to numerous outbreaks of Salmonellosis during 1960s and 1970s *(61)*. One of the current primary regulatory means to assure the safety of US beef are the food safety audit and inspection led by USDA FSIS. The food safety audit observes whether a food processing plant complies with good food safety practices *(66)*, and more than 70% of US ground beef comes from audited plants *(66)*. The inspection conducted by FSIS is mandatory for slaughter and meat processing plants. There are about 8,000 FSIS personnel working 6,200 federally inspected slaughters and processing facilities *(89)*.

USDA has mandated all slaughter and processing plants to incorporate a new risk-control program, the Hazard Analysis and Critical Control Point (HACCP) system to facilitate reduction of foodborne illness *(64)*. HACCP comprises seven principles: (a) conduct a hazard analysis; (b) identify critical control points; (c) establish critical limits; (d) establish critical control point monitoring requirements; (e) establish corrective actions; (f) establish record keeping procedures; and (g) establish verification procedures for HACCP. The USDA FSIS authorizing regulation for HACCP also mandated several procedures for meat-producing establishments, including Sanitation Standard Operating Procedures (SSOPs), microbial testing for generic *E. coli* and *Salmonella*, and pathogen reduction performance standards for

Salmonella (90). Methods of statistically evaluation over the effectiveness of HACCP have been proposed (*16*, *34*). Although HACCP is widely adopted in a number of both developed and developing countries *(80)*. HACCP may not address the inherent variability of risk during food processing *(34)*. Therefore, quantitative microbial risk assessment (QMRA) was introduced to HACCP to overcome this limitation *(14)*.

QMRA describes the ways that microbial hazards transfer to hosts and causes harms to hosts quantitatively, and generally includes four steps: hazard identification, exposure assessment, does-response assessment, and risk characterization *(44)*. An example of a QMRA project might be helpful to intuitively illustrate how QMRA works. The FSIS has recently published a risk assessment report regarding *Listeria monocytogenes* in Retail Delicatessens (Deli stores) *(1)*, which is a fairly comprehensive project that involves collaboration among multiple agencies. In this project, the behaviors of employees in deli stores were observed and the probabilities and frequencies of these behaviors were then modeled. The types of behaviors are very specific, including hand washing, sanitization of slicer, use of gloves, contact between gloves and non-food surfaces, and so on. Each of these employee behaviors may result in changes in the concentration of the bacteria, which is described by predictive models. There were different types of predictive models, including growth models, inactivation models, and transfer models were used for quantification of bacterial growth in refrigerator, reduction of bacteria due to sanitization on slicer, and number of bacteria transferred from gloves to meat

chub, respectively. Data on number and type of consumers, amount of product consumed, transport time, and dose-response models were available in databases, which can be used to estimate the probability and expected number of illness after consumption of deli products with certain concentration of the bacteria. The expected number of illness was estimated through simulation changing some inputs (e.g., initial concentration of the bacteria, types of deli products), using probabilities and frequencies employees' behaviors obtained from observation, predicted change of bacterial concentration during food handlings as given by predictive models, and using database of consumption data and dose-response models. The utility of certain critical control points can be determined based on the simulation results, which can be then used to modify the HACCP program. For example, the project mentioned above found that pre-slicing ready-to-eat products once per day (in the morning) will significantly increase the risk of illness; and that extra sporadic cleaning does not significantly help reduce the risk *(1)*.

I. 4. Predictive Modeling

Traditionally, quantification of bacterial number relies heavily on laboratory test, which can be costly, time- and labor-consuming. Although rapid detection methods, such as qPCR, have been introduced, they may be expensive or require additional procedures such as enrichment to complete the quantification *(92)*. In contrast, predictive models can make quick assessments at low cost *(61)*. Use of

predictive models is consistent with the idea that microbial testing should not be relied upon as a routine measure for HACCP *(87)*. Predictive modeling is an indispensable part of QMRA. Predictive modeling can help in preliminary hazard analysis, identification and establishment of critical control points *(53)*. Predictive models can also be used to estimate the impact on consumer safety or product quality once problematic products are already released to market *(59)*. Predictive models include models for cross-contamination, inactivation models for sanitation or sterilization, growth models for bacterial growth, etc. The research in thesis focuses on growth models.

A growth model can be subdivided into two categories: growth/no growth models and quantitative models. The growth/no growth model determines a boundary between growth and no growth under the effects of multiple factors (e.g. temperature, pH, water activity). It is a useful tool to help design hurdle techniques for microbial control. Logistic regression is the most commonly used tool for the development of growth/no growth model, which is shown in Eq. (I. 4. 1). The right hand part of the equation, $f(temp, pH, a_w \dots)$, can be linear or non-linear, as long as the regression coefficients are statistically significant. An example of growth/no growth model for *Salmonella* in liquid medium is shown in Eq. (I. 4. 2) *(41)*.

$$\ln\left(\frac{P}{1-P}\right) = f(temp, pH, a_w \dots) \qquad \text{(I. 4. 1)}$$

where P is the probability of growth, and 1-P is the probability of no growth.

$$\ln\left(\frac{P}{1-P}\right) = a_1 + a_2 T + a_2 pH + a_3 bw + a_4 T \cdot pH + a_5 T \cdot bw + a_6 pH \cdot bw + a_7 T \cdot pH \cdot bw + a_8 T^2 + a_9 pH^2 + a_{10} bw^2 \quad \text{(I. 4. 2)}$$

where $a_1, a_2 \ldots a_{10}$ are regression coefficients; T is temperature; bw is the square root of the complement of a_w.

Development of a quantitative growth model is typically composed of three stages: primary model, secondary model, and tertiary model. A primary model tells how the bacterial population changes at certain condition as time elapses. A secondary model can be built based on primary models, it describes how the parameters of growth (e.g., exponential growth rate, lag time duration) evolve as intrinsic (e.g., water activity, pH, concentration of solutes) or extrinsic factors (e.g., temperature, atmosphere gas composition) change. A tertiary model can be thought of as the computer interface that incorporates the developed secondary models, which enables users to input initial bacterial population and various intrinsic or extrinsic factors and then obtain the predicted bacterial population.

Bacterial growth is commonly thought to be composed of four phases, which are lag phase, exponential phase, stationary phase, and death phase. Death phase is usually excluded from a growth model. A primary model is to fit the growth data of a bacterium using sigmoidal-shape curve that mimics the growth curve using non-linear regression techniques. The commonly used mathematical functions for

primary models include but are not limited to modified Gompertz (100), logistic model *(35)*, and Baranyi model *(4)*, which are shown in Eq. (I. 4. 3), Eq. (I. 4. 4), and Eq. (I. 4. 5), respectively. The Baranyi model expresses the gradual improvement in the physiological state and some believe therefore that this model makes more biological sense *(61)*. The development of a secondary model for *Salmonella* on raw ground beef using four different primary models (logistic, modified Gompertz, Baranyi, and Huang model) revealed that there was no significant difference in terms of goodness of fit *(38)*. Another study comparing different primary models (three-phase linear, Baranyi, and Gompertz model) reported that, despite of better robustness of a three-phase linear model, the three models had similar goodness of fit *(38)*.

$$x(t) = x_0 + (x_{max} + x_0)\exp\left[-\exp[-B(t - M)]\right] \quad \text{(I. 4. 3)}$$

$$x(t) = x_0 + \frac{x_{max} - x_0}{1+\exp\left[-B(t-M)\right]} \quad \text{(I. 4. 4)}$$

where x_0 is the initial $\log_{10}$(CFU/g) concentration of the bacterium; $x(t)$ is the $\log_{10}$(CFU/g) concentration at time t; B is the maximum relative growth rate (h^{-1}) at $t=M$; M (h) is the time when the absolute growth rate reaches the maximum.

$$y(t) = y_0 + \mu_{max}F(t) - \ln\left(1 + \frac{\exp[\mu_{max}F(t)]-1}{\exp[y_{max}-y_0]}\right) \quad \text{(I. 4. 5)}$$

where $y(t)$ is ln(CFU/g) concentration of the bacterium at time t; y_0 is the initial concentration ln(CFU/g); μ_{max} is the maximum specific growth rate; v is rate of increase of the limiting substrate and is assumed to equal to μ_{max}; h_0 is assumed to be constant and equal to $\mu_{max}\lambda$; $\lambda=M-1/B$; $F(t) = t + \frac{1}{v}\ln\left(e^{-vt} + e^{-h_0} - e^{(-vt-h_0)}\right)$.

The specific growth rate (r_{max}) from Baranyi model can be obtained from $\mu_{max}/ln10$, whereas the r_{max} from modified Gompertz model and logistic model can be derived from Eq. (I. 4. 6) and Eq. (I. 4. 7) *(38)*.

$$r_{max} = \frac{x_{max} - x_{min}}{e} \times B \quad \text{(I. 4. 6)}$$

$$r_{max} = \frac{x_{max} - x_{min}}{4} \times B \quad \text{(I. 4. 7)}$$

The lag phase duration (λ) derived from modified Gompertz model and Baranyi model can be calculated by Eq. (I. 4. 8), and the λ for logistic model is calculated from Eq. (I. 4. 9) *(38)*.

$$\lambda = M - \frac{1}{B} \quad \text{(I. 4. 8)}$$

$$\lambda = M - \frac{2}{B} \quad \text{(I. 4. 9)}$$

As noted above, a secondary model is used to map conditional factors to the growth kinetics, i.e., growth rate or initial lag phase duration. Although conditional factors can be many, the simplest secondary models use only temperature because temperature is the primary factor affecting bacterial growth. Thus modeling the effect of temperature on growth rates or initial lag phase duration enables a dynamic quantification during a series of food handlings that are subject to temperature changes. One of the mostly used secondary models for temperature is modified Ratkowsky model *(98)* and is shown in Eq. (I. 4. 10). Primary models are typically built under isothermal conditions, thus a series of data points representing

different temperature are needed to develop a secondary model.

$$r_{max} = a(T - T_{min})^2(1 - \exp[b(T - T_{max})]) \quad \text{(I. 4. 10)}$$

where T is temperature (˚C); T_{min} and T_{max} are the minimum and maximum growth-allowed temperatures; a and b are constants.

Eq. (I. 4. 8) and (I. 4. 9) imply that lag phase duration is related to growth rates, thus it can be computed once the specific growth rates are derived from Eq. (I. 4. 5).

Table I. 4. 1. lists some growth models of *Salmonella* on either laboratory media or food products. Table I. 4. 2. shows some growth models for different bacteria on beef or beef products. A laboratory media-based model is more versatile that it can be used to predict growth on a relatively wide range of food products; whereas food-based model is more specific and can only be used to predict similar food products. Studies comparing the performances of the two are limited.

Tertiary models make primary and secondary models more accessible and user-friendly to non-expert users. Some tertiary models developed by governmental agencies or research institutes include Pathogen Modeling Program (Food Safety Research Unit, USDA), Food MicroModel (Ministry of Agriculture, UK), Growth Predictor (Institute of Food Research, UK), Seafood Spoilage and Safety Predictor (Danish Institute for Fisheries Research), and ComBase (Food Standards Agency, Institute of Food Research, UK; USDA-Agricultural Research Service, US) *(33)*. ComBase (http://www.combase.cc/) is a powerful tool in predictive microbiology. It can predict the growth of 14 microorganisms and thermal inactivation of 7

pathogens under dynamic temperature profile, as well as non-thermal survival of 2 pathogens. The growth predictor allows inputs of initial level, physiological state, temperature, pH, and water activity/sodium chloride concentration. Our research focuses on the validation of the ComBase model for *Salmonella*.

Table I. 4. 1. Development of *Salmonella* growth models on different media.

Source	Medium	Parameters
(27)	Egg yolk	Temp
(31)	Liquid eggs	Temp
(37)	Chicken	Temp
(45)	Laboratory media *	Temp, pH, ethanol conc., a_w
(40)	Cantaloupe	Temp
(46)	Scrambled egg mix	Temp
(47)	Lab medium	A_w, pH
(67)	Cooked chicken	Temp
(68)	Ground chicken breast	Temp
(69)	Ground chicken	Temp
(70)	Chcken frankfurters	Temp
(71)	Raw chicken skin	Temp
(72)	Chicken at low temperature	Temp
(74)	Laboratory media	Temp, NaCl, pH
(77)	Baby spinach leaves	Temp
(83)	Ready-to-eat lettuce	Temp
(86)	Liquid whole egg	Temp
(92)	Ground sterile pork	Temp
(97)	Chicken meat	Temp, a_w

* growth/no growth model.

Table I. 4. 2. Growth models of different bacteria on beef or beef products.

Source	Bacterium	Product	Parameters
(2)	*Salmonella, Satphylococcus aureus, E. coli O157:H7*	Raw beef	Temp
(4)	*Klebsiella, Pseudomonas, E. coli*	Ground beef	Temp, gaseous permeability,

			pH
(23)	*Salmonella*	Sterile lean beef	Temp
(7)	*Latic acid bacteria, Enterobacteriaceae, Pseudomonas, psychrotrophic bacteria*	Refrigerated beef	Temp
(39)	*Clostridium perfringens from spores*	Cooked cured beef	Temp
(36)	*Clostridium perfringens*	Cooked uncured beef	Temp
(12)	*Salmonella*	Ground beef	Temp
(48)	*Salmonella*	Minced beef	Temp, inoculum size
(43)	Spoilage bacteria	Ground beef	Temp, pH

I. 5. Challenges and Validation of Predictive Models

Challenges in the development of predictive models have been noted. The standard method for data collection of building primary models is total viable count, which can be quite labor-intensive, requiring in some cases more than a hundred growth curves be sampled *(53)*. More automated methods are claimed to have a greater risk of misinterpretation *(53)* and thus may not replace total viable count. Moreover, the application of predictive models in QMRA was questioned by Nauta (2002), who pointed out that predictive models should ideally give probability distributions, instead of point estimates, to better serve the needs of QMRA *(63)*, since QMRA use probabilities and give results in the form of probabilities, whereas more predictive models only gives deterministic answers.

A variety of factors may influence the accuracy of prediction on food products, such as background microflora. It has been noted that microbial competition

appears to limit the maximum population density (MPD) of the species present in the system; meanwhile it does not seem to significantly affect the lag time or growth rate of each species *(19)*. This MPD limiting phenomenon is called the Jameson effect *(82)*. For example, the growth rate of *L. monocytogenes* co-cultured with natural biofilm microflora was not reduced, but the growth of *L. monocytogenes* terminated as the microflora reached the stationary phase *(28)*. Ignoring the Jameson effect can lead to an overestimation of the modeled microorganism by a large scale. For example, it was found that unconstrained exponential growth models (i.e., where the background microflora is ignored) resulted in counts that were 10^6-10^7 higher per serving of meat products than a constrained model *(17)*. It has been demonstrated that microbial interaction is more predominant than other selective factors. For example, the simultaneous growth of *E. coli* O157:H7 and ground beef background microflora the followed Jameson effect, but were barely impacted by selective factors (i.e., bile salts, novobiocin, high temperature) *(95)*. By modeling the ratio between *L. innocua* and *L. monocytogenes*, researchers found that interspecies inhibitory interaction, rather than growth advantage of *L. innocua*, is the reason why *Listeria innocua* overgrows in an enrichment broth designed for *L. monocytogenes (20)*. Similarly, the maximum population density of *L. monocytogenes* Scott A in broth was suppressed by the presence of either the bacteriocin- or non-bacteriocin producing *Carnobacterium piscicola* strain *(15)*. A study of the competitive growth of *L. monocytogenes* with *Lactobacillus sakei* MN in meat gravy discovered that the

inter-species competition changes with temperature *(78)*.

Methods have been proposed to model the co-culture between competitive species. The Lotka-Volterra model Eq. (I. 5. 1) is used to model two-species competition *(76)*. Another generally used model is Jameson-effect model, which is shown in Eq. (I. 5. 2) *(19)*. Vereecken et al. (2000) had proposed graphical and analytical approaches to evaluate the microbial interaction models between two-species *(94)*.

$$\begin{cases} \frac{dN_1}{dt} = r_1 N_1[1 - \frac{N_1 + \alpha_{12} N_2}{TMD_1}] \\ \frac{dN_2}{dt} = r_2 N_2[1 - \frac{N_2 + \alpha_{21} N_1}{TMD_2}] \end{cases} \quad \text{(I. 5. 1)}$$

where N_1 and N_2 are the population densities of species 1 and 2; r_1 and r_2 are the monspecific growth rates; TMD_1 and TMD_2 are the maximum population densities under monospecific condition; α_{12} and α_{21} are the interspecific competition coefficients.

$$\begin{cases} \frac{1}{N_1(t)} \frac{dN_1(t)}{dt} = \mu_{max1} \cdot \alpha_1(t) \cdot f(t) \\ \frac{1}{N_2(t)} \frac{dN_2(t)}{dt} = \mu_{max2} \cdot \alpha_2(t) \cdot f(t) \end{cases} \quad \text{(I. 5. 2)}$$

where $\alpha(t) = \begin{cases} 0, if\ t < lag\ time \\ 1, if\ t \geq lag\ time \end{cases}$; $f(t)$ is inhibition function, an example of inhibition function is $f(t) = (1 - \frac{N(t)}{N_{max}})$; and $N_1(t)$ and $N_2(t)$ are the population densities of species 1 and 2, respectively.

The lag time duration not only reflects the environmental conditions, but also imparts the physiological state of the inoculum. Although a few predictive models (e.g., ComBase) do take physiological state of the inoculum as an independent

variable, it is difficult to link the temperature history and inoculum size to the physiological state. Therefore, lack of information about the state of the inoculum may affect the performance of a predictive model. Gay et al. (1996) reported that a combination of pre-incubation cold temperature and low inoculum size significantly shifted the growth curve of *L. monocytogenes (25)*. Augustin et al. (2000) observed the lag time of *L. monocytogenes* was extended when the inoculum was stressed by starvation *(3)*. Besse et al. (2006) discovered a significant effect of inoculum size of *L. monocytogenes* on lag phase duration and maximal population density *(9)*. The inoculum size also affected the boundary of temperature, a_w, and pH for the growth/no growth model for *L. monocytogenes (42)*. This concern about the lack of inoculum information becomes more significant when the actual starting concentration for pathogen contamination in foods is relatively small, since predictive models are typically developed with higher starting inocula *(61)*.

Another challenge for predictive modeling is intermediate lag phase. Intermediate lag phase can be induced if the modeled bacteria are subjected to abrupt environmental changes. Research on the growth of *Lactobacillus plantarum* under fluctuating temperature illustrated that a more accurate growth model should be able to predict additional lag if temperature shift occurs during both lag phase and exponential phase *(99)*. This research also showed that shifts around minimum growth-allowed temperature was more likely to induce lag than milder shifts. Attempts have been made to model intermediate lag as a function of

temperature-related parameters. To investigate the effect of temperature shift on growth kinetics, researchers have developed a model to describe the parameter h_0 (the product of growth rate by the lag phase duration) as a function of pre-incubation temperature and growth temperature for *L. monocytogenes* *(21)*. A systematic study on quantification of lag using *E. coli* focused on the effect of three quantities on lag: the amplitude of temperature shift, the pre-shift temperature level, and the post-shift temperature level *(88)*. The results generally suggested that (a) a temperature jump less than 5˚C probably does not cause intermediate lag, whilst a temperature jump greater than 10˚C probably does; (b) roughly speaking, a temperature jump of 10˚C at lower temperature causes longer intermediate lag than higher temperature. Similar to temperature shift, an abrupt osmotic shift can also induce lag phase in many foodborne bacteria including *Salmonella* (*48, 47, 57*). Given the fact that currently most available modeling methods merely focuses on initial lag, and that modeling intermediate lag is relatively difficult *(88)*, predictive models should be used with cautions for the scenarios with abrupt environmental changes.

In addition to the factors mentioned above, naturally present antimicrobials, structure of the food matrices, potential physic-chemical changes of conditions can also cause deviation from prediction, so predictive models should be validated before use. To date, there is no standard method for model validation. Ross (1996) developed accuracy and bias factors (can be seen in Eq. (II. 4. 1) and Eq. (II. 4. 2)) for

model evaluation *(81)*, which were modified by Baranyi (1999) *(5)* (Eq. (I. 5. 3) and Eq. (I. 5. 4)). Some researchers considered a prediction accurate if the difference between predicted and observed value was within 0.3 log CFU *(32, 10)*. Others may use root mean square error (RMSE) *(92)* or relative error (%RE) *(43)* to assess the performance of a model, which can be seen in Eq. (I. 5. 5) and Eq. (I. 5. 6).

$$A_f = \exp\left\{\sqrt{\frac{\sum_{k=1}^{m}(\ln f(x^{(k)}) - ln\mu^{(k)})^2}{m}}\right\} \quad \text{(I. 5. 3)}$$

$$B_f = \exp\left\{\frac{\sum_{k=1}^{m}(\ln f(x^{(k)}) - ln\mu^{(k)})}{m}\right\} \quad \text{(I. 5. 4)}$$

where $\mu^{(1)}$,$\mu^{(2)}$...$\mu^{(m)}$ are observations; $f(x^{(k)})$ is the predicted value corresponding to the k^{th} observation point.

$$RMSE = \sqrt{\frac{\sum(predicted - measured)}{N}} \quad \text{(I. 5. 5)}$$

$$\%\text{RE} = \frac{\text{observed} - \text{predicted}}{\text{observed}} \times 100 \quad \text{(I. 5. 6)}$$

In summary, *Salmonella* is a significant safety hazard for ground beef consumption. To reduce the risk of *Salmonella*, HACCP program has been extensively employed in meat slaughters and processing plants to assure meat safety and quality. Predictive models enable the quantification of the food safety hazards from farm to table, and thus become a complementary tool for HACCP. Predictive models, however, faces some factors that are not usually incorporated into the model development, such as background microbiota, insufficient knowledge for inoculum, and intermediate lag. Therefore, model validation, as the major topic of this thesis, becomes critical to realizing the value of predictive models.

Chapter II - **VALIDATION OF COMBASE MODEL FOR *SALMONELLA* IN GROUND BEEF UNDER MULTIPLE CYCLIC TEMPERATURE FLUCTUATIONS**

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II. 1. Abstract

Salmonella is one of the most common foodborne pathogens in the United States. ComBase is a predictive database that can be used to predict microbial behavior in various and changing environmental conditions. This research is an extension from our laboratory and is to validate ComBase for *Salmonella* in ground beef under multiple temperature-changing cycles (i.e., three cycles with or without holding time at 5˚C for 2.5 h in between, and six cycles with or without holding time at 5˚C for 24 h in between; each cycle included 2.5 h heating from 5˚C to 37˚C and 2.5 h cooling from 37˚C to 5˚C). Five rifampcin-resistant serotypes of *Salmonella* were used for this research, i.e., *S. Copenhagen*, *S. Montevideo*, *S. Typhimurium*, *S. Saintpaul*, and *S. Heidelberg*. The *Salmonella* cocktail was inoculated into 300 g of ground beef, which was then separated into 5 g samples and subjected to water bath for four different temperature profiles. Spread plates were conducted on tryptic soy agar plus rifampcin at certain time intervals. Predictions corresponding to the temperature profiles were generated in ComBase, which were then compared to the observed data. The accuracy factors and bias factors range from 1.27 to 1.74 and 1.27 to 1.78, respectively. We speculated that the major reason causing the deviation from the model was the intermediate lag phase induced by low temperature. Further research may be needed to very this speculation.

II. 2. Introduction

The Center of Disease Control (CDC) estimates that over 1.2 million salmonellosis cases occur annually in the U.S. annually, which causes $2.3 to 11.3 billions of economic loss *(54)*. Approximately 7% of those salmonellosis cases are attributed to beef *(54)*. Recently, *Salmonella* outbreaks due to ground beef occurred in 2011, 2012, and 2013 *(16)*. The 2011 outbreak included 7 states and 20 cases; 9 states were involved during the 2012 outbreak and 46 individuals were infected; the 2013 outbreak included 6 states and 22 infected individuals *(16)*.

Time consuming and expensive challenge test are the primary means to quantify microbial growth in food products, which are fairly time-consuming *(92)*. An alternative of assurance for microbiological safety is predictive modeling. Typically, primary models are developed for a microorganism of interest under certain constant environmental condition (pH, temperature, salt concentration, etc.). Mathematical functions with sigmoidal shape, e.g., Gompertz, logistic, Baranyi and Roberts' model, are usually used to fit the growth curves to develop primary models, and each generally provides an accurate means to predict microbial growth *(26)*. Juneja et al. (2009) developed a model for *Salmonella* in ground beef using logistic, modified Gompertz, Baranyi, and Huang models, and found that there was no significant difference with respect to model performance among the four models *(38)*. Based on primary models, secondary models are built to map a specific environmental condition to growth parameters (maximum growth rate and/or duration of lag phase). Predictions for microbial growth under changing

environmental factors can be made with secondary models. A tertiary model allows input of a variety of environmental parameters and generates pertinent predictions based on secondary models. ComBase is a tertiary model that is publicly available online (http://www.combase.cc/). The name *ComBase* derives from combined database or common database, which is created by merging Pathogen Modeling Program (PMP) in the U.S. and Food MicroModel (FMM) in the U.K., as well as data from collaborating institutes and literatures *(55)*.

Predictive models should be validated before being applied to food products. Models are usually developed in laboratory media, which may not be consistent in real life food products. The food matrix, competitive microflora, and extreme environmental conditions can all cause deviation from model prediction.

Researchers generally assumes that bacterial growth rates adapt instantaneously to temperature change if the changes are within the range for the growth of the bacteria *(12)*. But it has been noted that adaptation-related delay of growth occur if the already growing cells are subjected to a new temperature *(10)*. Also, most models focus on the effect of environmental conditions on the maximum growth rate with less emphasis on lag phase *(43)*, so temperature fluctuations across minimum growth-allowed temperature may expose the inaccuracies in a model.

This research is an extension of a research conducted by McConnell and Schaffner (2014) *(52)*, who validated ComBase for *Salmonella* in ground beef under

one cycle of temperature change (from 4.4 to 15.6, 26.7, or 37.8˚C over 4, 6, and 8 h). They reported that good agreements were observed when maximum temperatures were lower (15.6 and 26.7˚C), whereas the model was fail-safe when the maximum temperature was 37.8˚C. This research seeks to further validate ComBase for *Salmonella* in ground beef with more frequent and extreme temperature fluctuations to give a more conclusive evaluation on the performance of the model.

II. 3. Materials and Methods

Methods are modified based on prior research conducted in our laboratory *(18)*. Rifampicin (rif) -resistant *Salmonella* strains (listed in Table II. 3.) were used. Prior research has shown that antibiotic-resistant and –nonresistant strains are not significantly different with respect to growth rate in ground beef *(52)*. Ground beef (20% fat) used in the experiments were purchased from a local supermarket no more than one day before use.

II. 3. a. Inoculation

The five strains were each grown separately in tryptic soy broth with 50 μg/ml of rif (TSB+rif) for 8 h at 37°C. The strains were combined in equal volume to form a cocktail and 1 ml cocktail was centrifuged (accuSpin Micro 17 Microcentrifuges, ThermoFisher, Grand Island, NY) at 5×g for 5 min. Supernatant was discarded and pellet was re-suspended with 0.15% peptone water. The centrifugation procedure

was repeated three times and the final bacterial suspension was diluted 1:10^{-3}, from which 1 ml was inoculated into 300 g of ground beef, to yield a starting concentration of 3 log (CFU/g). The inoculated ground beef was kneaded in a sterile sample bag (24 oz., Nasco Whirl Pak, Salida, CA) for 10 min. Preliminary experiments showed that 10 min kneading would result in homogenous distribution of the inoculum in 300 g ground beef. The ground beef was separated into 5 g portions contained in small sterile sample bags (1 oz., Nasco Whirl Pak, Salida, CA). The 5 g samples were securely submerged in programed water bath (Chiller Recirculating Water Bath RTE 17 and RTE 221, Thermo-NESLAB, Portsmouth, NH) and were subjected to the desired temperature profile.

Table II. 3. *Salmonella* strains used in this study.

	Strains				
USDA strain identification	S21	S24	S25	S26	S40
serotype	Copenhagen	Montevideo	Typhimurium	Saintpaul	Heidelberg

II. 3. b. Temperature profiles

The temperature profile was programmed to rise linearly from 5°C to 37°C in 2.5 h and to drop linearly from 37°C back to 5°C in the same time, constituting one cycle of temperature change. This experiment included three and six cycles in the temperature profile. The three-cycle temperature profile had 0 or 2.5 h holding time at 5°C in between; whereas the six-cycle temperature profile had 0 or 24 h holding

time at 5°C in between.

II. 3. c. Growth assay

Samples were assayed for *Salmonella* concentration approximately every two hours during three-cycle temperature profile. For the six-cycle temperature profile, two tests were conducted at the beginning, the middle, and the end of each cycle. Samples were diluted 1:50 with 0.15% peptone buffer and were homogenized by a stomacher (Stomacher Lab Blender 400, Cooke Laboratory Products, Alexandria, VA). Additional dilutions were made according to expected *Salmonella* concentration and sampling time. Samples were plated on tryptic soy agar plus rif (TSA+rif) in duplicate and *Salmonella* colonies were enumerated after 24 h incubation at 37°C. An uninoculated ground beef sample was plated on TSA+rif at the beginning and the end of the experiment as a negative control. Appropriately diluted first sample and final samples with were plated on TSA to enumerate total plate count (TPC).

II. 3. d. Predictive modeling

The ComBase online model (http://www.combase.cc/) was used to generate dynamic growth prediction for *Salmonella* spp. The physiological state, initial level (log), pH, and NaCl (%) were set to be as default, 3, 5.7 and 0.5, respectively. The temperature profile was set as above. The observed data were adjusted slightly to

have an initial *Salmonella* level of 3 log (CFU/g) to facilitate comparison with model predictions. Since the ComBase model for *Salmonella* does not allow temperature below 7°C, any temperature points below that limit were adjusted to be 7°C. Accuracy and bias factors *(81)* were calculated with the observed and predicted data to evaluate the performance of the ComBase model.

II. 4. Results

The results are seen in Table II. 4. 2. Holding time indicates the length of time when temperature was held at 5˚C between two cycles. The resulting accuracy and bias factors can also be seen in Table II. 4. 1. While Figure II. 4. 1., Figure II. 4. 2., Figure II. 4. 3., and Figure II. 4. 4. depict the results from experiment of 3 cycles without holding, 3 cycles with holding, 6 cycles without holding, and 6 cycles with holding, respectively.

The TPC present in the ground beef were high at the beginning and end of all experiments: 6.65±0.28 and 7.07±0.36 (95% CI) logs CFU/g, respectively. Although such a high level of background microflora might be thought to inhibit the growth of *Salmonella*, prior research in our lab *(52)* and elsewhere *(22)* indicated a minimal impact.

It can be seen from Figure II. 4. 1. and Figure II. 4. 2. that prediction and observation agreed well in the first temperature cycle, and this continued until the middle of the second cycle. Since temperature between the end of first cycle and the

beginning of second cycle was below 10˚C, and it has been reported that the lag duration for *Salmonella* in ground beef at 10˚C was as high as 2,784 h *(10)*, we speculate that the *Salmonella* in that period were experiencing physiological changes due to low temperature and consequently additional lag was induced, as suggested by Swinnen et al. (2004) *(88)*.

Development of ComBase was based on primary and secondary models, and this methodology assumes that maximum growth rate adjusts to new temperature instantaneously. If the temperature abruptly shifts to below the minimum growth-allowed temperature for a sufficient amount of time, temperature-induced lag may be induced and if the model does not take this into account, this may be the key reason why the difference prediction and observation increased after the first temperature cycle in Figure II. 4. 1. and Figure II. 4. 2.

Similarly, in the 6 cycles experiments (Figure II. 4. 3. and Figure II. 4. 4.), prediction and observation agreed well in the first two hours, and the deviation increased at the rest of the sampling points. It should be noted that both the maximum predicted concentrations in Figure II. 4. 3. and Figure II. 4. 4. were 8.51 log CFU/g, which is the maximum concentration that *Salmonella* can grow. The difference between prediction and observation at the fourth cycle was approximately as high as 3 log CFU/g, and the final differences were to 3.03±0.24 and 4.37±0.43 log CFU/g for Figure II. 4. 3. and Figure II. 4. 4., respectively.

The closer the accuracy factor is to 1, the more accurate the model. The closer

the bias factor is to 0, the less biased the model. By comparing the accuracy and bias factors of the four experiments, general trends regarding cycle number and low temperature exposure can be seen. The 6-cycle experiments have higher accuracy and bias factors than the 3-cycle experiments, which means the model is, under these temperature profiles, less accurate and more biased with more temperature cycles. The 6-cycle plus 24-h-holding experiment had higher accuracy and bias factors than the one without holding, which suggests that experiencing low temperature amplifies the deviation between prediction and observation. This was not the case for the 3-cycle experiments, where accuracy and bias factors for 3-cycle with holding were lower than that without holding. We suspect that this is because the holding time (2.5 h) in the 3-cycle experiment was too short to cause a physiological difference in the cells.

Some researchers considered a prediction accurate if the difference between final predicted and observed concentration (Δlog CFU/g) was within 0.3 log CFU *(11 2)*. Although the specific value of this criterion can vary, the result of Δlog CFU/g in this study suggests that ComBase was does not provide an accurate prediction for the tested temperature profiles. Interestingly, the accuracy factors in Table II. 4. 1. were lower than the average accuracy factor (2.11) reported in previous study *(52)*, whereas the final differences between the observed and predicted increase in the current study were generally less than 2 log CFU/g.

Accuracy factor = $10^{[\frac{1}{n}\sum|\log(\frac{predicted}{observed})|]}$ Eq. (II. 4. 1)

Bias factor = $10^{[\frac{1}{n}\sum\log(\frac{predicted}{observed})]}$ Eq. (II. 4. 2)

Table II. 4. 1. Result of accuracy and bias factors under different temperature profiles. Each cycle includes heating from 5°C to 37°C in 2.5 h and cooling from 37°C back to 5°C in 2.5 h. Holding time is the length of time when temperature is held at 5˚C between two cycles. Δlog CFU/g is the mean difference within 95% confidence interval (CI) between the final observed and final predicted value of log CFU/g.

Number of cycles	Holding time (h)	Accuracy factor	Bias factor	Δlog CFU/g
3	0	1.23	1.23	2.30±1.87
3	2.5	1.18	1.16	2.22±1.32
6	0	1.41	1.40	3.03±0.24
6	24	1.74	1.74	4.37±0.43

Table II. 4. 2. TPC in different experiments. Initial and final TPC are the TPCs present in the ground beef in the beginning and in the end, respectively. ΔTPC is derived from subtracting final TPC by initial TPC. The values of initial TPC, final TPC, and ΔTPC are the 95% confidence interval (CI) of their means.

Number of cycles	Holding time (h)	Initial TPC (log CFU/g)	Final TPC (log CFU/g)	ΔTPC (log CFU/g)
3	0	6.36±0.47	6.70±0.74	0.35±0.48
3	2.5	6.52±2.43	7.34±3.12	0.81±0.70
6	0	6.77±0.27	6.82±0.43	0.05±0.52
6	24	6.97±0.06	7.41±0.06	0.44±0.02

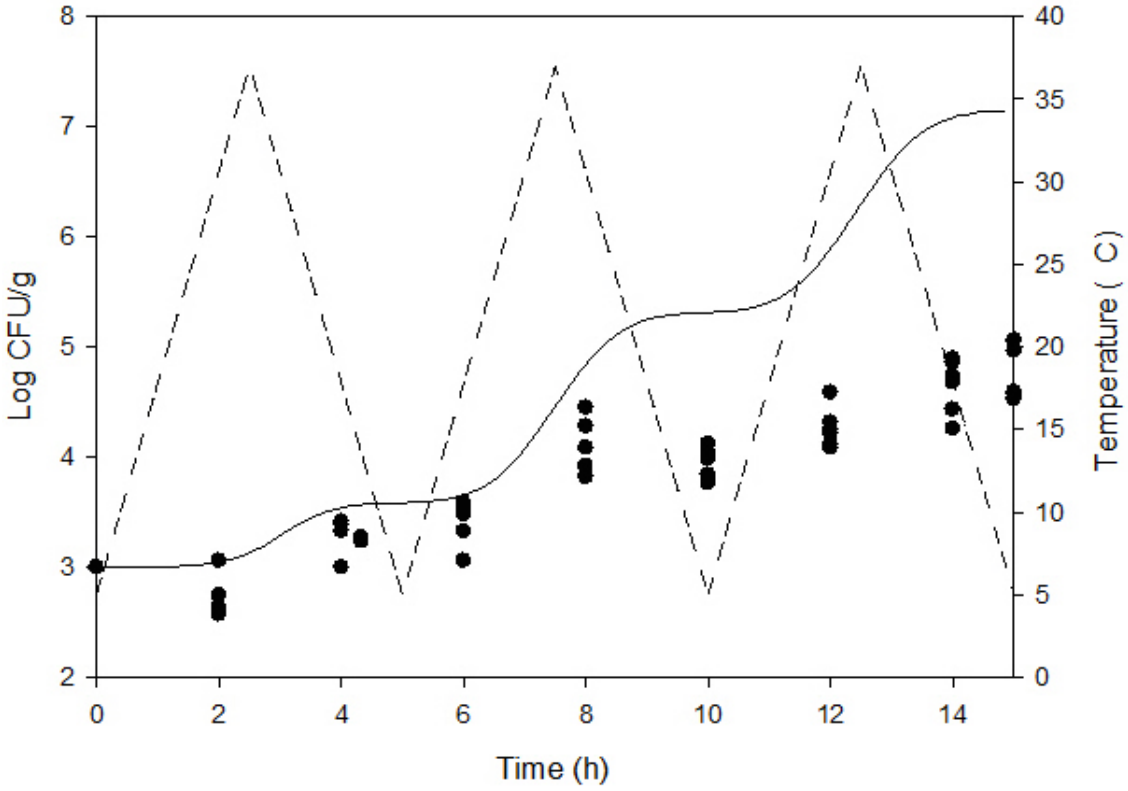

Figure II. 4. 1. *Salmonella* growth in ground beef under 3 cycles of temperature change without holding at 5˚C between each cycle. Dashed line, solid line, and solid circles represent the temperature profile, predicted concentration, and observed concentration, respectivel.

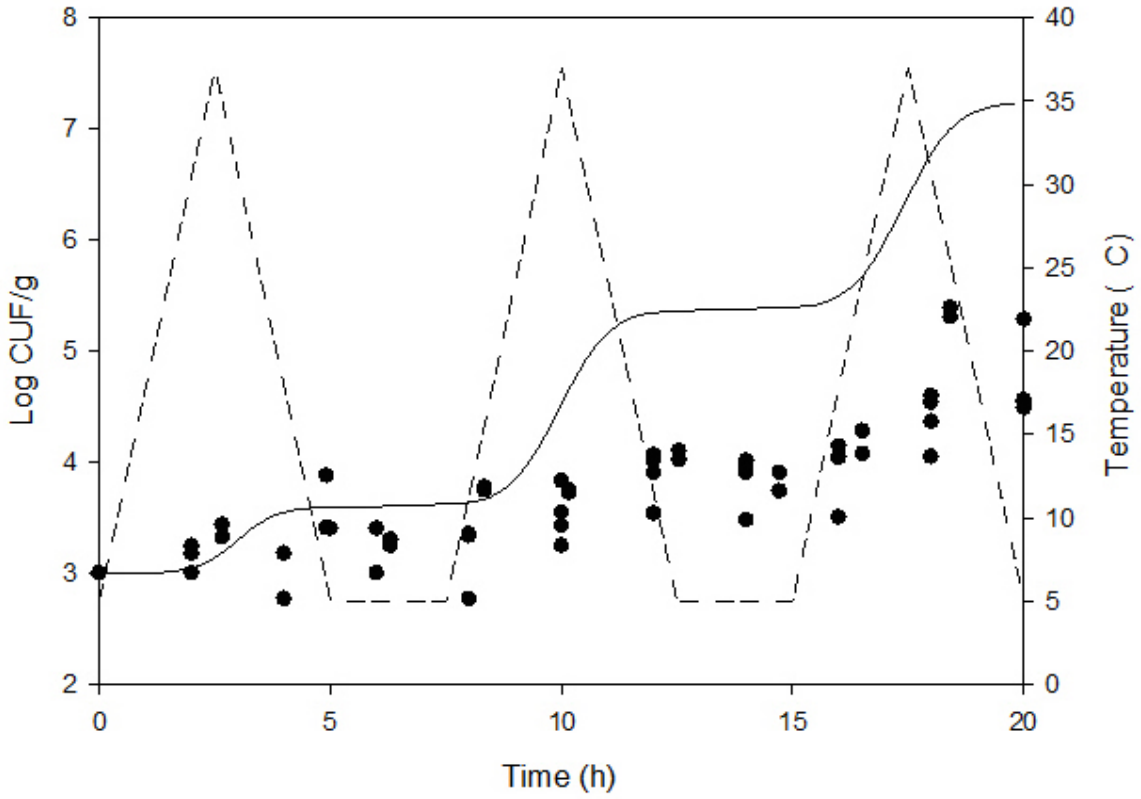

Figure II. 4. 2. *Salmonella* growth in ground beef under 3 cycles of temperature change with holdings at 5˚C between each cycle. Dashed line, solid line, and solid circles represent the temperature profile, predicted concentration, and observed concentration, respectivel.

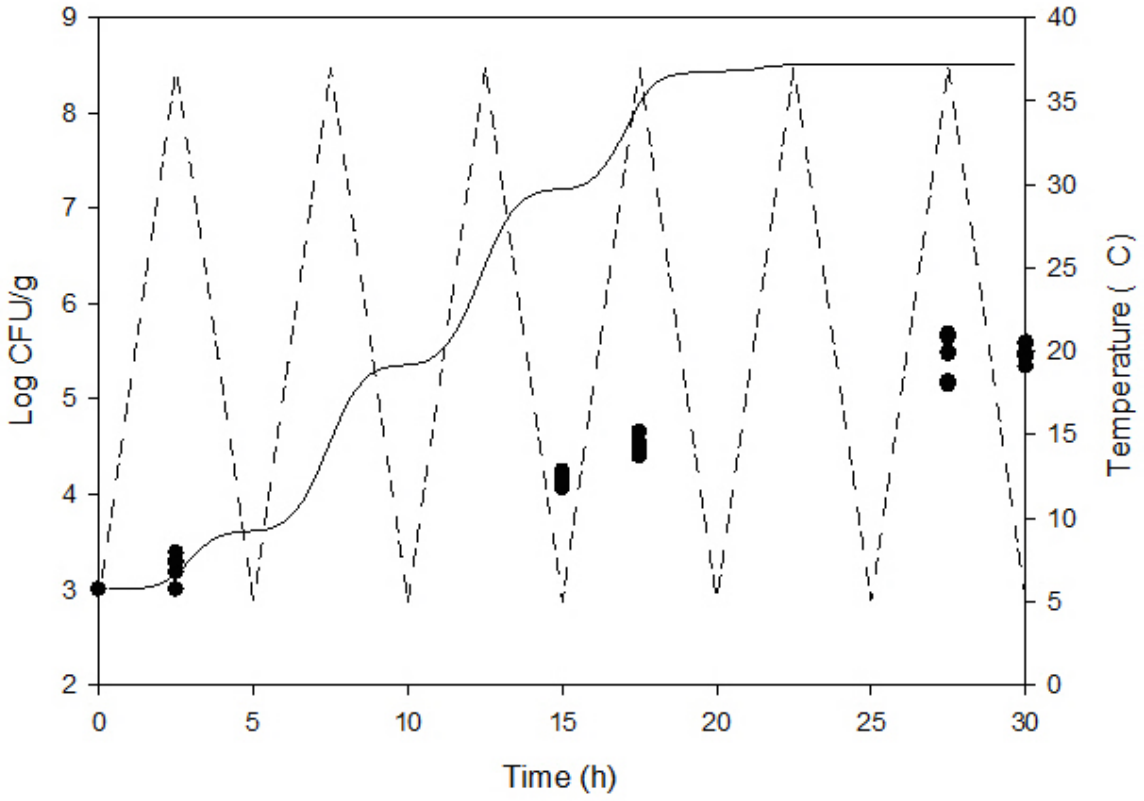

Figure II. 4. 3. *Salmonella* growth in ground beef under 6 cycles of temperature change without holding at 5˚C between each cycle. Dashed line, solid line, and solid circles represent the temperature profile, predicted concentration, and observed concentration, respectivel.

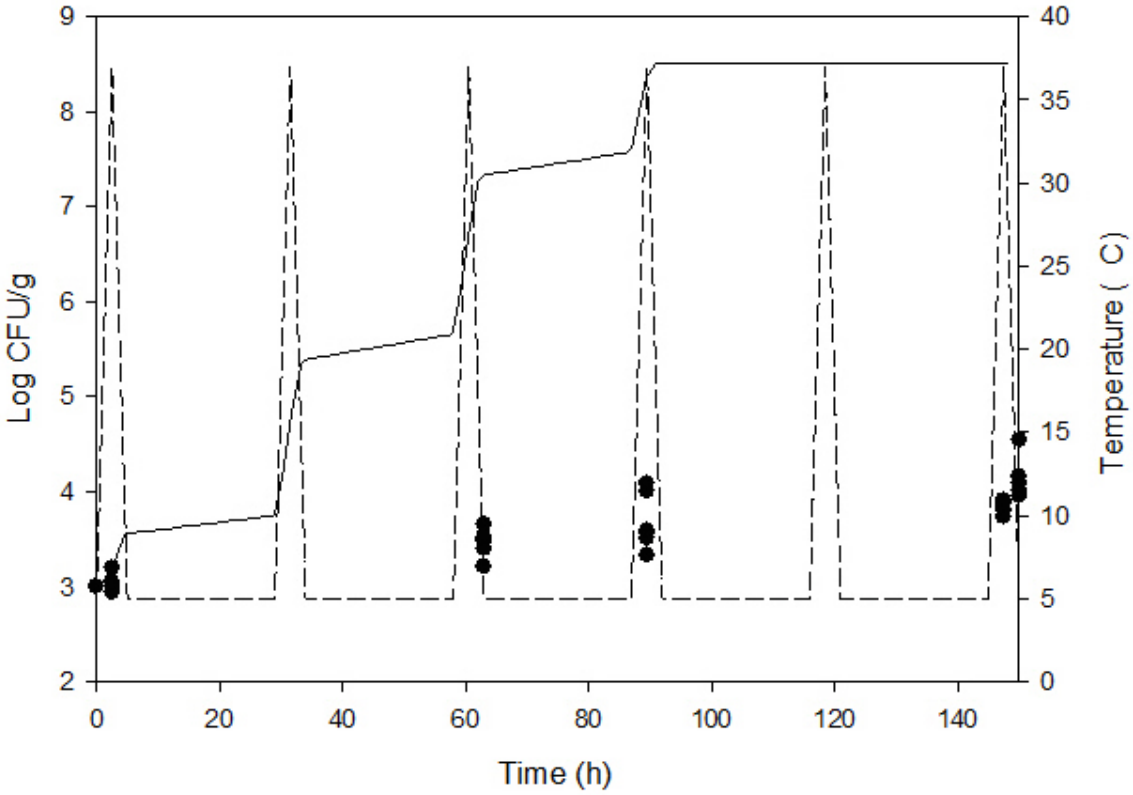

Figure II. 4. 4. *Salmonella* growth in ground beef under 6 cycles of temperature change with holdings at 5˚C between each cycle. Dashed line, solid line, and solid circles represent the temperature profile, predicted concentration, and observed concentration, respectivel.

II. 5. Discussion

Many studies of growth of *Salmonella* in meat or laboratory medium have been performed, and some include model development. Mackey and Roberts (1979) reported that *Salmonella* did not grow at 7-8˚C on beef and that the mean generation times on beef at 10˚C, 12.5˚C and 15˚C were 8.1 h, 5.2 h and 2.9 h, respectively *(49)*. Dickson et al. (1992) developed a model for the lag and generation time of *Salmonella*, and subsequent validation studies showed no significant difference between observed and predicted *Salmonella* concentration in the scenario that lean or fatty tissues of beef were cooled from 40°C to 10°C in 3.3 or 5 h *(23)*. Mann et al. (2004) tested the critical limits of temperature and time for *Salmonella* and background microflora on ground pork. They observed that *Salmonella* and background microflora showed significant log increase in *Salmonella*-inoculated ground beef held at room temperature after 6 h and 8 h, respectively; whereas, at refrigerated temperature, no significant growth of *Salmonella* was observed and it took 24 h for microflora to show significant growth *(51)*. These researchers also found that it took more time for microflora to grow in uninoculated sample than in inoculated sample *(51)*.

A predictive tool called THERM (Temperature History Evaluation for raw Meats) was developed to predict pathogen behavior qualitatively (growth/no growth) and quantitatively in meats *(32)*. Validation studies for THERM showed that, despite of good qualitative performance, it was inaccurate (observed and predicted growth

differed by >0.3 log CFU) with respect to quantitative prediction in 58% experiments for *Salmonella* in meat *(32)*. Borneman et al. (2009) reported that the accuracy (within ±0.3 log CFU) of THERM prediction for *Salmonella* serovars, *Escherichia coli* O157:H7, and *Staphylococcus aureus* in meat products ranged from 51.4 to 67.2% *(10)*.

Performances of PMP, FMM, and ComBase *Salmonella* models have been investigated. Oscar (2013) validated PMP prediction for *Salmonella* at various constant temperatures on chicken skin that had been frozen for 6 days *(73)*. He found that the experimental results generally agreed with PMP predictions. Bovil et al. (2001) subjected *Salmonella*-medium suspension to rapid temperature increasing, decreasing, or fluctuating profiles and found no cell death or induced lag phase due to temperature shock and that the experimental results agreed well with FMM *(12)*. McConnell and Schaffner (2014) have found that ComBase was accurate or fail-safe for in predicting *Salmonella* growth in ground beef in dynamic temperature conditions *(52)*.

Some research has also been conducted on periodic or fluctuating temperature profiles. Mitchell et al. (1995) modeled *Salmonella* growth by evaluating growth rates at various isothermal medium conditions with different combinations of pH and sodium chloride concentrations *(60)*. Their study subjected *Salmonella* cultures to sinusoidal temperature cycles from 4 to 22˚C in 60, 120, or 240 min, and the result showed good agreement between experimental and predicted result, and, in

most cases, the growth of *Salmonella* responded instantaneously to temperature change without lag phase being induced *(60)*. Pin et al. (2011) modeled *Salmonella* concentration throughout the pork supply chain under fluctuating conditions, and the model they used (Baranyi and Roberts model) did not include lag phase, which resulted in bias and fail-safe prediction *(75)*. Velugoti et al. (2011) developed a model to predict growth of *Salmonella* in sterile ground pork and validated it under increasing (from 2 to 44˚C in 24 h), decreasing (from 45 to 7˚C in 10 h), or sinusoidal (between 10 to 30˚C in 40, 120, 240, and 480 min) temperature profiles *(92)*. They found that most observed data points of increasing and decreasing agreed with prediction well (within 0.5 log difference); whereas deviation was enlarged if the sinusoidal temperature profile stayed below minimum growth temperature for longer period *(92)*.

In our research, the major reason causing experimental deviation from prediction may be low temperature-induced lag phase. It is commonly believed that duration of lag is dictated by pre-inoculation physiological state and post-inoculation environment *(6)*. In those experiment used to develop growth models, the post-inoculation environment is standardized, thus the predicted duration of lag is only dependent on pre-inoculation physiological state of the bacteria *(6)*. However, Swinnen (2004) have pointed out that, besides initial lag, intermediate lag can be induced by sudden environmental changes and should be considered in predictive modeling *(88)*. There are many factors that may influence

intermediate lag qualitatively and quantitatively, including environmental conditions, growth state, inoculum size, etc., and thus very complicated to model *(88)*.

Another important factor influencing the accuracy of predictive model is the competitive natural microflora present in the food matrix. The spoilage flora in ground meat (predominantly *Pseudomonads* and *Brochothrix thermosphacta*) can grow up to 10^9 CFU/g at the end of shelf life *(42)*. Pathogens generally stop growing once the dominant microflora reaches the maximum population density, which is known as the Jameson effect *(62)*. However, Dickson and Olson (2001) found that removal of microflora in ground beef by irradiation did not give *Salmonella* competitive advantage to grow in ground beef *(22)*. Koutsoumanis et al. (2005) developed a dynamic model for spoilage microflora in ground meat and their validation studies indicated good performance of the model in the scenario that temperature changed periodically from 0 to 10, 15, or 20˚C *(42)*. Møller et al. (2013) modeled the effect of microflora on growth of *Salmonella* in fresh pork and noted that the Jameson effect was temperature dependent *(62)*.

II. 6. Conclusions

ComBase was consistently fail-safe in the fluctuating temperature profiles we tested. It was noted that more temperature fluctuations and more exposure to low temperature could increase the deviation from the model predictions. While

background microflora is one possible explanation for the model deviation, prior work in our lab and elsewhere indicate background microflora is not the key factor responsible for the deviation from model predictions. We suggest that low-temperature-induced intermediate lag phase does influence the performance of the model.

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