12-2016

Modeling for thermal resistance of non-O157 shiga toxin producing Escherichia coli in ground beef

Jagpinder S. Brar
Purdue University

MODELING FOR THERMAL RESISTANCE OF NON-O157 SHIGA TOXIN PRODUCING ESCHERICHIA COLI IN GROUND BEEF

by

Jagpinder S Brar

A Dissertation

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Doctor of Philosophy

Department of Food Science

West Lafayette, Indiana

December 2016

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF DISSERTATION APPROVAL

Dr. Manpreet Singh, Chair

Department of Food Science

Dr. Arun Bhunia

Department of Food Science

Dr. Bruce Applegate

Department of Food Science

Dr. Jolena Waddell

Department of Animal Science, Tarleton State University

Approved by:

Dr. Carlos Corvalan

Head of the Departmental Graduate Program

I would like to dedicate my work to my loving wife, Mandeep Brar, my mom, dad, sister and the newest addition to the family, my nephew Aval Grewal.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my advisor Dr. Manpreet Singh for his motivation, patience and his continuous support throughout my project. All the food safety knowledge that I have acquired during this time and this journey could not have been possible without his guidance. I sincerely appreciate him for sending me to different conferences and workshops to enhance my understanding of food safety and nurture my interests in the field. I would also like to thank my committee members Dr. Arun Bhunia, Dr. Bruce Applegate and Dr. Jolena Waddell for their suggestions and recommendations to improve my research project.

Furthermore, I want to thank all my friends in Singh lab who have helped me in my research project, including Matthew Bailey, Rhonda Taylor, Sydney Coakran, Carmen Velasquez, and Estefania Novoa Rama. In addition, I would like to thank one of my dearest friend Lucky Mehra for providing his expertise in statistics and helping me in data analysis. This project would not have completed on time without all their assistance and support. Finally, I would like to extend my special appreciation to my parents, my wife, my sister and my friends for all their love and constant support.

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ABSTRACT

Author: Brar, Jagpinder, Singh. Doctor of Philosophy
Institution: Purdue University
Degree Received: Fall 2016
Title: Pathogen Modeling for Thermal Resistance of Non-O157 Shiga Toxin Producing *Escherichia coli* in Ground Beef.
Major Professor: Manpreet Singh.

Predictive models in microbiology are used for estimating the growth or survival of microorganism in a set of environmental conditions. A validated predictive model provides an alternative to extensive survival and shelf life studies. In this study, a predictive inactivation model for non-O157 shiga toxin producing *Escherichia coli* (STEC) in ground beef was developed. Six strains of non-O157 STEC; *E. coli* O26:H1, *E. coli* O45:H2, *E. coli* O103:H2, *E. coli* O111:H8, *E. coli* O121:H9, and *E. coli* O145: non-motile, has similar pathogenicity as *E. coli* O157:H7 and can cause serious food borne illnesses. These pathogens are considered as an adulterant in meat products. The thermal behavior these non-O157 STECs was studied in laboratory media as well as in ground beef with varying fat content. There was no significant difference in the heat resistance among the strains, therefore, a cocktail of the strains was used for ground beef study. Ground beef fat content levels of 5, 10, 15, 20, 25, and 30% were used. Survival curves were generated between surviving population against time during heat treatment at five temperatures 55, 60, 65, 68, 71.1ºC. The shape of survival curves was analyzed by statistical analysis software (SAS®) to identify the best fitting primary model. The survival of these pathogens was modeled as a second order polynomial function of fat content of ground beef and temperature of cooking. The accuracy factor of the developed model was 11.43%, which is in the acceptable limit of 25%. The model was successfully validated for predicting process lethality in ground beef obtained from three grocery stores.

CHAPTER 1: INTRODUCTION

Shiga toxin producing *Escherichia coli* (STEC) or Enterohemorrhagic *E. coli* (EHEC) can cause gastrointestinal illnesses, bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS) in humans (Anderson et al., 2009). The infectious dose of STECs can be as low as 10-100 CFU/g and the incubation time approximately 2-4 days (Paton et al., 1996). Young, old, immunocompromised, and pregnant populations are vulnerable to STEC infection and are a major concern in meat industry as approximately 75% of the STEC outbreaks are linked with meat and meat products (Nguyen and Sperandio, 2014). Scallan et al., (2011) estimated 175,905 infections and 3,673 hospitalizations caused by STECs annually in the US. *E. coli* O157:H7 is the most frequent STEC strain causing food related outbreaks, however, the number of outbreaks linked with non-O157 STECs is increasing. A total of 1,113 incidences of *E. coli* infections in the United States have been reported from 2006-2013, of which, 49.6% of the cases were caused by *E. coli* O157 and 50.4% of them were caused by non-O157 STECs, resulting in 286 hospitalizations and 4 deaths (Crim et al., 2014). Six strains of non-O157 STECs, *E. coli* O26:H1, *E. coli* O45:H2, *E. coli* O103:H2, *E. coli* O111:H8, *E. coli* O121:H9, and *E. coli* O145: non-motile, contribute to the majority of the non-O157 STEC infections (Gould, 2009). To eliminate these pathogens from food products, USDA has a zero-tolerance policy for *E. coli* O157 and these six strains of non-O157 STECs (USDA, 2011).

FSIS requires a minimum of 5-log CFU/g reduction of pathogens during processing in ready to eat (RTE) meat products as a preventive control (FSIS, 2001).

Understanding of thermal behavior of target pathogens is critical in designing the processing conditions to ensure the required process lethality. The rate of thermal inactivation varies with not only the temperature but also with the intrinsic properties of the food (Juneja et al., 1997). Juneja and Eblen (2000) found an increase in the heat resistance of *Salmonella* Typhimurium as the fat content of ground beef increased. The authors speculated that fat provides a protective layer for pathogens and hence it takes longer to kill pathogens at higher fat content. Similarly, other intrinsic factors like pH, water activity, and moisture content can also impact the survival of pathogens in food. Hence, studying impact of intrinsic factors is vital for ensuring microbial safety of food.

Pathogen modeling provides an alternative to extensive pathogen survival studies and quantifying the impact of environmental conditions and intrinsic factors of food (Baranyi et al., 1994). The USDA has developed a pathogen modeling protocol (PMP) containing various predictive models to provide an estimation of growth and/or survival of different pathogens and spoilage organisms and to assist processors to design process conditions for adequate lethality (USDA, 2016). A scientific validation is required for each specific food product and target pathogen before applying predictive models (USDA, 2016). Juneja et al. (2009) developed a predictive model for inactivation of *E. coli* O157 as a function of tea leaf and apple skin powder for ground beef. Similarly, Skandamis et al. (2000) developed and validated a predictive model for inactivation of *E. coli* O157:H7 in homemade eggplant salad with environmental factors of pH, temperature and oregano essential oil concentration. Understanding of design of experiments, and statistical analysis are very important to develop an accurate predictive model. The thermal behavior of the pathogens of interest are studied and the data generated are used

for building a predictive model. Primary modeling analysis is performed to understand the distribution of survival curves; surviving population versus exposure time, and the impact of environmental factors are included in the secondary modeling analysis. Combinations of both primary and secondary models are used to estimate the population of target pathogen within the specification of a given set of intrinsic and extrinsic conditions (Baranyi et al., 1994). The potential pathogenicity of non-O157 STECs, low infectious dose, and high prevalence in meat products make it important to develop an inactivation model for these pathogens. Hence, thermal inactivation of non-O157 STECs has been modeled as a function of temperature and fat content of ground beef in this research. This predictive model for inactivation of non-O157 STEC will provide the information required for successful elimination of these pathogens in ready to eat meat products. The following were the main objectives of the research

1) To study the thermal behavior of six non-O157 STEC strains individually in laboratory medium at 55, 60, 65 and 71.1°C
2) To study the impact of fat content of ground beef on the heat resistance of non-O157 STEC at 55, 60, 65, 68 and 71.1°C
3) To develop a mathematical predictive model for thermal inactivation of non-O157 STEC in ground beef
4) To validate the model developed in the objective 3

CHAPTER 2: REVIEW OF LITERATURE

2.1 General Overview

Escherichia coli is a gram negative, non-spore forming mesophilic bacteria, and has optimal growth conditions of 4.5-9 pH, 37 ± 2°C and <5% salt content. Pathogenic *E. coli* can cause gastrointestinal illness, Hemorrhagic colitis, Hemolytic Uremic Syndrome (HUS), nausea and self-limiting watery diarrhea (Tarr, 1995). Based on their mechanism of pathogenesis, *E. coli* has been divided into five groups; enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC) or Shiga toxin producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC) and enteroaggregative *E. coli* (EAEC) (Mathusa et al., 2010).

The nomenclature of *E. coli* strains is based on their O and H antigen: O antigen is the somatic antigen and H antigen is flagellar antigen (Sheng et al., 2008). The O classification is based on repeats of oligosaccharide units, a part of outer membrane lipopolysaccharide (LPS) (Perry et al., 1986). H-antigen consists of flagellin polymer present in the flagellar filament, which helps in the motility of the bacterium. The N-terminal and C-terminal of flagellin are conserved, however, the middle section is variable and yields different H-antigen (Lino et al., 1988). A total 53 flagellar antigen groups have been described for *E. coli* (Starr, 1986).

2.2 Shiga-toxin Producing *Escherichia coli* (STEC)

The pathogenicity of STECs is mainly attributed to shiga toxin genes (*stx*$_1$ and/or *stx*$_2$) and the *E. coli eae*, which codes for intimin allowing attachment and effacing.

Intimin is required for attachment of STEC cells to the intestinal cells and Shiga toxins damage the host cell by blocking its protein synthesis mechanism (Sandvig and van Deurs, 2000). Expression of *eae* gene is critical for the pathogenesis of STECs as Stx cannot invade host cells without attachment by intimin. The presence of Stx_2 further increases the virulence of the organism and makes it more likely to cause HUS and bloody diarrhea (Mathusa et al., 2010).

STECs can be further classified into five seropathotypes, A to E, based on the pathogenicity and frequency of occurrence. Type A are the most virulent pathogens including strains of O157:H7 and O157:H^- (non-motile). Seropathotype B contains non-O157 strains that have *eae*, stx_1, and/or stx_2. Six major strains of type B, also called the "Big Six", are O26:H1, O45:H2, O103:H2, O111:H8, O121:H9, and O145: H^-. Type C can sometimes cause HUS but not frequently associated with outbreaks, type D causes diarrhea and cannot cause HUS, and type E lacks intimin (*eae*) gene, required for invasion of *E. coli* and are non-pathogenic for humans (Karmali et al., 2003). Seropathotype A, O157:H7 and O157: H^-, has been associated with major outbreaks with different food products in the United States. Young, old, immunocompromised and pregnant population are more vulnerable to STEC infection. One of the major outbreak in 1993 associated with ground beef infected 732 people and caused death of 4 children (Golan et al., 2004). As a result of this outbreak and the public health risks that *E. coli* O157:H7 can pose, the United State Department of Agriculture (USDA) declared *E. coli* O157 as an adulterant and has a "zero tolerance" policy against it, which was further extended to six non-O157 STEC strains (USDA, 2011). Seropathotype B, Non-O157 STECs, are the STEC strains that do not have O157 as their somatic antigen. More than

200 non-O157 STEC strains have been associated with illness worldwide (Brooks et al., 2005). However, the big six strains are the most common occurring non-O157 STECs. About 82% of the human isolates from diseases collected by FoodNet from 2000-07 (n=803) belong to these six strains (Gould, 2009). The infectious dose of non-O157 STECs can be as low as *E. coli* O157 (Paton et al., 1996) and the disease onset time for non-O157 STECs is 3-4 days but it can be as low as 1-2 days (Mathusa et al., 2010). Four of the 'Big six' strains, O26, O103, O111, and O145, possess both stx_1 and stx_2 and therefore more virulent than others (Erickson and Doyle, 2007).

Non-O157 STEC infection has been linked with various types of foods like sausage, iceberg lettuce, milk, raw meat and poultry products. Contamination of beef carcasses with STECs is a major concern in the meat industry as these pathogens are naturally present in the intestine of ruminants (Bettelheim, 2001). Some researchers have found the prevalence of non-O157 STECs higher than *E. coli* O157 in beef carcasses. Beutin et al. (1997) found 63.2% of cattle feces positive with *E. coli* and all of the 33 strains collected were non-O157 STECs. Hussein (2007) found non-O157 STEC prevalence rate of 2.1 to 70.1% in different beef processing plants. Non-O157 STECs have been linked with various outbreaks in meat products. In 2007, an outbreak was caused by *E. coli* O26:H11 in beef sausage infecting 20 people. Among them, one patient developed bloody diarrhea and others reported mild symptoms. Recently, two multistate outbreaks in a fast food chain restaurant caused by non-O157 STEC infected 60 people in 14 states and 22 patients were hospitalized (CDC, 2016). It is suspected that contaminated meat was the main cause of these outbreaks, however, the exact sources could not be determined.

2.3 Heat resistance of STEC

The survival of *E. coli* at higher temperature is attributed to heat shock sigma factor σ^{32} encoded by *rpoH* gene. σ^{32} is very unstable at the optimum growth temperatures but becomes stable as the temperature increases and promotes transcription of heat shock genes (Nagai et al., 1991). Bukau et al., (1993) observed a 15-fold increase in the heat shock proteins (HSPs) in *E. coli* when the temperature was increased from 30 to 42°C. The HSPs consists of chaperones systems, which help in stabilizing and folding of proteins and protect them from heat damage. Heat resistance of *E. coli* O157 has been very widely studied in different laboratory media and food products like fluid milk (D'Aoust et al., 1998); beef and chicken (Juneja et al., 1997); apple cider (Ugarte-Romero et al., 2006) and liquid white (Geveke, 2008), however, limited data is available for thermal behavior of non-O157 STECs. Juneja et al. (1997) studied the thermal behavior of *E. coli* O157 in ground beef. The survival curves, surviving microbial population versus time, were generated at 55, 57.5, 60, 62.5, and 65°C. Decimal reduction time (D-value), was 21.13 min at 55°C, which decreased to 4.95, 3.17, 0.93 and 0.39 min for 55, 60, 62.5, and 65°C. The survival data in different environmental conditions can be used to develop a predictive model. The distribution of data in survival curves is very important to understand for developing a predictive model. In the next section, the basic principles of predictive modeling and the tools used for selection of a model, and validation of a model will be covered.

2.4 Predictive Modeling

Predictive models are widely used in the food industry to estimate the growth or death of pathogenic microorganisms in the storage or processing conditions, respectively.

The construction of a predictive model is divided into two main stages: Primary and Secondary modeling. As most of the scientific studies, a careful design of experiments is very important for developing an effective model. The estimations of predictive models can only be made by interpolation of data (Baranyi et al., 1996), therefore, the minimum and maximum values of environmental factors should be considered in the experimental design.

2.4.1 Design of Experiments

2.4.1.1 Complete factorial design

In complete factorial design, experiments are performed to investigate the effect of each factor level and data is collected in multiple replicates at each level of each factor. The advantage of this design is that it helps in determining the impact of factors with highest accuracy. However, this also means that a higher volume of experiments needs to be performed. For example, if there are four factors and three levels per factor, two biological replicates; a total to 3^4x2=162 experiments would be needed. This design is best suited for the conditions where number of factors is less and higher accuracy is demanded. Dalgaard et al. (1997) used complete factorial design to design an experiment for understanding the impact of temperature and carbon dioxide on shelf life of packed fish. Similarly, Chhabra et al. (1999) used a complete factorial design to quantify the effect of fat, pH and processing temperature on thermal inactivation of *Listeria monocytogenes* in milk. Three levels for each factor were selected and a complete factorial designs yielded 3x3x3x3=81 experiments with three replicates at each factor and level. In the present study, a complete factorial design to estimate the impact of two factors; fat and temperature, on heat resistance of non-O157 STECs was used. There

were six levels of fat and four levels of temperature and three biological replicates were performed. Hence, making the total number of 72 experiments (6x4x3).

2.4.1.2 Fractional factorial design

In fractional factorial design the number of experiments is reduced by omitting some factors and levels. A statistical software is used to minimize the impact of omitted experiments on the response variable. The goal of this experimental design is to obtain statistically similar information for the factors by performing lesser experiments as compared to a complete factorial design. Juneja and Eblen (1999) used fractional factorial design to develop a predictive model including the impact of temperature, pH, NaCl and sodium pyrophosphate on thermal inactivation of *L. monocytogenes*. There were four main factors and three levels for each factor and three replications per combination. The researchers would have to perform 3^4x3= 247 experiment runs for a complete factorial designs but they successfully reduced the number of experiment to 47 by using a fractional factorial design.

2.4.1.3 Central composite design

In this design, two levels: minimum and maximum are considered for each factor and at least one experiment is performed at the central intersection of all factors. Hence, making the total number of $2^k + 2k + n_o$, where k is the number of factors and n_0 is the number of experiments at central portion ($\geq$1). For example, to investigate the impact of three factors on the response variable, a minimum of 15 experiments will be required. Lebert et al. (1998) developed a predictive growth model for *Listeria monocytogenes* in meat broth. The impact of three factors, pH, temperature and NaCl, was studied for this

experiment. The temperature range was 4 to 14°C, a_w was 0.98-1.00 and pH ranged from 5.8 to 6.2. A total of 10 growth curves were generated based on the central composite experimental design as compared to 3^2x3=27 growth curves for a complete factorial design. Similarly, Geurzoni et al. (2002) also used central composite design to study the impact of pH, pressure treatment and NaCl concentration (%w/w) on survival of *S.* Enteritidis in egg-based products.

2.4.2 Primary modeling

After design of experiments and data collection, primary modeling is the first step of data analysis. The objective of primary modeling is to define the distribution of the data with highest accuracy and minimum residual sum of squares. It is very important to select the best performing primary model in order to have reliability in the secondary model. The following are the different types of primary models defined in the literature that help in understanding the behavior of survival curves.

2.4.2.1 Log-linear model

The most common and widely used model in thermal inactivation studies is the log-linear model, which assumes that the survival curves follow first order kinetics and population reduction is directly proportional to the time (Stumbo, 1973). This is the simplest primary model for thermal inactivation of microorganisms. The other major assumption of log-linear model is that all cells of a culture have similar thermal tolerance and they respond similarly to heat. The equation for log-linear model is given below.

$$log\ N_t = \quad log\ N_0 - b * t$$

$$D = \quad -\frac{1}{b}$$

Where, N_t = Bacterial population at a given time;
N_0 = Initial bacterial population at the target temperature;
t = time;
b = slope of the line;
D = Decimal reduction time (D-value).
N_t, N_o and t are known based on the experiments and b is estimated by using a curve fitting software.

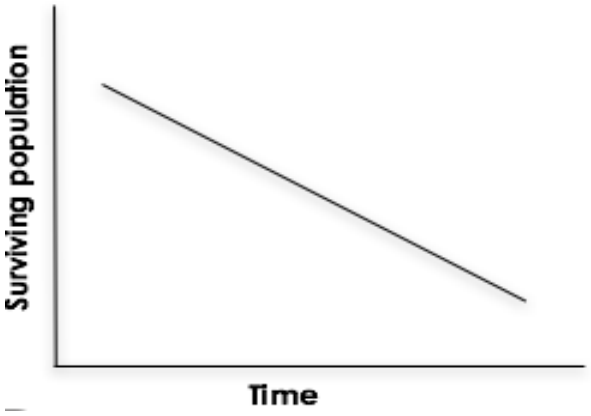

Figure 2.1 Log-linear inactivation model

Figure 2.1 shows a typical behavior of the log-linear primary model with bacterial population in log scale on the y-axis and time on the x-axis. Decimal reduction time (D-value) is calculated by talking a negative inverse of the slope. The data can further be extrapolated to calculate z-values (Stumbo, 1973). Various researches have used log-linear model to calculate D, and z-values of different microorganism in the past (Juneja et al, 1997; Juneja et al., 1999; Murphy et al., 2002; Luchansky et al., 2013). However, in most cases, survival curves deviate from the log-linear model hence creating a necessity to look for other primary models to define the distribution. The reason of the deviation could be the differential heat resistance of the cells, environmental factors that could act as a protective layer for the cells, differential stress response of the cells etc. Various non-linear models have been used by researchers in the past to define the data distribution of survival curves.

2.4.2.2 Log-linear with tail

The major assumption of this model is that a certain portion of the surviving population is very resistant to the treatment and it survives for a longer time period (figure 2.2; Geeraerd et al., 2005). This model was initially designed for bacterial spores but later adopted for vegetative cells for mild heat or other treatments. Greenacre et al. (2003) used this model for acid tolerance response of *Listeria monocytogenes* and *Salmonella enterica*. Lactic and acetic acids were used to adapt the pH of the growth media and the survival curves followed the log-linear with tail reduction. Similarly, Marquenie et al. (2003) applied this model for pulsed white light treatment for inactivation of fungi, *Monilia fructigena* and *Botrytis cinerea*. The survival curves showed a complete fit to the log-linear with tail model.

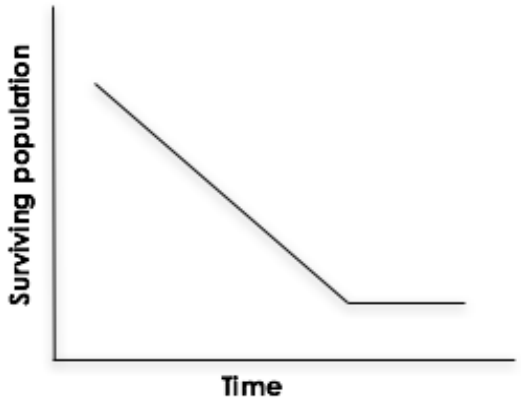

Figure 2.2: Log-linear with Tail inactivation model

$$\log_{10} N_t = \log_{10}((N_0 - N_{res})e^{-k*t} + N_{res})$$

Where, N_t = Bacterial population at a given time;
N_0 = Initial bacterial population at the target temperature;
t = time;
k= inactivation rate;
N_{res}=remaining heat resistant surviving population
N_t, N_o and t are known based on the experiments and k and N_{res} estimated by using a curve fitting software.

2.4.2.3 Biphasic Model

Biphasic inactivation of microorganisms also called Cerf model, shows two separate linear regions in the survival curve (Cerf, 1977), indicating two regions of different heat resistance in the population (figure 2.3). Microorganisms are assumed to have two death rates and hence two D-values for each set of population. Humpehson et al. (1998) studied the cause of biphasic inactivation of *Salmonella* Enteritidis PT4 in nutrient broth (NB) and reported that the initial cell concentration impacts the death kinetics. The survival curves obtained from less than 7-logCFU/ml initial concentration were linear and more than 7-logCFU/ml showed two phases of linear regions. By extrapolating the data, it was determined that 1 in 10^5 cells had higher heat resistance. Another reasons for tailing effect could be protective effect from the debris of dead cells, localized locations with low water activity, and induction of heat shock proteins (Allan et al., 1988; Cerf, 1977).

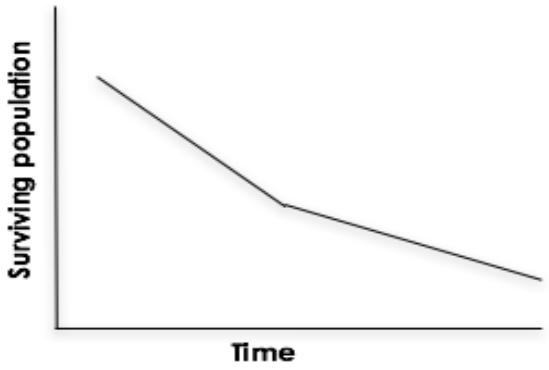

Figure 2.3: Biphasic inactivation model

$$\log_{10} N_t = \log_{10} N_0 - \log_{10}(f e^{-k_1 t} + (1-f)e^{-k_2 t})$$

$$D_1 = \frac{1}{k_1}$$

$$D_2 = \frac{1}{k_2}$$

Where, N_t = number of cells at a given time;
N_0= initial population;
f = portion of population that is more heat resistant;
k_1, k_2 = death rates of both populations respectively;
D_1, D_2 = D-values for both regions
N_t, N_o and t are known based on the experiments; f, D_1 and D_2 are estimated by using a curve fitting software

2.4.2.4 Modified Gompertz model

Gompertz model (figure 2.4) is used to understand the sigmoidal shape survival curves. The sigmoidal shape or inverted 'S' shape survival curves show significant deviation from the log-linear model. Two asymptotes are formed in this curve: upper and a lower asymptote (Bhaduri et al., 1991). Upper asymptote represents the lag phase and the lower asymptote represents the tailing effect. The center part of the Gompertz curves follow the first order kinetics with log-linear death rate. If the asymptotes are not present, then Gompertz model is similar to log-linear model. Linton et al. (1995 and 1996) and Bhaduri et al., (1991) used modified Gompertz model to understand the thermal inactivation behavior of *L. monocytogenes* in various food products. These authors reported that Gompertz model showed better representation of data distribution of survival curves than that of linear first order kinetics model.

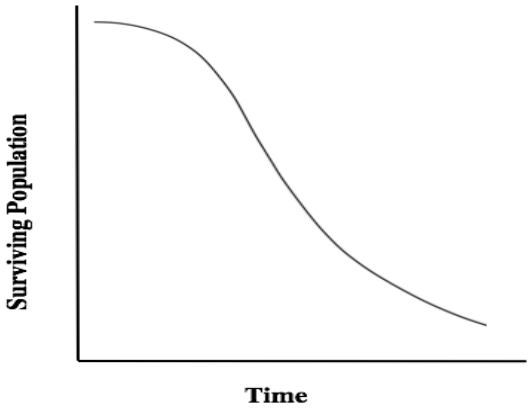

Figure 2.4: Gompertz inactivation model

$$\text{Log}\frac{N}{N_0} = A - Ce^{-e^{-B(t-M)}}$$

$$\mu_{max} = \frac{BC}{e}$$

$$t_{lag} = M - \frac{1}{B} + \frac{(\log N_0 - A)}{\mu_{max}}$$

Where, N_t = number of cells at a given time;
N_0= Initial population;
A = value for upper asymptote;
M = time at which absolute death rate is maximal;
B = relative death rate at M;
C = difference in the value between upper and lower asymptote;
μ_{max} = maximum death rate;
t_{lag} = lag phase of the survival curve.
N_t, N_o and t are known based on the experiments and A, B, C and M are estimated by using a curve fitting software

2.4.2.5 Sigmoidal model

The standard sigmoidal curve (figure 2.5) and the equation to define the curve are shown below. This model is also used for inverted 'S' shaped curve with longer lag phase and a tail. In this model, a and b are the function of lag period (shoulder), rate of inactivation, and the tail.

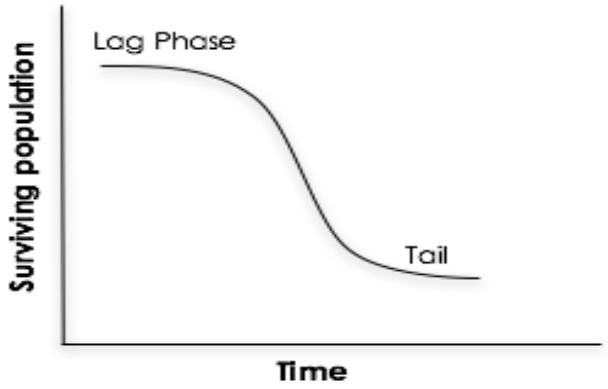

Figure 2.5: Sigmoidal inactivation model

$$\log_{10} N_t = \log_{10} N_0 - \log_{10}(1 + e^{a+b\log(t)})$$

Where N_t = number of cells at a given time;
N_0= initial population;
t = time in min.;
a, b = constants that define the shape of the curve

2.4.2.6 Weibull Model

Weibull model consists of two main parameters, b and n, that defines the slope and the shape of the curve respectively. For n=1, the Weibull function is same as the log-linear model. For n>1, the survival curve shows downward concavity and for n<1 an upward concavity is shown by the survival curves (figure 2.6a and 2.6b). An upward concavity of the Weibull model represents that the surviving cells have adapted to the hot temperature and shown more resistance initially. Similarly, downward concavity represents the tailing effect and shows that the surviving population and a downwards concavity represents that the remaining cells are more resistant to heat in the later phase of heat treatment (Van Boekel., 2002).

The equation defining the Weibull model is shown below.

$$\log_{10} N_t = \log_{10} N_0 - b * (t)^n$$

Where, N_t = number of cells at a given time;
N_0= Initial population;
b =function of slope of the curve;
n= function that defines the shape of the curve.
N_t, N_o and t are known based on the experiments and b and n are estimated by using a curve fitting software

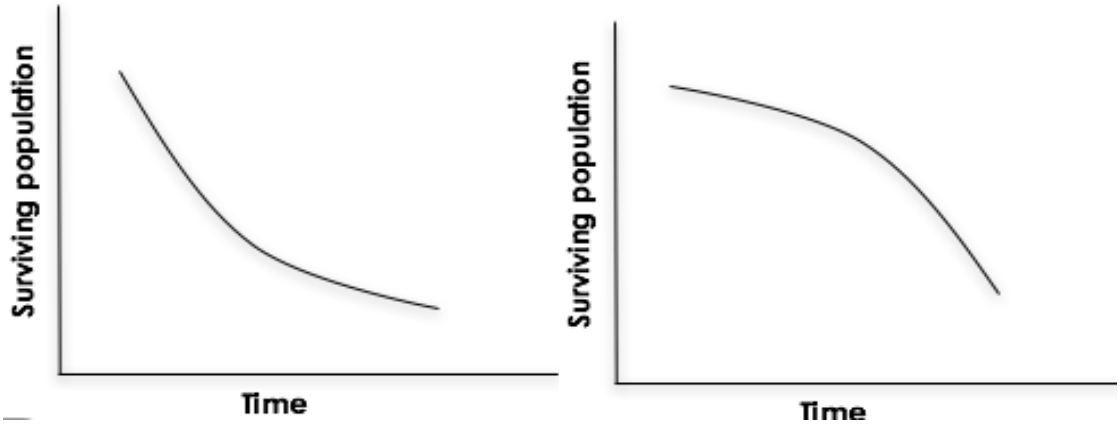

Figure 2.6 Weibull inactivation model (a) when n>1 (b) when n<1

Chen (2007) studied the high pressure inactivation of *Vibrio parahaemolyticus*, *L. monocytogenes*, *E. coli* O157:H7, *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Staphylococcus aureus* in milk. The author observed that Weibull model with downwards concavity (n>1) was the best fit for the distribution of survival curves. In another study, Couvert et al. (2005) also used Weibull model to understand the survial cuvres of *Bacillus pumilus* A40 spores at 89, 92, 95, 98, 101 and 104°C. All the survival curves also showed a downward concavity with n>1.

2.4.2.7 Mixed Weibull model

A mixed Weibull model (figure 2.7) is an extension of the existing Weibull model. This model assumes the co-existence of two portions with different heat resistance, and both populations follow Weibull model. The upper portion of the curve follows the Weibull model with upward concavity and the lower portion follows the

Weibull model with downwards concavity (Coroller et al., 2006). Coroller et al. (2006) studied the inactivation of *L. monocytogenes* and *S.* Typhimurium and observed that the survival curves showed two different heat resistance patterns. The authors developed the following mixed Weibull equation.

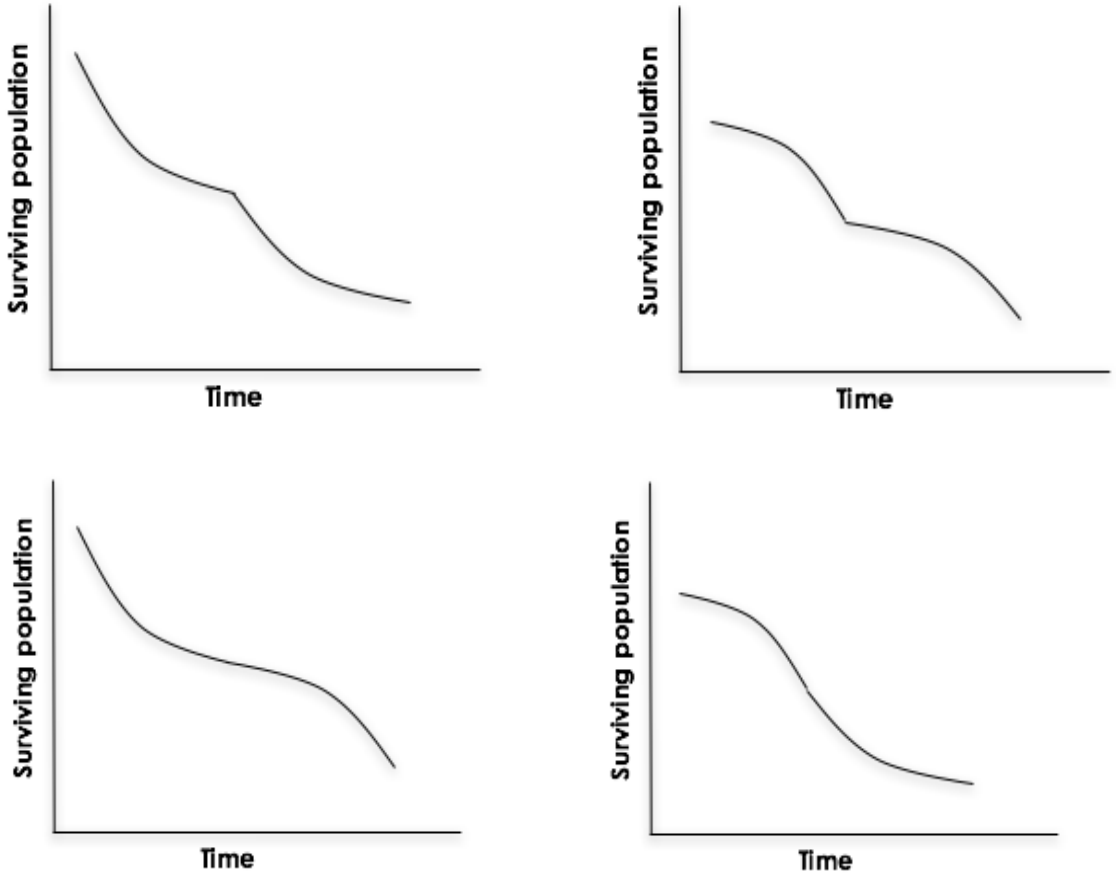

Figure 2.7: Four different possibilities of modified Weibull model.

$$N_t = \frac{N_0}{1 + 10^a}\left[10^{\left(\frac{t}{\partial_1}\right)^n + a} + 10^{\left(\frac{t}{\partial_2}\right)^n}\right]$$

$$a = \log_{10}\left(\frac{f}{1-f}\right)$$

Where, N_t = number of cells at a given time;
N_0= Initial population;
t = time

f= portion of population which has different heat resistance;
a = transformation of f
∂_1, ∂_2 = decimal reduction times for sub-population 1 and 2 respectively;
n= function of the shape of curve for each sub-population

2.4.2.8 Baranyi Model

Baranyi model (figure 2.8) was originally developed to understand growth of microorganisms in different environmental conditions (Baranyi and Roberts, 1994). Later on, researchers tried to fit this model for survival curves data and modified it to represent thermal inactivation (Baranyi et al., 1996). Baranyi model very commonly uses primary model for survival curves (Xiong et al., 1999, Pal et al., 2008, Farakos et al., 2013). u_{max}, h_0 and N_{min} are the functions of deactivation rate, lag phase and tailing of the curve. is the maximum The equation for Baranyi inactivation model is given below.

$$\log_{10} N_t = \log_{10} N_0 + u_{max} t + \frac{1}{u_{max}} \log(e^{-u_{max} \cdot t} + e^{-h_0} - e^{-u_{max} t - h_0})$$

$$- \log_{10} \left(1 + \frac{e^{u_{max} + \frac{1}{u_{max}} \log(e^{-u_{max} \cdot t} + e^{-h_0} - e^{-u_{max} t - h_0})} - 1}{e^{(\log_{10} N_{min} - \log_{10} N_0)}} \right)$$

Where, N_t = number of cells at a given time;
N_0= Initial population;
t = time,
u_{max}= maximum kill rate;
N_{min} = minimum population after the treatment;
h_o = function lag phase or shoulder;

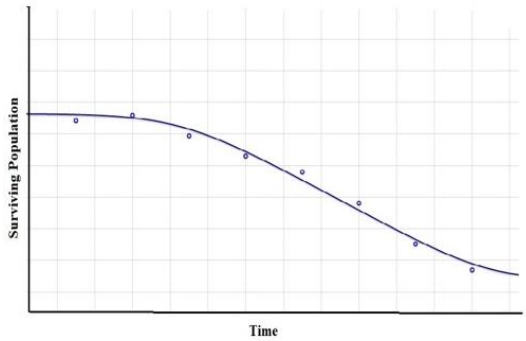

Figure 2.8: Baranyi inactivation model

A Microsoft Excel extension, DMFit, has been developed by the Institute of food research which uses Baranyi Model as a primary model and estimate growth/death parameters of the curves (Institute of Food Research, 2016). DMFit has been used in many publications for curve fitting data analysis (Koutsoumanis et al., 1999; Aljarallah et al., 2007; Luchansky et al., 2013).

2.4.3 Best Performing Primary Model

In predictive modeling, it is assumed that only one primary model defines the distribution of data, i.e. best fitted in the distribution and then its parameters are estimated (Burnham and Anderson, 2004). Secondary model is developed on the basis of primary model. Hence, it is very important to find the best performing primary model for a given set of data. Various performance measuring statistical tools can be used to identify the primary model. Following are the most common accuracy measurement criterion used for estimating the best performing primary model.

2.4.3.1 Residual sum of squares (RSS)

The residual sum of square (RSS) is calculated by adding the square of the difference between observed value and prediction value. Higher RSS values indicates that the predicted values are different from the observed values. Therefore, a lower RSS value is desired for the best fitted model. An RSS value of 0 would indicate the model is a complete fit and an RSS value is also used for parameter estimation of a given primary model for a survival curve. The parameters that yields minimum RSS value are selected.

$$RSS = \sum_{i=1}^{n} \left(y_i - f(x_i)\right)^2$$

Where, y_i= observed value at *i*
$f(x_i)$= predicted value at *i*

2.4.3.2 Akaike Information Criteria

Akaike information criteria (AIC) is a function of RSS and number of parameters to be estimated by the model. The model selection based on AIC numbers works on the principal of parsimony. If two or more models have similar RSS value, then the model with minimum number of parameters is selected (Akaike 1981). As the number of parameters increases, the error related to each parameter also increases and reduces the accuracy of the model. Hence, a penalty for number of parameters to be estimated has been included in the AIC formula. The following equation is used for calculating AIC value of a primary model

$$AIC = \quad n * \log_{10}\left(\frac{RSS}{n}\right) + 2k$$

Where, n = number of data points on the curve;
RSS = residual sum of squares;
k = number of parameters.

2.4.3.3 Akaike's weights (w)

The AIC number is a good criterion to measure best performing model for a single surviving curve. However, in order to identify primary model in a survival curve with multiple environmental factors, a weighted value of AIC is taken. The following equations are used to calculate combined AIC weight for each primary model and the model with highest weight is selected for further analysis (Burnham and Anderson, 1998). Minimum AIC value for one survival curve is subtracted from all other AIC values resulting in a Δ_i of 0 for the best fitting model for the curve. Similarly, Δ_i is calculated for other survival curves and a net AIC weightage is calculated. An AIC weight of 10 means that there is five times more confidence in choosing this model over the model with *w* of 2 (Link et al., 2006).

$$\Delta_i = AIC_i - minAIC$$

$$w_i = \frac{e^{(-0.5\Delta_i)}}{\sum_{r=1}^{R} e^{(-0.5\Delta_r)}}$$

Where, AIC_i= AIC number of model *i*
minAIC= minimum AIC among all primary models.
Δ_i = AIC difference for model *i*
R= number of primary models

2.4.3.4 Bayesian information criterion

Bayesian information criterion (BIC) is directly proportional to the log-likelihood function of model and can also be used to differentiate best performing models among a group (Schwarz, 1978). Minimum BIC value is preferred for a best fitted model.

$$BIC = \quad -2 * ln\,(L) + k * ln\,(n)$$

Where, L = maximized value of likelihood function;
k=number of parameters;
n= number of data points;

2.4.3.5 Accuracy and Bias factors

Baranyi et al. (1999) proposed Accuracy factor (A_f) and Bias factor (B_f) to test the performance of a predictive model. These factors are used to measure average deviation of the prediction from the observed data point. A_f can further be used to measure percentage discrepancy (D_f), that tells the average error of prediction. A D_f of less than 25% is desired for a good predictive model (Ross et al., 2000). B_f provides the information if the model is overestimating or underestimating the predicted value. The A_f and B_f of 1 shows that the model is predicting the exact value as the experimental data. The major difference between A_f and B_f formulas are that the B_f formula uses the value with sign of Log (N_{model}/N_{data}) value, whereas A_f uses the absolute value. B_f >1 shows

that model is overestimating and B_f<1 shows the model is underestimating the predicted variable.

$$\%B_f = sgn(B_f) * (B_f - 1) * 100$$

$$B_f = 10\text{^}\left[\frac{\sum_1^n\left(\log\frac{N_{model}}{N_{data}}\right)}{n}\right]$$

$$sgn(B_f) = \begin{pmatrix} +1 & if & B_f > 0 \\ 0 & if & B_f = 0 \\ -1 & if & B_f < 0 \end{pmatrix}$$

$$A_f = 10\text{^}\left[\frac{\sum_1^n\left|\left(\log\frac{N_{model}}{N_{data}}\right)\right|}{n}\right]$$

$$\%D_f = (A_f - 1) * 100\%$$

Where, $LogN_{model}$ = predicted value
$LogN_{Data}$ = observed value
n= number of data points

2.4.3.5 F test

The F-test can also be used to test the performance of a primary model. The mean square error of the model is calculated and compared with the mean square error of data. The f-value is compared with the F-table of 95% confidence interval with degree of freedom of the model and the data. The following equations are used for the analysis.

$$MSE_{data} = \frac{\sum_{i=1}^{m}\sum_{i=1}^{k}\left(average\log_{10}N^i - \log_{10}N^{ij}\right)^2}{n-m}$$

$$MSE_{model} = \frac{\sum_{i=1}^{n}\left(\log_{10}N^i_{observed} - \log_{10}N^i_{fitted}\right)^2}{n-s}$$

$$f = \frac{MSE_{model}}{MSE_{data}}$$

Where, $N_{observed}$ i is the observed population value

N_{fitted} *i* is the fitted population level
n is the number of data points,
s is the number of parameters of the model,
m is the number of time points (sampling times),
k is the number of replicates at each time point,
average *Ni* is the mean value of the population at time point *i*,
Nij is the population at time point *i* for specific replicate *j*

2.4.4 Secondary Modeling

Secondary modeling analysis is performed to incorporate the impact of environmental factors in the equation of best performing primary model. Response surface modeling (rsm) has been widely used for the secondary modeling analysis for predictive modeling (Buchanan et al., 1994; Aouadhi et al., 2013; Wang et al., 2014). This multiple regression analysis defines the output variable as a second degree polynomial function of input variable. Wang et al. (2014) developed a predictive model inactivation of *Vibrio parahaemolyticus* in acidic electrolyzed water on cooked shrimp by using this method. Similarly, Aouadhi et al. (2013) used rsm for modeling of inactivation of *Bacillus sporothermodurans* spores in high hydrostatic pressure with combination of mild heat. The following equation is developed as secondary model for each primary model parameter.

$$x_1 = ß_0 + ß_1E_1 + ß_2E_1^2 + ß_3E_2 \ldots\ldots\ldots\ldots\ldots + ß_nE_1E_m + e$$

Where, x_1 is a parameter of best performing model;
E_1, E_2…E_m: Environmental factors
e= random error
$ß_1$ to $ß_n$= coefficients of the model

2.5 References

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CHAPTER 3: THERMAL INACTIVATION OF SHIGA TOXIN PRODUCING *ESCHERICHIA COLI* IN GROUND BEEF WITH VARYING FAT CONTENT

Abstract

Decimal reduction time (D-value) was calculated for six non-O157 shiga toxin producing *Escherichia coli* (STEC), in laboratory medium and ground beef at 55, 60, 65, 68 and 71.1°C. For laboratory medium, overnight grown cultures were divided into 10ml sample bags and heated in a water bath for a specific time based on temperature. Survival curves were generated by plotting the surviving bacterial population against time and linear-log primary model was used to estimate the D-values from survival curves. z-values were calculated by plotting the log D-values against temperature. Similarly, for ground beef, six fat contents, 5, 10, 15, 20, 25, and 30% were used. Inoculated meat was divided into 5-g sample bags and submerged in water bath set at specific temperatures (55, 60, 65, 68, and 71.1°C). Results showed no significant differences ($p>0.05$) in the D-values between the six strains of STECs in laboratory medium at all temperatures. There was a negative correlation between fat content of ground beef and D-values at 55°C. However, at temperatures greater than 60°C, there was no impact ($P>0.05$) of fat content of ground beef on the thermal resistance of non-O157 STECs. The data generated can be helpful for the meat industry to develop predictive models for thermal inactivation of non-O157 STECs in ground beef.

3.1. Introduction

Heat treatment is typically a critical control point (CCP) as part of the overall food safety system for cooked and ready to eat (RTE) meat and poultry products. To make this process successful in eliminating and/or reducing pathogens, it is important to understand the heat resistance of target bacteria. Decimal reduction time (D-value) and the temperature raised to reduce D-value by one tenth (z-value) are critical parameters that help decide the processing limits to ensure safety of meat products (Stumbo, 1973). The F-value, defined as time taken to kill a known population of microorganisms, is calculated from the D and z-values and is used to obtain standard operating conditions (SOC) for meat processing. Hence, the understanding of D, z and F-values is important to ensure food safety of cooked and RTE meat and poultry products.

A major outbreak of *Escherichia coli* O157:H7 (ECO157) in 1993 lead to the United States Department of Agriculture (USDA) enforcing zero tolerance of this pathogen in ground beef in the United States. This multistate outbreak infected 732 people, of which 4 children died and 178 patients developed hemolytic uremic syndrome (HUS; Golan et al., 2004). Like *E. coli* O157:H7, non-O157 Shiga toxin producing *E. coli* (STEC) strains have the potential to cause bloody diarrhea, which can further develop into HUS, especially in young, old, pregnant, and immunocompromised populations. More than 200 other serotypes of non-O157 have been isolated from infections worldwide (Brooks et al., 2005). However, six serotypes, distinguished based on their O antigen, are most common. These six strains have resulted in approximately 71% of the infections in the US from 1993 to 2002 (Brooks et al., 2005) and are also known as ‘Big Six’; *E. coli:* O26:H1, O45:H2, O103:H2, O111:H8, O121:H9, and O145:

non-motile. Scallan and others (2011) estimated about 110,000 illnesses caused by the non-O157 group in the US.

Various foods such as dairy, leafy vegetables, game meat, beef, pork, fruit and nuts have been linked to non-O157 outbreaks in the US (Gierke et al., 2014). In 2010, two of the big six, *E. coli* O103 and *E. coli* O145, were isolated from an outbreak caused by consumption of undercooked venison in Minnesota (CDC, 2010), which infected 29 high school students and two of them were hospitalized. In a multistate outbreak related to the non-O157 group, consuming contaminated romaine lettuce infected 27 people, of which 14 were hospitalized and three developed HUS (Taylor et al., 2013). Pulsed field gel electrophoresis (PFGE) confirmed the presence of *E. coli* O145:Non-motile strain causing the outbreak. Recently, two outbreaks caused by non-O157 STEC in a fast food chain restaurant infected 60 people in 14 states, hospitalizing 22 (CDC, 2016). Even though the exact ingredient that caused this outbreak is not known yet, it is expected that consumption of undercooked meat was the cause. Whole genome sequencing (WGS) and DNA fingerprinting analysis confirmed the presence of *E. coli* O26 in stool samples of the patients. In another study, the Connecticut Department of Health reported 51% (n=403) laboratory confirmed STEC infections were caused by non-O157 STECs (CDC, 2007).

More non-O157 outbreaks were observed in the last decade because of increased surveillance methods and more strain specific tests being performed. The actual number of infections could likely be higher than reported, as only 4% of the laboratory in the US actively screen for non-O157 group infections (Kalchayanand et al., 2012). Multiple outbreaks, wide variety of food vehicles, and potential pathogenicity of the non-O157

group have resulted in the zero-tolerance policy of USDA-FSIS in raw and non-intact beef. These pathogenic strains are considered as an adulterant in beef products (USDA, 2011), therefore eliminating *E. coli* O157:H7 and the non-O157 STECs from beef and beef products is very critical to the meat industry.

Thermal inactivation of pathogens during meat processing could be affected by various intrinsic and extrinsic factors; fat content being one of them. Juneja and Eblen (2000) studied the impact of fat content in ground beef on the thermal inactivation of *Salmonella* Typhimurium. An increase in the death rate, indicating a lower D-value, was observed in beef with higher fat content at 55, 58 and 62°C. However, a longer lag period was observed with more fat, which increased the overall D-value along with increased fat content of ground beef. The researchers concluded that fat acts as a protective barrier for cells and hence caused a longer lag period. In another study, Kotrola et al., (1997) did not observe any significant impact of fat content on the thermal inactivation of *E. coli* O157 in ground turkey meat. The thermal behavior of *E. coli* O157:H7 has been very well studied and documented in various food products and conditions; however, there is limited information available for the non-O157 group in literature. Given the contrasting reports that are available on behavior of *E. coli* O157:H7 and the non-O157 STECs, and to bridge the gap of knowledge, this study was conducted to determine the thermal inactivation parameters for non-O157 STECs in laboratory medium and ground beef with varying fat contents at 55, 60, 65, 68 and 71.1°C.

3.2. Materials and Methods

3.2.1 Bacterial Strains

The bacterial strains used for this study were obtained from American Type Culture Collection (ATCC). The six strains used were *E. coli* O26:H1 ATCC BAA 2196 (ECO26), *E. coli* O45:H2 SJ9 (ECO45), *E. coli* O103:H2 87.1368 (ECO103), *E. coli* O111:H8 ATCC BAA 179 (ECO111), *E. coli* O121:H9 ATCC BAA 2221 (ECO121), and *E. coli* O145:Non-motile ATCC BAA 2192 (ECO145). Nalidixic acid resistance (NAL^+) was induced to differentiate the cells from the background flora of ground beef. Bacterial strains were grown overnight in Tryptic Soy Broth (TSB; Neogen, Lansing MI) at 37°C to target populations of 8-9 log CFU/ml and 100µl of the inoculum was transferred to two tubes for each strain containing 10ml of TSB supplemented with 5ppm of NAL, and incubated at 37°C for 24h. Following this, 100µl of the inoculum was transferred to another 10ml of TSB supplemented with 10ppm of NAL and this process was repeated until a 50ppm NAL resistance was induced in the strains. The NAL^+ strains were stored on slants of plate count agar (PCA; Neogen, Lansing, MI) supplemented with 50ppm of NAL at 4°C for future use. Fresh slants were prepared every six weeks by repeating the above process.

3.2.2 Growth Curve

Each strain of non-O157 STEC was grown in TSB supplemented with 50ppm of Nalidixic acid (NAL; Fisher BioReagents, Fair Lawn, NJ) overnight at 37°C. Cultures were then serially diluted to 10^{-6} and 1ml of the culture was used to inoculate 99ml of TSB + NAL. Following this, 2ml of the sample was taken every hour and the bacterial

population was enumerated by serial dilution in 0.1% peptone water (PW; Neogen, Lansing, MI) and plating onto PCA supplemented with 50ppm of NAL. A 100µl portion of the sample was used to measure absorbance at 600nm wavelength using an Epoch spectrophotometer (BioTech, Winooski, VT). The data were collected for up to 24h and the increase in the absorbance of light was co-related with the increase in the cell concentration. The data were analyzed with curve fitting software DMFit (Institute of Food Research, Colney, UK) to estimate the growth parameters.

3.2.3 Laboratory Medium

For the first part of this experiment, the D-values of these pathogenic strains was studied in TSB. A 10µl loop from slants was used to inoculate 200ml TSB supplemented with 50ppm NAL and incubated overnight at 37°C. Following this, 10ml of the inoculum was transferred into sterile bags (3”x5”, Fisher Brand, Fisher Scientific, Waltham, MA) and a total of 12 bags were prepared for each temperature exposure and submerged in a thermostatic water bath (Model: Haake A25B, Fisher Scientific, Waltham, MA) set at 55, 60 and 65°C with an immersion circulator (Model: Haake AC150, Fisher Scientific, Waltham, MA). Bags were removed from the water bath at a fixed interval (10 min. for 55°C, 1 min. for 60°C and 15s for 65°C) and cooled immediately in an ice water bath. Temperature of the inoculated TSB in bags was monitored by inserting a K-type thermocouple connected with a temperature data logger (Model: HH806AU, Omega Engineering, Stamford, CT). After cooling, serial dilutions were made in 0.1% PW and plated onto PCA supplemented with 50ppm NAL. Plates were then incubated for 48h to provide time for recovery of heat-treated cells, and the

bacterial population at each time interval was calculated (log CFU/ml) and plotted against the exposure time.

3.2.4 Ground Beef

For the second part of this experiment, the heat resistance of non-O157 STECs in ground beef with varying fat content was studied. Ground beef was obtained from the Purdue University Meat Lab. Meat was trimmed to remove all visible fat and then the required amount of beef fat was added to make the desired fat content of 5, 10, 15, 20, 25 and 30% (%w/w). Meat was ground three times to obtain a homogenous distribution of fat and pouches containing 100g of ground beef were prepared and stored at -20°C. Meat was thawed at 4°C for 24h before use. A cocktail of NAL^+ non-O157 STECs was used in this study to inoculate ground beef. NAL^+ phenotype helped to select against background flora of meat. A cocktail of all six strains was prepared and after 24h of incubation, cells were washed twice with 0.1% PW after centrifugation (Model: Sorvell Legend XTR, Thermo Scientific, Waltham, MA) at 4700xg, for 10 min. at 4°C and re-suspended in 1ml of 0.1% PW. The washed cells were homogenized to dissolve the pellets, and all strains were mixed together to prepare the cocktail. One ml of the cocktail was used to inoculate 100g of ground beef to achieve a target initial population of ca. ~log 7-8 CFU/g of ground beef. After inoculation, ground beef pouches were hand massaged for 2 min. for homogenous distribution of cells and maintained at room temperature for 30 min. for attachment of cells to the meat. Small pouches (figure 3.1, 7.6 x 12.7 cm or 3”x5”) containing 5 ± 0.05g of inoculated meat were heat sealed, and flattened to a target 1-2 mm thickness to facilitate even distribution of heat during thermal treatment. The pouches were submerged in a thermostatic circulating water bath (Model: Haake A25B, Fisher Scientific, Waltham, MA)

set at four temperatures: 55, 60, 65 and 71.1°C as showin in figure 3.2. Bags were removed from the water bath at a fixed time interval (7.5 min. at 55°C, 30s at 60°C, 5s at 65°C, 2s at 68°C and 1s at 71.1°C) and cooled in ice water for instant cooling. In order to enumerate the survival population of bacteria, meat from bags was aseptically transferred to filter bags (7 oz., Nasco Whirl-Pak, Atkinson, WI) followed by addition of 5ml (1:1) of 0.1% PW. Filter bags were homogenized in a Stomacher (Stomacher 400, Steward Limited, West Sussex, UK) at 260 rpm for 60s, serially diluted in 9ml of 0.1% PW and then plated onto PCA supplemented with 50 ppm NAL. Plates were incubated for 48 h at 37°C to provide recovery time for heat injured cells and the populations were reported as log CFU/g of ground beef.

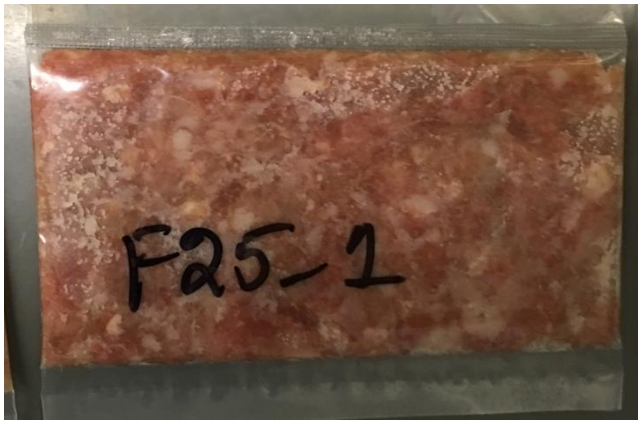


Figure 3.1 A pouch (7.6 x 12.7 cm) containing 5-g inoculated ground beef used for thermal resistance study.

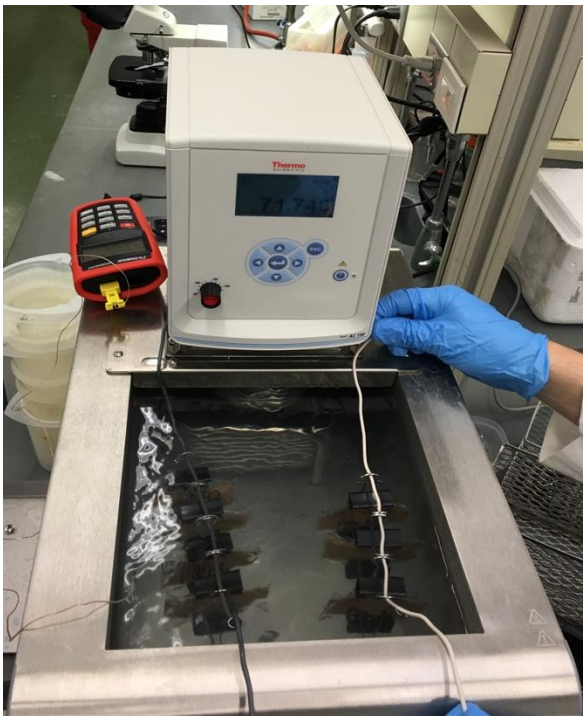


Figure 3.2: Pouches containing inoculated ground beef submerged in a water-bath with a thermocouple and datalogger to monitor temperature.

3.2.5 Fat content of ground beef

Fat content of the ground beef samples was measured by Soxhlet extraction with petroleum ether (ACS, VWR International, Radnor, PA). The samples were weighted (approx. 2-3 g) in a filter paper, and dried in a convection oven (Binder, Cole-Parmer, Vernon Hills, IL) at 105°C for 18-24h (AOAC, 1995 Method 939.60). Dried samples were transferred into a Soxhlet apparatus and heating temperature was set to obtain condensation rate of 4-5 drops/s. The extraction was performed for 6 h followed by overnight drying of samples at 105°C. Three replications for each sample were performed. Sample weight, pre-extraction and post-extraction weights were measured the following equation was used to calculate fat content:

$$Fat\ content\ (\%) = \left(\frac{B - C}{A}\right) * 100$$

Where, A = sample weight (g);
B = weight of filter paper prior to extraction (g);
C = weight of filter paper after extraction (g).

3.2.6 D-values and Statistical analysis

Survival curves were generated for each experiment set by plotting log survival population against exposure time. A log-linear primary model, in which the log number of the bacterial population decreases linearly with time, was used to calculate D-value:

$$\log_{10} \frac{N_t}{N_o} = \quad b * t$$

$$D = \quad -\frac{1}{b}$$

Where N_t = bacterial population at a given time;
N_0 = Initial bacterial population at the target temperature;
t = time
b = slope of the line
D = decimal reduction time (D-value)

A minimum of five data points with a coefficient of regression (r^2) more than 0.90 were used to estimate the slope of the curve. Three replicates for each experiment were performed After calculating the D-values from the curves, analysis of variance of the data was performed using SAS® (SAS Institute, Cary, NC, USA) with a 95% confidence interval. The z-values were computed by plotting log of D-values against the temperature. The negative inverse of the slope of this plot was taken to compute z-values (time taken to reduce D-value by one log).

3.3. Results and Discussion

3.3.1 Growth Curve

The bacterial cells from the stationary phase were harvested and used in this study as they have been reported to be more heat resistant than the cells in the exponential phase (Gauthier and Clement, 1994). Growth curves were generated for each strain of non-O157 STECs to identify the lag, log and stationary phases of growth. Figure 3.3 illustrates the growth pattern of each strain of non-O157 STEC. DMFit analysis showed a complete fit of Baranyi and Roberts primary growth model (Baranyi and Roberts, 1994) with the coefficient of regression as 0.97, 0.97, 0.97, 0.98, 0.98, and 0.99 for ECO26, ECO45, ECO103, ECO111, ECO121, and ECO145, respectively. The growth parameters for the six strains are shown in Table 3.1. Based on these results bacteria were grown for 18 to 24h at 37°C before making a cocktail of the six non-O157 STECs to target the cells in the stationary phase of their growth.

3.3.2 Laboratory media

Thermal inactivation of each strain of non-O157 was studied in TSB supplemented with 50 ppm of NAL. The survival curves at 55, 60, and 65°C for each strain of non-O157 STEC are shown in figure 3.4. When the overnight grown cultures were heated at 55°C, an average lethality of 6.13, 5.17, 5.44, 5.57, 5.01, and 4.65 log CFU/ml of bacterial population was observed for ECO26, ECO45, ECO103, ECO111, ECO121, ECO145, respectively after 60 min. of heating. Similarly, the lethality at 60°C was 6.05, 4.50, 6.38, 5.93, 4.98, and 5.88 log CFU/ml after 9 min. of heating and at 65°C, lethality was 3.51,

5.32, 4.41, 4.50, 3.08, 5.59 log CFU/ml after 1.5 min. of heating for ECO26, ECO45, ECO103, ECO111, ECO121, ECO145, respectively.

The D-values, calculated by fitting primary log-linear model to the survival curves, are shown in figure 3.5. The average D-value, irrespective of strain, at 55°C was 17.96 min. and it reduced significantly ($p<0.05$) to 1.58 min. at 60°C and then further reduced ($p<0.05$) to 0.46 min. at 65°C. The $D_{55°C}$ values were 20.92, 19.68, 19.51, and 19.69 min. for ECO45, ECO103, ECO121 and ECO145. respectively and there was no significant difference ($p<0.05$) among these strains. D-values for ECO26 and ECO111 were recorded as 14.37 and 13.63 min. respectively, and they were significantly lower ($p<0.05$) than the other four strains. The results indicate that ECO45, ECO103, ECO121 and ECO145 were more heat resistant than ECO26 and ECO111 and longer heat treatment is required to eliminate these pathogens from food at 55°C. At 60 and 65°C, there was no significant difference ($p>0.05$) in the heat resistance of all six of these non-O157 STECs. The $D_{60°C}$ was 1.63, 1.77, 1.40, 1.54, 1.61, and 1.58 min. for ECO26, ECO45, ECO103, ECO111, ECO121 and ECO145, respectively. The $D_{65°C}$ values were 0.44, 0.45, 0.55, 0.45, 0.53, and 0.33, respectively. The results indicate that in laboratory medium, it would take 2.25, 2.75, 2.25, 2.65, and 1.65 min. for a 5D treatment, reducing 5 log CFU/ml population, of ECO26, ECO45, ECO103, ECO111, ECO121 and ECO145, respectively at 65°C.

Based on the D-values at three different temperatures, z-values were computed. The z-value was also used to estimate D-values at unknown temperatures by extrapolating the graph (Stumbo. 1973). The z-values were 6.6, 6.0, 5.8, 6.6, 6.4, and 5.6°C for ECO26, ECO45, ECO103, ECO111, ECO121 and ECO145, respectively. The

results show that an increase of 6.6°C can reduce the D-value to one tenth for ECO26. Hence the D-value of ECO26 can be estimated at 0.044 min. (2.64s) at 71.6°C.

3.3.3 Ground Meat

There was no significant difference ($p>0.05$) in the thermal behavior of single strain non-O157 STECs, hence a cocktail containing all six strains was used to study their heat resistance in ground beef. Fat content of the ground beef samples was 8.03 ± 1.524, 11.97 ± 0.999, 17.66 ± 0.737, 21.70 ± 1.586, 28.52 ± 0.325, and 31.04 ± 0.786% for F5, F10, F15, F20 F25 and F30 respectively. Survival curves generated from the inactivation of non-O157 STECs in ground beef are shown in figure 3.6. Sample bags containing 5g of ground beef were prepared and subjected to heat treatment as described in the methods section 2.4. The average come-up time, was 31.2, 32.2, 27.2s, and 18.2s for 55, 60, 65 and 68°C, respectively, irrespective of the fat content of the meat. Luchansky et al., (2013) observed a quicker come-up time, 9.5, 8.1 and 8.1s for 54.4, 60 and 65.6°C, for non-O157 STEC strains in ground beef, however, this could be attributed to the use of 3g meat pouches as compared to 5g pouches that were used in our study.

The D-values decreased significantly ($p<0.05$) when the temperature increased from 55 to 68°C (table 3.2). The $D_{55°C}$ ranged from 11.69 to 15.93 min. in 5 to 30% of fat content of ground beef. There was a significant decrease ($p<0.05$) in the D-value when the fat content increased from 5% to 10%. However, no significant difference was observed when the fat content was increased from 10 to 25%. A further significant decrease ($p<0.05$) was observed from 25% to 30% fat content. Overall, the D-values showed a negative correlation with fat content of ground beef at 55°C, indicating that low heating times are required at higher fat content to get similar lethality at 55°C. Vasan et

al., (2014) compared the heat tolerance of non-O157 STECs with *E. coli* O157:H7 and observed a significant decrease ($p<0.05$) in the D-values of *E. coli* O45:H2 from 29.26 min. to 21.07 min. when the fat content of the ground beef was increased from 7% to 27%. There was no impact of fat content on the heat tolerance of other five of the 'Big six' non-O157 STEC strains. However, some researchers have shown that heat resistance of organisms increases with the fat content of ground beef. Juneja and Eblen (2000) studied the heat resistance of *Salmonella* Typhimurium in ground beef with varying fat content and observed a 'shoulder' or 'lag period' in the survival curve, followed by a log-linear phase. The researchers suggested that the fat content act as a protective layer and caused the shoulder/lag-period effect. The lag-period was added to the D-values to estimate the time for 5-D (5-log reduction) process. 'Lag period' increased significantly ($p<0.05$) from 4.43 to 28.12 min. when the fat content was increased from 7 to 24% but the D-values decreased from 3.22 to 1.61 min. for the same increase in fat content and hence increased the heat resistance of *S.* Typhimurium. In our study, lag-phase in the survival curves was not significant ($p>0.05$) irrespective of fat content and hence a negative correlation was observed.

At 60°C, a total of 3.99 log CFU/g lethality was calculated in 1.5 min. irrespective of the fat content. There was no significant difference ($p>0.05$) in heat resistance of the non-O157 STECs due to change in the fat content of ground beef. The $D_{60°C}$ values were 1.15, 1.16, 1.06, 1.11, 0.91, and 1.12 min. for 5, 10, 15, 20, 25, and 30% fat content of ground beef, respectively. Similarly, at 65 and 68°C, there was no significant ($p>0.05$) impact of fat content on the D-values. The $D_{65°C}$ values were 0.14, 0.14, 0.12, 0.11, 0.10, and 0.09 min. and the $D_{68°C}$ values were 0.05, 0.05, 0.06, 0.07,

0.06, and 0.05 min. for 5, 10, 15, 20, 25, and 30% fat content of ground beef. The average lethality irrespective of fat content was 3.94 and 3.66 log CFU/g at 65 and 68°C in 30 and 14s. Our results are in agreement with a study conducted by Luchansky et al. (2013), where it was reported that there were no significant differences in $D_{60°C}$ values of non-O157 STECs in ground beef with 7 and 30% fat content.

The z-values of non-O157 STEC strains and the coefficient of regression of the plot between log D-value v/s temperature are shown in table 3.3. The coefficient of regression for the z-value curves was greater than 0.98 and ranged from 5.28 to 5.60°C for all the fat contents. The z-values calculated for this data are in the proximity of z-values reported for *E. coli* O157 in the literature. Osaili et al. (2006), observed the z-value of 5.2°C in ground beef. Similarly, Juneja et al. (1997), observed z-value of 6.0°C for a four strain cocktail of *E. coli* O157 in ground beef with 10% fat. In this study, an attempt was made to collect thermal inactivation data at 71.1°C in ground beef, however, due to lower initial bacterial numbers because of a higher kill rate during the come-up time, the data could not be collected. The survival curves are shown in figure 3.7. This data confirms that 71.1°C (160°F) is a lethal temperature for non-O157 STECs in ground beef. The data collected in this study is useful to enhance our knowledge about the heat resistance of the non-O157 STECs and further provide information to develop predictive models for thermal inactivation of non-O157 STECs in ground beef. These predictive models that are then generated from various data sets such as the one generated on our study can prove to be useful for meat processers to set up their standard operating conditions for safer processing of RTE meat products.

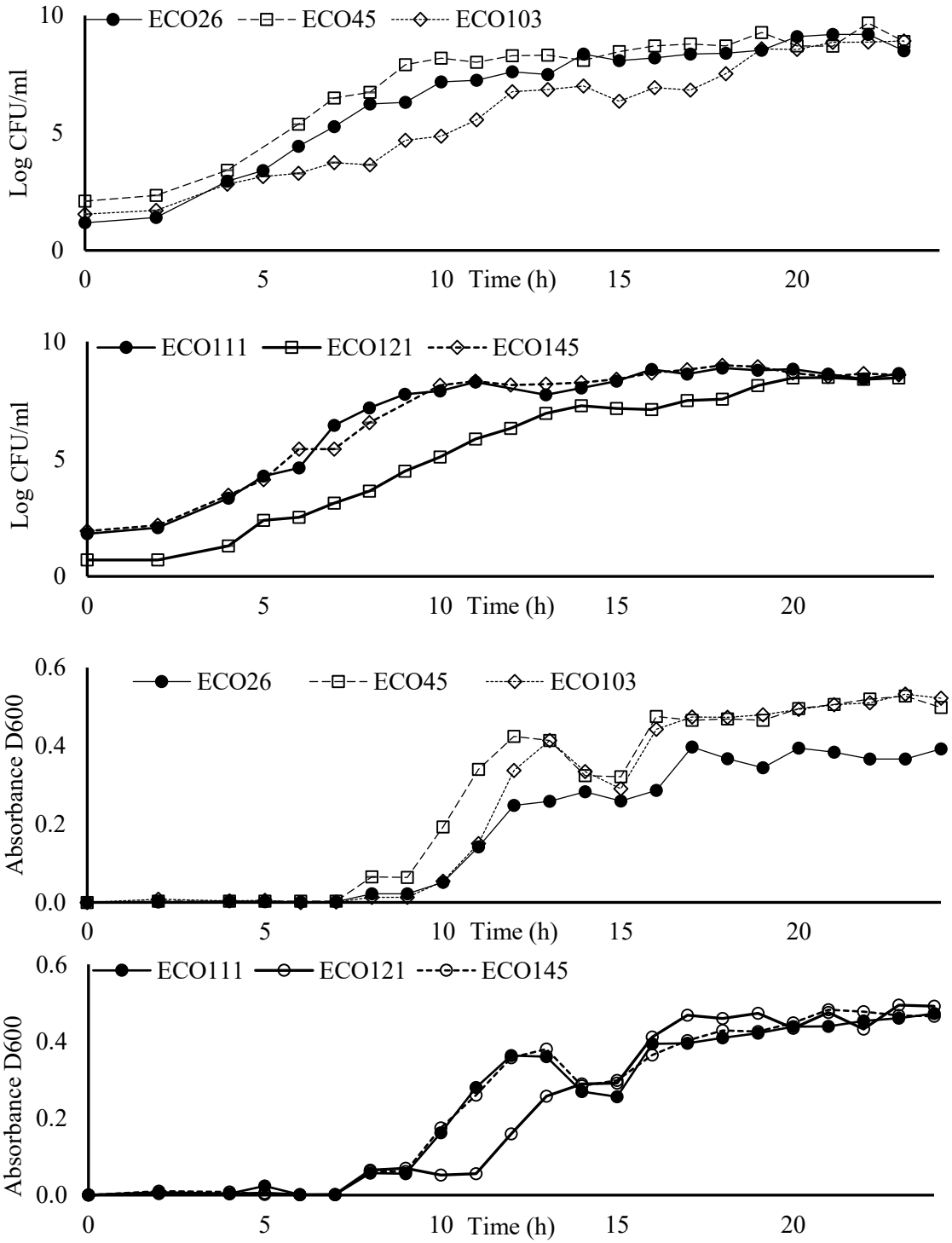


Figure 3.3: (a) Growth curves and (b) absorbance at 600nm light for six non-O157 STEC strains in TSB+NAL (50ppm) at 37°C.

Table 3.1 Growth parameters for non-O157 STEC strains grown in Tryptic Soy Broth (TSB) supplemented with 50ppm Nalidixic Acid (NAL) at 37°C

	ECO26	ECO45	ECO103	ECO111	ECO121	ECO145
Initial conc. (log CFU/ml)	0.92 ± 0.39	1.99 ± 0.34	1.39 ± 0.40	1.79 ± 0.28	0.46 ± 0.29	1.86 ± 0.23
Lag time (h)	0.67 ± 0.94	2.29 ± 0.77	0.78 ± 1.50	2.57 ± 0.53	2.33 ± 0.76	2.15 ± 0.49
Max Rate (h/(log CFU/ml))	0.64 ± 0.05	0.88 ± 0.10	0.39 ± 0.03	0.97 ± 0.09	0.59 ± 0.03	0.81 ± 0.06
Final conc. (log CFU/ml)	8.64 ± 0.13	8.68 ± 0.11	9.11 ± 0.37	8.49 ± 0.09	8.20 ± 0.13	8.59 ± 0.07

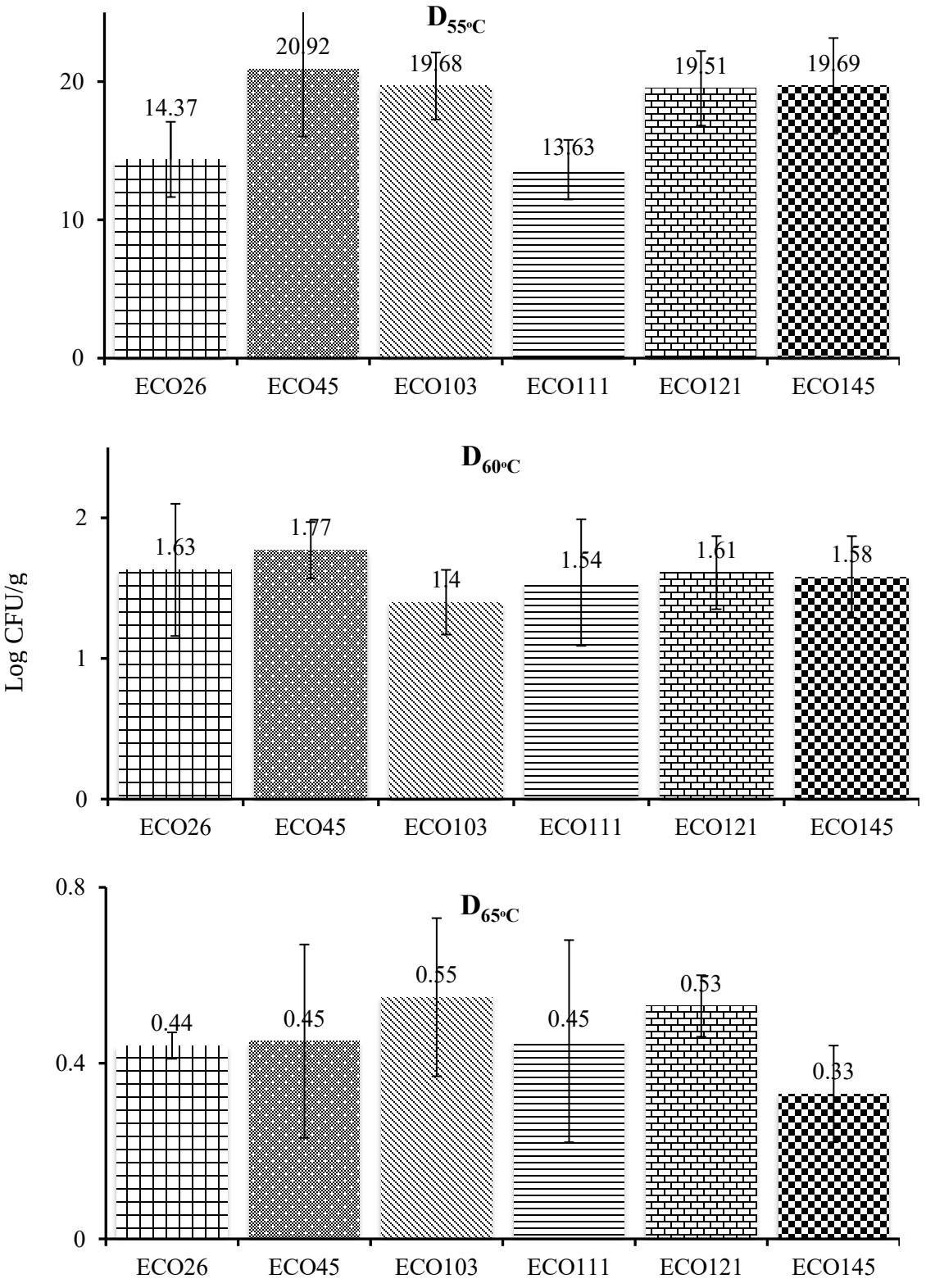
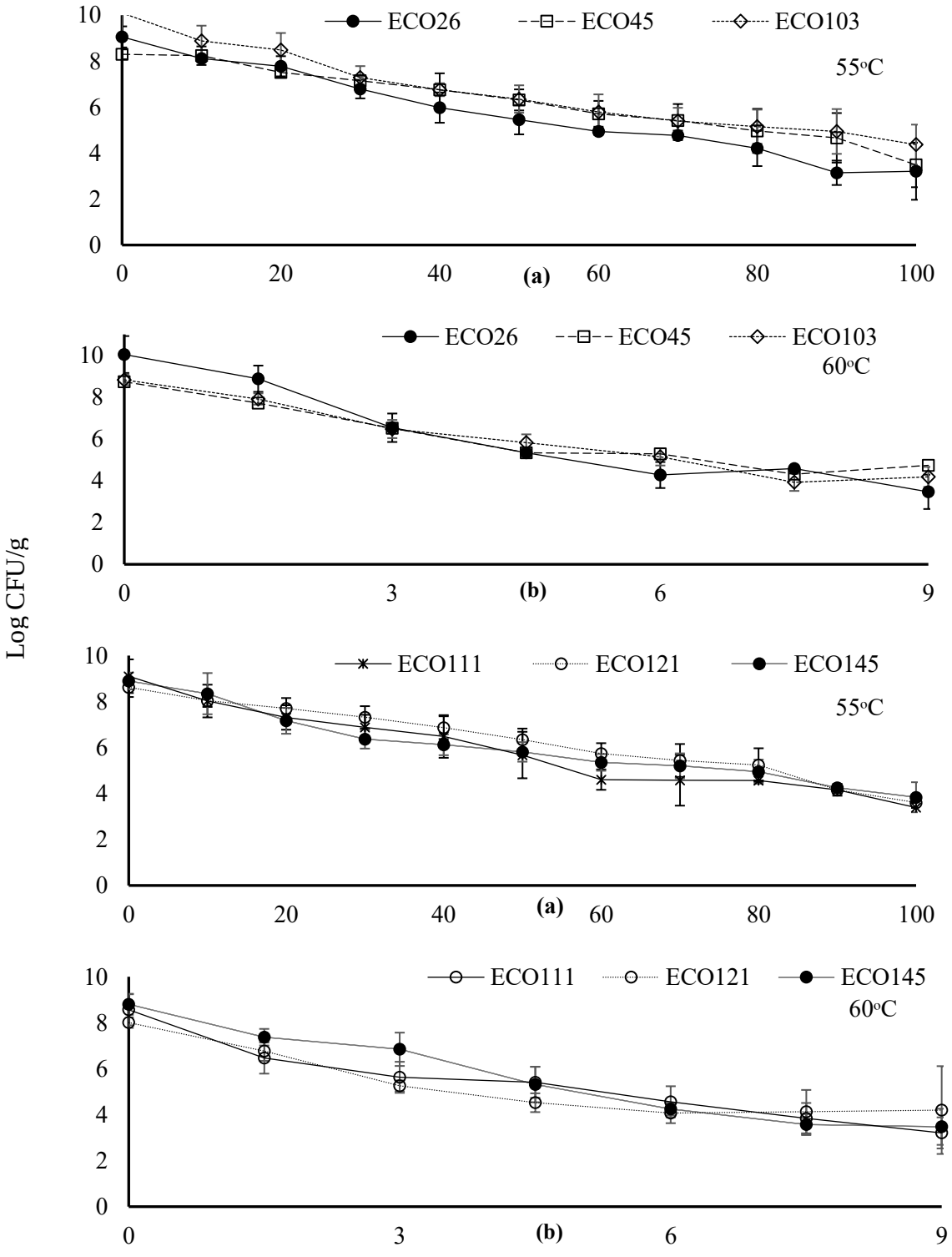


Figure 3.4 Decimal reduction value (D-value) for non-O157 STEC strains grown individually in Tryptic Soy Broth (TSB) medium


ECO26
ECO45
ECO103
55°C
60°C
ECO111
ECO121
ECO145
(a)
(b)
Log CFU/g
Time (min.)

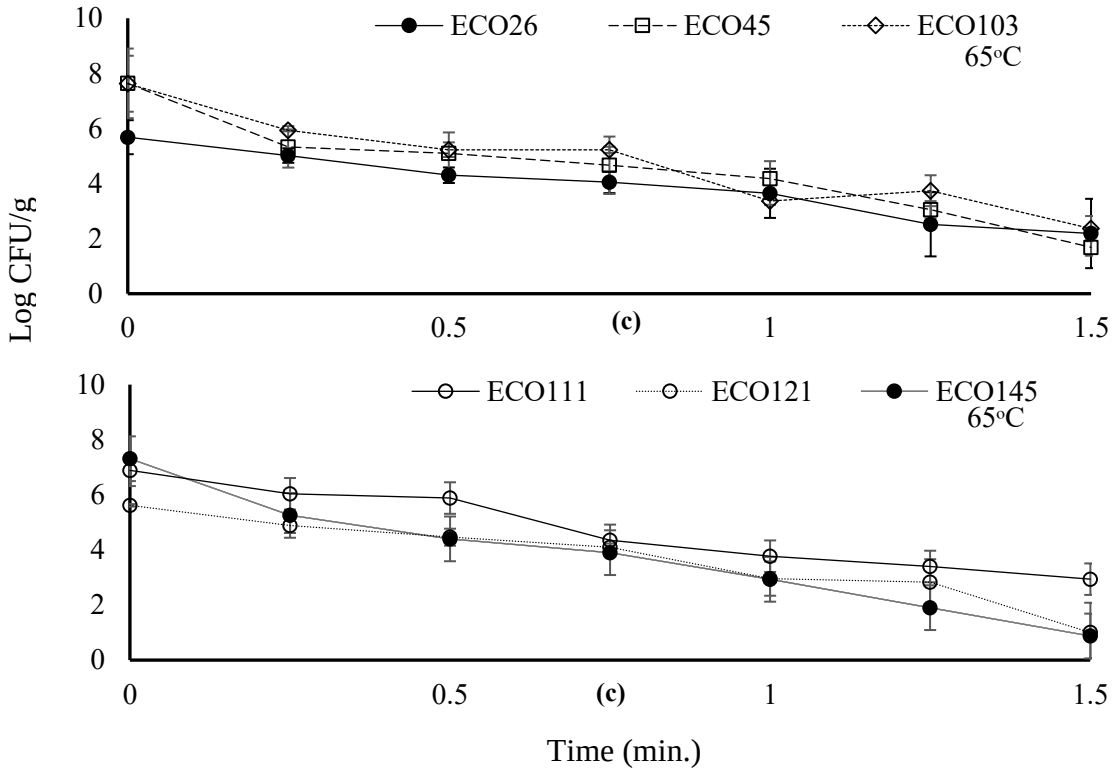
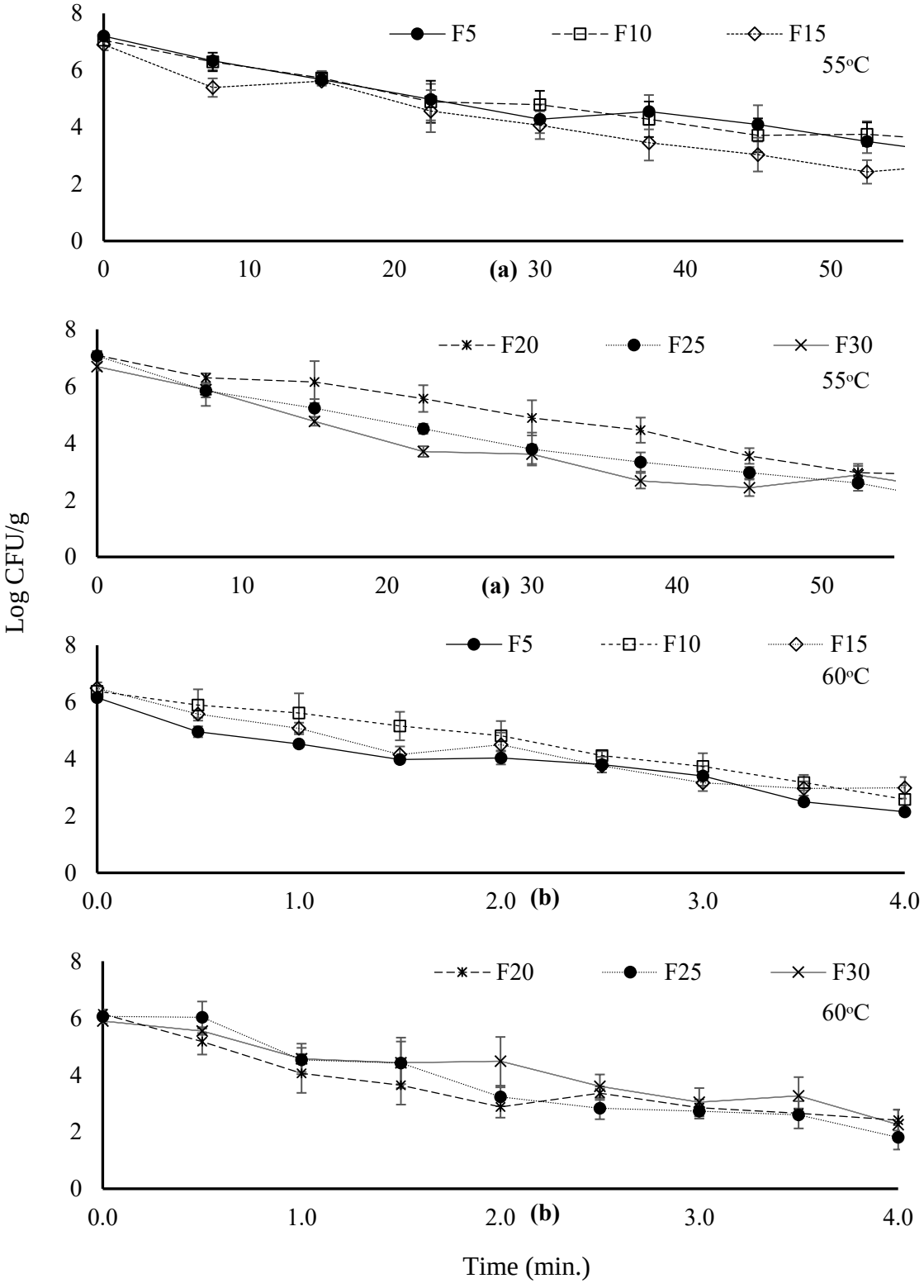


Figure 3.5: Survival curves for non-O157 STEC strains in Tryptic Soy Broth (TSB) at (a) 55°C, (b) 60°C and (c) 65°C


F5
F10
F15
55°C
(a)
F20
F25
F30
55°C
Log CFU/g
F5
F10
F15
60°C
(b)
F20
F25
F30
60°C
Time (min.)

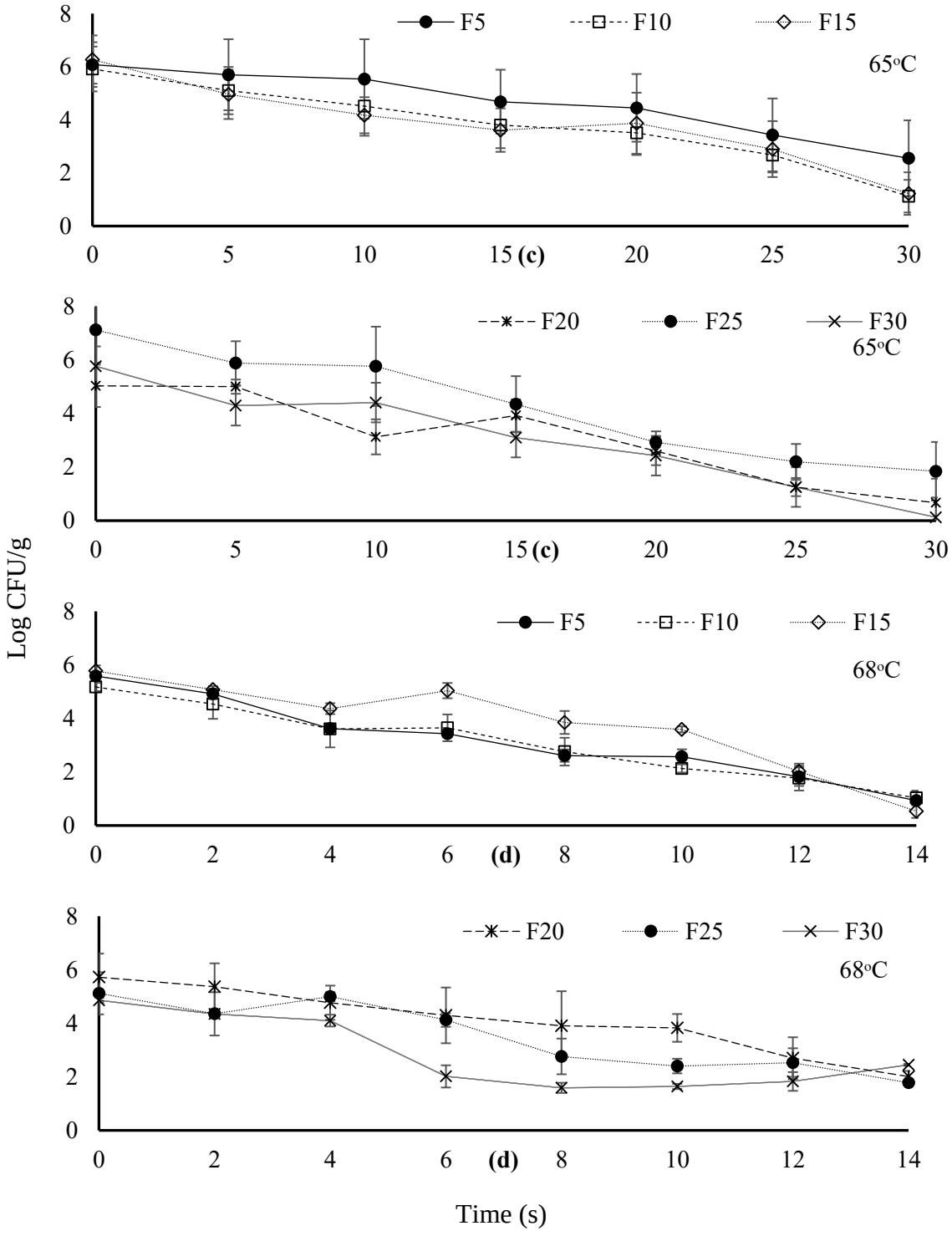


Figure 3.6: Survival curves for a cocktail of six non-O157 STEC strains in ground beef with fat content of 5% (F5), 10% (F10), 15% (F15), 20% (F20), 25% (F25) and 30% (F30) at (a) 55°C, (b) 60°C, (c) 65°C and (d) 68°C.

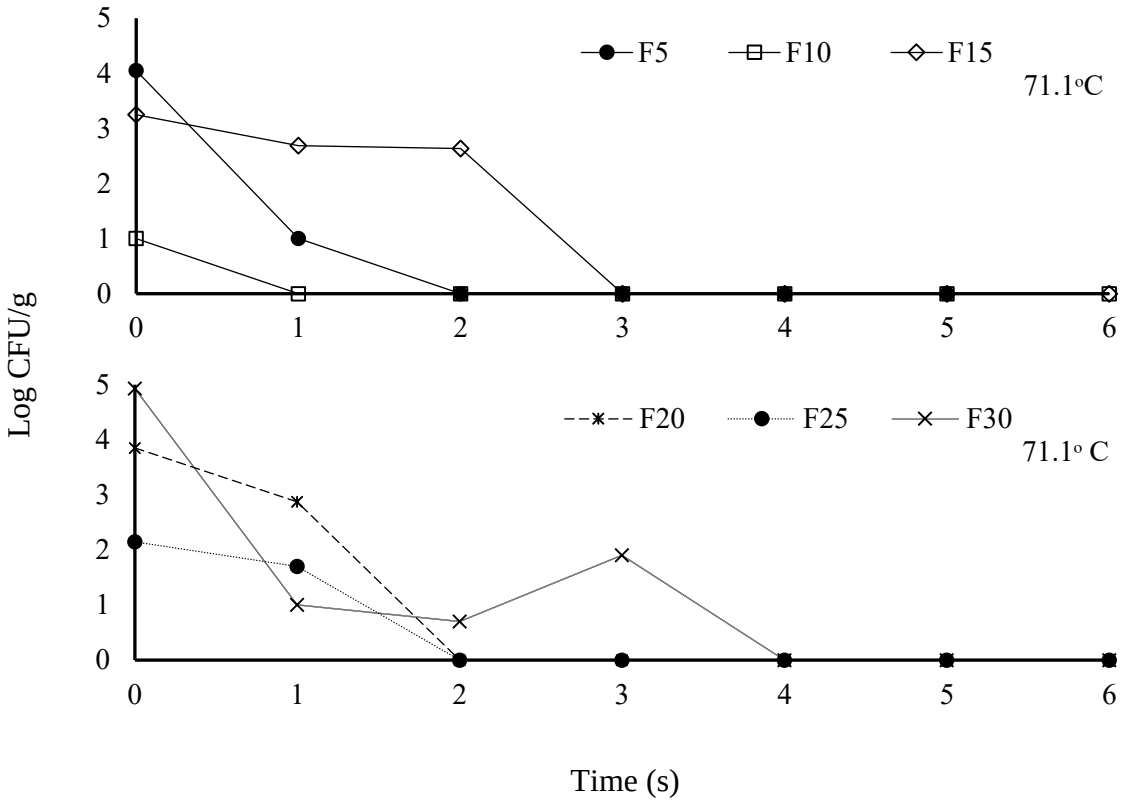


Figure 3.7: Survival curves for a cocktail of six non-O157 STEC strains in ground beef with varying fat content at 71.1°C.

Table 3.2 Decimal reduction time (D-value) of a cocktail of six strains of STEC in ground beef with varying fat content at 55, 60, 65 and 68°C.

Temperature	Fat content (%)	D-value (min.)	RMSE	r^2
55°C (131°F)	5	15.93 ± 0.44^{a}	0.44	0.91
	10	13.87 ± 0.40^{b}	0.40	0.91
	15	$12.75 \pm 0.17^{b, c}$	0.17	0.91
	20	$12.40 \pm 0.74^{b, c}$	0.74	0.94
	25	$12.66 \pm 0.35^{b, c}$	0.35	0.94
	30	$11.69 \pm 0.91^{c, d}$	0.91	0.91
60°C (140°F)	5	1.15 ± 0.04^{e}	0.04	0.92
	10	1.16 ± 0.14^{e}	0.14	0.92
	15	1.10 ± 0.08^{e}	0.08	0.90
	20	1.10 ± 0.13^{e}	0.13	0.90
	25	0.91 ± 0.05^{e}	0.05	0.93
	30	1.12 ± 0.10^{e}	0.10	0.92
65°C (149°F)	5	0.14 ± 0.01^{f}	0.12	0.94
	10	0.14 ± 0.01^{f}	0.12	0.92
	15	0.12 ± 0.01^{f}	0.11	0.94
	20	0.11 ± 0.01^{f}	0.11	0.93
	25	0.10 ± 0.02^{f}	0.08	0.90
	30	0.09 ± 0.01^{f}	0.08	0.94
68°C (154.4°F)	5	0.05 ± 0.01^{g}	0.45	0.87
	10	0.05 ± 0.01^{g}	0.14	0.89
	15	0.06 ± 0.01^{g}	0.39	0.83
	20	0.07 ± 0.01^{g}	0.25	0.88
	25	0.06 ± 0.02^{g}	0.34	0.82
	30	0.05 ± 0.01^{g}	0.24	0.86

a-g: values with no common letter indicate significant difference at 95% confidence interval.

Table 3. 3 z-values and coefficient of regression calculated for a cocktail of six non-O157 STEC strain in ground beef with varying fat content.

Fat content (% w/w)	z-value (°C)	Coefficient of regression (r^2)
5	5.17	0.99
10	5.32	1.00
15	5.55	0.99
20	5.60	0.98
25	5.47	0.98
30	5.28	0.98

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CHAPTER 4: A PREDICTIVE MODEL FOR THERMAL INACTIVATION OF NON-O157 SHIGA TOXIN PRODUCING *ESCHERICHIA COLI* IN GROUND BEEF.

Abstract

A mathematical model to predict the thermal inactivation of non-O157 shiga toxin producing *Escherichia coli* (STEC) in ground beef was developed in this study. The input parameters were temperature and fat content of ground beef. Survival curves for a cocktail of non-O157 STECs in ground beef at four temperature levels: 55, 60, 65 and 68ºC and six fat contents: 5, 10, 15, 20, 25 and 30% were generated. Nine primary models: Log-linear, Log-linear with tail, Biphasic, Sigmoidal, four factor Sigmoidal, Baranyi, Weibull, Mixed Weibull, and Gompertz models were tested for betterment of fit. The model with the highest Akaike's weight was selected as the best performing primary model. Data analysis showed the Weibull model to be the best fitted to define the distribution of survival curves. The parameters of Weibull model were estimated using non-linear mixed model in SAS® and response surface modeling was used to develop a second order polynomial regression to quantify the impact of fat in ground beef and cooking temperature on the heat resistance of non-O157 STECs. The secondary model developed was successfully validated by comparing the predicted lethality (log CFU/g) with the observed value for 10 and 27% fat content of ground beef at 58 and 62ºC. Process lethality obtained from experimental data was in the accuracy range of the predictive model.

4.1 Introduction

Pathogen modeling provides an alternative to intensive and extensive tests used to determine shelf life and food safety (Baranyi et al., 1994). A validated predictive model for pathogens helps in estimating their behavior in various environmental conditions by interpolation within the experimental limits (Baranyi et al., 1996). Therefore, the experiments should be designed carefully to cover extremes of environmental factors impacting survival/growth of pathogens. The process of constructing a predictive model is divided into two parts: Primary modeling and secondary modeling. Primary modeling analysis is performed to identify the best model for defining the distribution of survival curves and secondary modeling quantifies the impact of environmental factors on primary modeling parameters (Whiting and Buchanan., 1993).

Log-linear primary model with first order kinetics is very commonly used for thermal inactivation of pathogens (Stumbo, 1973; Tomlins and Ordal, 1976), however, deviation from log-linear curves has been reported by various researchers (Bhaduri et al., 1991; Linton et al., 1996; Coroller et al., 2006). Sigmoidal model (Augustine et al., (1998), Gompertz (Linton et al., 1995), four factor sigmoidal, Baranyi models (Baranyi et al, 1996) have been widely used for survival curves with "S" shape containing a lag phase and a tail; Weibull (Van Boekel., 2002) and Mixed Weibull (Coroller et al., 2006) models for concave and convex curves; Biphasic (Cerf, 1977) and log-linear with tail for two proportions of populations, each following a different log-linear model. To compare primary models, different statistical tools have been used in literature such as mean square errors (MSE), coefficient of regression (R^2), Akaike's information criteria (AIC), Bayesian information criteria (BIC), and residual sum of squares (RSS) (Farakos et al.,

2013; den Besten et al., 2006, Vega et al., 2016, Juneja et al., 2016). Response surface modeling (RSM), a quadratic model used to optimize the response variable (Box and Wilson, 1992), has been widely used for secondary modeling in predictive microbiology (Aouadhi et al., 2013; Beatty and Walsh, 2016).

Six strains of non-O157 shiga toxin producing *Escherichia coli* (STEC) are considered as an adulterant in non-intact beef products (USDA, 2011). These strains, *E. coli* O26:H1, *E. coli* O45:H2, *E. coli* O103:H2, *E. coli* O111:H8, *E. coli* O121:H9, and *E. coli* O145:Non-motile, attribute to more than 80% of the non-O157 STEC human infections (Gould, 2009) and have been linked with contamination in meat products in the recent past (CDC, 2010; CDC, 2016). It has been estimated that the non-O157 STECs cause 110,000 illnesses per year in US (Scallan et al., 2011). Therefore, it is important to understand processing parameters to control these non-O157 STECs in beef and beef products. The challenge with determining the level of control at various parameters is that it requires extensive experimentation that forces processors to rely on estimations that can be accurate if predictive modeling is used. A predictive model for thermal inactivation of non-O157 STEC can be used for designing processing times and temperatures to ensure food safety. In this study, a predictive model was developed and validated for thermal inactivation of non-O157 STECs in ground beef with varying fat content. The major objectives for this study was to (a) identify the primary model with goodness of fit in survival curves; (b) quantify the impact of fat content of ground beef and cooking time and temperature on parameters of primary model; (c) validate the developed predictive model with experimental data.

4.2 Material and Methods

4.2.1 Experiment design

The experiments were divided into two stages: Model development and validation. For model development, six non-O157 Shiga toxin producing *Escherichia coli* (STEC), *E. coli* O26:H1 ATCC BAA 2196, *E. coli* O45:H2 SJ9, *E. coli* O103:H2 87.1368, *E. coli* O111:H8 ATCC BAA 179, *E. coli* O121:H9 ATCC BAA 2221, and *E. coli* O145:Non-motile ATCC BAA 2192 were used. Four temperatures 55, 60, 65, and 68ºC and six fat contents 5, 10, 15, 20, 25, and 30% (%w/w) of ground beef were used in this study. Survival curves were generated between the surviving non-O157 STEC population (log CFU/g) verses time (min.) of heat exposure as described in chapter 3. A complete factorial design of experiment was performed with three replicates at each level, resulting in 6x4x3=72 survival curves. Primary modeling analysis was performed by using nlmixed program in SAS® to identify the best model to define the distribution for survival curves.

4.2.2 Primary Modeling

Nine primary models: Log-liner (LL), Log-linear with tail (LLT), Gompertz (GM), Biphasic (Bph), Weibull (WB), Mixed Weibull (MdWB), Sigmoidal (Sgm), four factor sigmoidal (FFSgm) and Baranyi (BRNI) were used for the analysis. Non-linear mixed program of SAS® Analytics software (Cary, NC) was used to fit primary models in the survival curves. The primary model parameters were estimated based on the minimum residual sum of square (RSS) values obtained by curve fitting. The coefficient of regression (r^2), root mean square error (RMSE) and RSS were calculated for each

survival curve. The primary models used for the analysis and their equations are shown in table 4.1.

4.2.3 Comparing primary models

Akaike's information criteria (AIC) values were calculated for the goodness of fit of primary models for each survival curve. The AIC number is directly proportional to log of residual sum of squares (RSS) of residuals with penalty added for additional number of parameters (Akaike, 1981). As per the principle of parsimony, the model with minimum parameters is preferred for estimation. Therefore, a penalty for number of parameters has been added in our estimations in this study. For the best performing model a minimum deviation between the predicted value and the experimental value (RSS) is desired, which is suggested by a lower AIC number. The equation for calculating AIC is given below.

$$AIC = \quad n * \log_{10}\left(\frac{RSS}{n}\right) + 2k \tag{4.1}$$

Where, n = number of data points on the curve;
RSS = residual sum of squares;
k = number of parameters to be estimated.

Akaike's weights (*w*) were calculated to compare the primary models and identify the best performing model for all survival curves. Equation 4.2 and 4.3 were used to measure AIC weights that provide the evidence in favor of one primary model to be the best fitted model (Burnham and Anderson, 1998). The sum of *w* for a model for all survival curves was the total weightage in favor of that model. The primary model with highest *w* was the best model to define the distribution of survival curves.

$$\Delta_i = AIC_i - minAIC \quad (4.2)$$

$$w_i = \frac{e^{(-0.5\Delta_i)}}{\sum_{r=1}^{R} e^{(-0.5\Delta_r)}} \quad (4.3)$$

Where, AIC_i= AIC number of model *i*
minAIC= minimum AIC among all primary models
Δ_i = AIC difference for model *i*
R= number of primary models

4.2.4 Secondary Modeling

Response surface modeling (rsm) was used to develop a secondary model to quantify the impact of environmental factors on thermal inactivation of pathogens by using SAS® analytical software (SAS, 1990). The parameters of best preforming primary models were expressed as a function of temperature and fat content of ground beef.

$$x_1 = ß_0 + ß_1 T + ß_2 T^2 + ß_3 f + ß_4 f^2 + ß_5 fT + e \quad (4.4)$$

Where, x_1 is a parameter of best performing model;
T= Temperature (ºC)
f= fat content of ground beef (%w/w)
e= random error
$ß_1$ to $ß_5$= coefficients of the model

4.2.5 Validation of model

For the second stage of the experiments, the survival population predicted by the model were validated against the experimental data. The developed model was used to estimate time taken for 3 and 5-log CFU/g reduction and bacterial population at the same time were enumerated. For the validation study, two fat content of ground beef 10 and 27% at both 58 and 62ºC were selected. Ground beef with 10 and 27% fat was procured from three different local grocery stores. Beef samples were stored at -20ºC, thawed at 4ºC for 24h before use. A cocktail of the non-O157 STECs was prepared for inoculating ground beef. The bacterial strains were grown at 37°C in Tryptic Soy Broth (TSB;

Neogen, Lansing MI) supplemented with 50 ppm of Nalidixic acid (NAL; Fisher BioReagents, Fair Lawn, NJ). After 18-24 h of incubation, cells were washed twice with 0.1% peptone water (PW, Neogen, Lansing, MI) after centrifugation (Model: Sorvell Legend XTR, Thermo Scientific, Waltham, MA) at 4700xg, for 10 min. at 4°C and re-suspended in 1ml of 0.1% PW. The washed cells were homogenized to dissolve the pellets, and washed cells of each strain were mixed together to prepare the cocktail. Fifty grams of thawed ground beef was inoculated with 500µl cocktail to target 7-8 log CFU/g of bacterial population on the meat and hand massaged for 2 min for homogenous distribution of cells. Small pouches containing 5-g of inoculated meat with dimensions 7.6 x 12.7 cm (3" x 5") were made, heat sealed and then flattened to target 1-2 mm uniform thickness. The pouches were submerged into an isothermal circulating water bath (Model: Haake A25B, Fisher Scientific, Waltham, MA) set at 58 and 62ºC. The temperature inside the sample pouches were monitored by K-type probe and a datalogger (Model: HH806AU, Omega Engineering, Stamford, CT). One pouch was taken out once the desired temperature was reached to calculated the starting population. Two more sample bags, one each at the predicted time for 3- and 5-log CFU/g reduction, were taken out from the water bath and immediately cooled down in an ice-water bath. Meat form the pouches was aseptically transferred into filter bags (7 oz., Nasco Whirl-Pak, Atkinson, WI) containing 5 ml (1:1 dilution) of 0.1% PW. Meat samples in the filter bags were homogenized in a stomacher (Stomacher 400, Steward Limited, West Sussex, UK) at 260 rpm for 1 min. The bacterial population was enumerated by serial dilution in 0.1% PW and plating onto Plate Count Agar (PCA, Neogen, Lansing, MI) supplemented with

50-ppm NAL. Three replicates were performed for each temperature and fat level resulting a total of 2x2x3x3=36 experiments.

The model performance was tested by calculating the accuracy factor (A_f) for %discrepancy (D_f) and bias factor (B_f) for the % bias. The A_f provides the confidence in the predicted data and B_f provides the information if the model is over estimating or underestimating the predicted values (Baranyi et al., 1999). The following equations were used to calculate validation parameters.

$$\%B_f = sgn(B_f) * (B_f - 1) * 100$$

$$B_f = 10\text{^}\left[\frac{\sum_1^n\left(\log\left(\frac{N_{model}}{N_{data}}\right)\right)}{n}\right] \quad (4.5)$$

$$sgn(B_f) = \begin{pmatrix} +1 & if & B_f > 0 \\ 0 & if & B_f = 0 \\ -1 & if & B_f < 0 \end{pmatrix}$$

$$A_f = 10\text{^}\left[\frac{\sum_1^n\left|\left(\log\left(\frac{N_{model}}{N_{data}}\right)\right)\right|}{n}\right] \quad (4.6)$$

$$\%D_f = (A_f - 1) * 100\%$$

The confidence interval around the predicted data was calculated by using the standard error of the residual values. Confidence interval was further used to estimate prediction width by using the following equation (Montgomery et al., 2006)

$$prediction\ width = \sqrt{(CI_i)^2 + \left(t_{\alpha/2,n-p} * RMSE\right)^2} \quad (4.7)$$

Where, CI_i= Confidence interval around estimated parameter i

4.3 Results and Discussion

4.3.1 Primary Model

Eight of the nine primary models showed convergence of data for all 72 survival curves and their corresponding parameters were estimated successfully. However, Gompertz model was only able to converge 36 out of 72 survival curves, showing that this model does not have goodness of fit (Table 8, Appendix B). The AIC values of each survival curve against eight primary models are shown in Table 4.2. For survival curve at 55°C and 5% fat content, AIC values were 13.8, 15.8, 17.8, 13.2, 16.4, 17.4, 12.4, and 16.4 for LL, LLT, Bph, Sgm, FFSgm, BRNI, WB and MdWB model, respectively. Minimum AIC values represent lower RSS and hence better fitting model (Akaike, 1981). Hence, WB has better goodness of fit for the first survival curve at 55ºC and 5% fat content followed by Sgm (13.2) and LL (13.8). However, for the third survival curve at the same temperature and fat combination, The LLT model has the lowest AIC value (2.2 vs 7.2 of Sgm and 14 of WB) and has better fitness. Therefore, different best fitted primary model for different survival curves were observed. Thermal inactivation of microbes is affected by temperature and intrinsic properties of food, which, in turn, affects the shape of survival curve. Hence, different models are used to define the distribution of data. To find the best fitting model for all survival curves, AIC values were further used to calculate Akaike's weights (w) (Equation 4.3).

High Akaike's weights (w) provide more confidence in the primary model and hence desired to select the best fitting primary model (Burnham and Anderson, 1998). Akaike's weight for all survival curves are shown in Table 4.3. For the 55ºC and 5% fat content, w values were calculated as 0.40, 0.33 and 0.23 for Sgm, LLT and WB models.

Hence, *w* values helped in selecting Sgm as the best performing model for survival curves at 55ºC and 5% fat content. Similarly, WB was the best performing model for 5/60 and 5/65 (Fat content/Temp) with *w* of 0.69 and 0.63, respectively. The combined *w* for all survival curves was 8.77 for WB followed by 4.78 for Sgm 4.70 for LLT, 2.90 for LL, 1.84 for MdWB, 0.57 for FFSgm and 0.44 each for Bph and BRNI. This analysis concludes that WB was two times (8.77/4.78; 8.77/4.70) better than Sgm and LLT, and approximately 20 (8.77/0.44) times better than the BRNI and Bph in defining distribution for survival curves at all temperature and fat content combinations. Therefore, WB model was the best performing primary model based on Akaike's weight's (*w*) analysis.

To further increase our confidence in selecting the best fitting primary model, the percentage discrepancies (D_f) were calculated (Equation 4.6) and observed to be minimum (11.43%) for WB model, indicating that the predicted data will be in the range of ±11.43% of the observed data. The D_f values were 16.77, 11.70, 12.41, 15.08, 37.99, 11.56, and 16.54 % for LL, LLT, Sgm, BRNI, FFSgm, MdWB and BpH, respectively. Based on both analyses, WB model was selected as the primary model for thermal inactivation of non-O157 STECs in ground beef. Juneja et al., (2014) also observed Weibull model as the best fitting primary model for thermal inactivation of *L. monocytogenes* in ground beef. A secondary model for thermal inactivation of *L. monocytogenes* as a function of NaCl, sodium pyrophosphate and sodium lactate on survival behavior of *L. monocytogenes* was developed. Similarly, Huang (2009) also found that Weibull model describes the distribution of survival of *L. monocytogenes* in ground beef. However, under dynamic conditions, where heating temperature was

gradually increased from 30-65ºC, modified Gompertz model was the best performing model. The equation for the WB model is given below.

$$log\ N_t = \quad log\ N_0 - b * (t)^n \tag{4.8}$$

Where Log N_t = population at time t
Log N_o= initial population
b= slope parameter
n= shape parameter

4.3.2 Parameters estimation of primary model:

The parameters of WB model, b and n for slope and shape respectively, were estimated with non-linear mixed program in SAS®. This program estimates the parameters that yields minimum residual sum of square values. The estimated parameters, root mean square error (RMSE) and coefficient of regress (r^2) of the WB model are shown in table 4.3. The r^2 values 60/72 curves were more than 0.85 for WB model, which further added confidence in choosing this model as the primary model. A square root transformation of the parameters was performed, which made $\sqrt{b}$ and $\sqrt{n}$ normally distributed along 2.40 ± 1.77 and 0.95 ± 0.20, respectively. The transformed data were used for further analysis.

4.3.3 Secondary Model

To predict the survival of non-O157 STECs in the range of temperature (55-68ºC) and fat content (8-31% w/w), the parameters of WB were defined as a second order polynomial function of fat content and temperature. The multiple regression analysis was performed by response surface modeling was used in SAS® (proc rsreg) for estimating the coefficients of equation 4.4 as shown in the material and method section. The fat content of the beef samples was measured by Soxhlet extraction method (Chapter 3). The

average measured fat content of ground beef was 8.03, 11.97, 17.66, 21.70, 28.52, and 31.04% and these values were used as an input in response surface modeling. The secondary models for estimates of b and n are shown in equation 4.9 and 4.10. A constant error term of 0.06911 and 0.00 were added to $\sqrt{b}$ and $\sqrt{n}$ respectively to make sum of residuals as zero, for satisfying the assumption of secondary modeling (Robinson, 2014).

$$\sqrt{b} = 57.309488 + 0.239032f - 2.020609T - 7.85\ X\ 10^{-4}f^2 - 3.532\ X\ 10^{-3}fT + 0.201106T^2 - 0.06911 \quad (4.9)$$

$$\sqrt{n} = -0.543841 + 3.2695\ X\ 10^{-2}f + 1.895\ X\ 10^{-2}T - 3.6\ X\ 10^{-4}f^2 - 3.12\ X\ 10^{-3}fT + 5.6864\ X\ 10^{-5}T^2 \quad (4.10)$$

Where; T= Temperature (ºC)
f= fat content of ground beef (%w/w)

A response surface plot was generated for predicted time taken to reduce 5-log CFU/g of non-O157 STECs as a function of fat content and temperature (fig. 4.3). As expected, the predicted time decreased with the increase of temperature. At lower temperatures, there is a downward slope of time for a 5-log CFU/g lethality suggesting that the heat resistance decreases with increase in fat content at low temperatures. However, a slight upward slope was observed in time with higher fat content, indication that the heat resistance would increase with fat content at higher temperatures according to the model.

Residual (observed values - WB prediction) plot and comparative plots are shown in figure 4.3. According to model assumptions, residuals should be random and normally distributed for a predictive model. The r^2 values of the residual plot was 0.16, showing that 84% of the residuals were random and there was no trend in the residual plots. The

residuals were normally disturbed with average of 0.00 and standard deviation of 1.04, hence satisfying residuals assumption of secondary modeling (Robinson, 2014).

4.3.4 Validation of predictive model

Validation of a predictive model is a critical step before using it for decision making (Jagannath and Tsuchido, 2003) and was validated with experimental data collected at the data points different from those used in developing the model. The predicted values of b and n (using equations 4.9 and 4.10), were 0.567 and 0.73 for 10% fat conent at 58ºC. By using these values in WB model (Equation 4.8), time taken for 3 and 5-log CFU/g reduction was calculated as 9.69 and 19.44 min. Similarly, b values were 0.706, 3.733 and 3.165 and n values were 0.771, 0.897 and 0.898 for 27/58, 10/62 and 27/62 (Fat content/Temp) combinations. Time predicted for 3 and 5-log CFU were 6.53, 12.66; 0.78, 1.39; and 0.94, 1.66 for 27/58, 10/62 and 27/62 combinations. Inoculated ground beef was exposed to heat for the predicted times and lethality was measured.

Bias factor (B_f) and Accuracy factor (A_f) (equations 4.6 and 4.7) are used to measure the performance of a predictive model. The major difference between both factors is that an absolute value is taken for A_f calculation. A_f=B_f=1 shows that the model is predicting response variable with 100% accuracy; B_f>1shows overestimated; and B_f<1 shows underestimated predicted value (Baranyi et al., 1999). Irrespective of the fat content, Bf values were 1.002, 1.003, 0.977, and 1.0039 for 55, 60, 65, and 68ºC. The overall bias factor was 0.971 for the model. Accuracy factor helps in deciding the percentage discrepancy (D_f) of the predicted values. The D_f of our model was 11.43% indicating that that the experimental values will be in the range of ±11.43% of the

predicted values. Skandamis and Nychas (2000) calculated D_f of 23.3% and bias factor of 3.3% for a predictive model for inactivation of *E. coli* O157 in eggplant salad with pH, temperature and oregano essential oil concentration as the input factors. Ross et al., (2000) established standards for measuring performance of a predictive model; B_f range of 0.95-1.10 is considered as 'good', 0.85-1.30 considered 'acceptable' and outside this limit is considered 'unacceptable' for inactivation models. The authors also estimated an increase 10-15% in D_f with addition of one input variable in the model. Hence, the acceptable limit for this model with two input parameters, fat content and temperature, would be less than 25-30%. D_f and B_f values of this model was 11.43% and 0.971 respectively, therefore satisfying the standards of predictive modeling.

Figure 4.4 shows the validation data for process lethality in ground meat obtained from three grocery stores (G1, G2 and G3) with 3 replications at each level resulting in a total of 9 validation data points per fat/temp combination. The prediction interval (PI) is the range in which the prediction values should be present for successful validation of a predictive model (Montgomery et al., 2006). PI of the model, calculated from equation 2.7, was ±1.71 logCFU/g. Figure 4.4 shows the WB predictions, %D and PI of the developed predictive model. Underestimation of surviving population (Overestimation of lethality) is the major concern that causes a failure of a microbiological inactivation model. For 10/58 (fat content/temp) combination, the experimental values were below the prediction line, which means the experimental lethality (Log N_o/N) was higher than the predicted lethality, hence not a food safety concern. Both 3 and 5 log CFU/g reduction values were in the range of accuracy factor for 27/58 and 10/62 for meat from all three grocery stores with model overestimating surviving population in 2/9 cases at 27/58.

However, none of the predicted population value was outside the upper limit of the model (-11.43% of predicted value), hence these data validate the predictive model.

Table 4. 1: Primary inactivation models used for curve fitting in survival curves

Model	Equation
Log-Linear	$log\ N_t = \quad log\ N_0 - k * t\ ;\ \ D = \quad -\frac{1}{k}$
Biphasic	$log\ N_t = \quad log\ N_0 - log(fe^{-k_1 t} + (1-f)e^{-k_2 t})$
Log-linear with tail	$log\ N_t = \quad log((N_0 - N_{min})e^{-k*t} + N_{min})$
Modified Gompertz	$log\ \frac{N}{N_0} = A - Ce^{-e^{-B(t-M)}};\ \mu_{max} = \frac{BC}{e}$
	$t_{lag} = M - \frac{1}{B} + \frac{(log\ N_0 - A)}{\mu_{max}}$
Baranyi	$log\ N_t = \quad log\ N_0 + u_{max}t + \frac{1}{u_{max}}\log(e^{-u_{max}\cdot t} + e^{-h_0} - e^{-u_{max}t - h_0}) - log\left(1 + \frac{e^{u_{max} + \frac{1}{u_{max}}\log(e^{-u_{max}\cdot t} + e^{-h_0} - e^{-u_{max}t - h_0})} - 1}{e^{(\log_{10} N_{min} - \log_{10} N_0)}}\right)$
Sigmoidal	$log\ N_t = \quad log\ N_0 - \log_{10}(1 + e^{a + b\log(t)})$
Four Factor	$Log\ N_t = Log\ N_0 - k_1 t^{n_1} k_2 t^{n_2}$
Sigmoidal	
Weibull	$log\ N_t - log N_0 = \quad -b * (t)^n$
Mixed Weibull	$N_t = \frac{N_0}{1+10^a}\left[10^{k_1 t^n + a} + 10^{k_2 t^n}\right]$
	$a = \log_{10}\left(\frac{f}{1-f}\right)$

N_o: Initial count; N_t: count at time t; t: time; k, k_1, k_2: inactivation rates; D:-D-value; N_{min}: remaining population after heat treatment; u_{max}: maximum inactivation rate; f: portion of heat resistant population; A, C, B, M, a, b, h_o, n, n_1, n_2: Model coefficients

Table 4. 2 Akaike's informational criterion (AIC) values of primary models for survival curves obtained at different fat content and temperatures

Fat	T (°C)	LL	LLT	Bph	Sgm	FFSgm	BRNI	WB	MdWB
5	55	13.8	15.8	17.8	13.2	16.4	17.4	12.4	16.4
5	55	17.3	15.4	21.3	11.8	13.2	20.1	9.2	13.2
5	55	23	2.2	27	7.2	18	20.4	14	24.4
5	60	13.2	15.2	17.2	15.6	15.2	17.2	11.2	15.1
5	60	14.5	14.7	18.5	10.2	7.5	18.4	3.5	7.5
5	60	13.5	15.1	17.5	13.9	13.6	17.5	9.6	13.6
5	65	7.9	3.5	11.9	7.3	35.3	11.3	-0.2	3.8
5	65	16.1	17.9	20.1	15.5	42.1	19.9	14.1	18.1
5	65	11.9	13.9	15.9	14.2	39.7	15.9	10.6	14.6
5	68	19.2	20.5	23.2	25	53.3	23.2	21.2	32.9
5	68	21.9	21.8	25.9	25.1	50.6	25.8	22.4	27.4
5	68	13.7	15.1	17.7	12.5	35.7	17.7	15.5	10.4
10	55	15	12.8	19	8.3	15.7	16.9	11.7	15.7
10	55	22.3	24.3	11.2	8.8	14.7	23	10.7	14.7
10	55	13.4	13.1	17.4	16.4	18.8	13.4	14.8	18.8
10	60	12.9	12.9	16.9	19.9	47.4	16.9	14.6	16.9
10	60	14.6	11.6	18.6	5.6	11.1	18.5	7.1	2.8
10	60	11.6	12.1	15.6	9.8	9.7	11.2	5.7	9.7
10	65	10	11.5	14	13	38.6	14	10.5	14.5
10	65	11	2.7	15	11.3	35.8	14.6	6.6	10.6
10	65	15.1	9	19.1	20.6	42.1	18.9	15.8	19.8
10	68	12.4	13.6	16.4	7.7	37.3	16.5	9.7	23.1
10	68	17.6	11.5	21.6	24	51	21.4	17.3	4.9
10	68	9.5	10.3	13.5	9.2	27.8	13.6	9.1	13.9
15	55	22.1	11.6	26.1	-0.3	3.4	22.2	-0.6	19.3
15	55	10.9	8.7	14.9	13.1	10.4	9.5	6.4	10.4
15	55	27	11.3	31	10.9	16.6	25.2	12.6	17.8
15	60	18.6	14.7	22.6	8.9	13.2	22.6	9.2	13.2

15	60	12.9	12.9	16.9	14	17.4	16.9	13.4	17.4
15	60	16.1	7	20.1	1.9	2.6	20	-1.4	2.6
15	65	12.6	13.4	16.6	18.4	39.3	16.5	14.5	19.7
15	65	10.8	12.7	14.8	15.6	41.3	14.8	12.8	16.4
15	65	13.8	15.1	17.8	16.9	40.4	17.8	14.5	18.8
15	68	24.2	25.2	28.2	19.7	43.7	28.2	11.6	27.7
15	68	24	25.5	28	24.9	49.7	27.9	22.6	26.6
15	68	21	23	25	25	46.4	25	22.7	26.7
20	55	11.4	8.8	15.4	6.6	3.5	12.9	-0.5	3.5
20	55	18.6	16.1	22.6	13.3	4.4	4.2	0.4	39.5
20	55	18.5	14	22.5	11.2	15.5	17.8	11.5	15.5
20	60	14.3	15.8	18.3	11.2	20.2	18.2	16.2	20.2
20	60	19.5	4.4	23.5	3	48.7	23.4	8.4	2.8
20	60	20.8	14.8	24.8	9.2	13.9	24.8	9.9	13.9
20	65	16	12.3	20	17	37.7	19.9	15.5	11.1
20	65	3.9	2.5	7.9	11.3	40.4	7.8	3.4	16
20	65	14.1	15.1	18.1	16.9	39.2	17.9	14.1	16
20	68	13.2	13.2	17.2	17.2	43.1	17.2	15.2	19.2
20	68	14.5	12.7	18.5	11.4	45.9	18.5	6.6	21.4
20	68	19.4	20.3	23.4	21.8	48.1	23.4	20.4	20.6
25	55	8.3	8.8	12.1	9.9	6.1	11.5	2.1	6.1
25	55	16.5	14	20.5	11	14.1	18.2	10.1	14.1
25	55	21.3	15.1	25.3	8.9	6.9	23.1	2.9	20.9
25	60	15.9	11.2	19.9	11.7	16.2	19.9	12.2	14.6
25	60	20.3	16.6	24.3	10.7	16.6	24.3	12.6	16.6
25	60	14.1	14.6	18.1	10.3	19.6	18.1	15.6	19.6
25	65	9.9	11.4	13.9	13.7	40	13.9	11.9	7
25	65	16.6	16.1	20.6	20	45	20.6	17	21
25	65	14.2	16.1	18.2	11.7	37.3	18	15.1	14
25	68	8	4.2	12	8.2	36.8	12	7.8	7.7
25	68	22.7	24.7	26.7	21.3	43.7	26.7	23.9	27.9
25	68	20.3	22.2	24.3	25	51.6	24.3	22.3	26.3
30	55	22.3	2.6	26.2	8.9	18.9	18.2	14.9	25.9

30	55	17.9	13.1	21.9	9.5	18.6	20.4	14.6	18.6
30	55	24.7	17.5	28.7	16	20.3	26.1	16.3	20.3
30	60	6.3	8.2	10.3	9.8	40.1	10.3	5.4	8.9
30	60	10.1	12	14.1	15.2	15.7	13.7	11.7	15.7
30	60	18.5	17.1	22.5	10.9	19.4	22.5	15.4	19.4
30	65	19.7	19.1	23.7	14.2	37.6	23.5	12.8	14.8
30	65	20	18.3	24	16.4	43.1	24	16.9	13
30	65	11.4	9.3	15.4	18.9	42.7	15.1	12.2	16.2
30	68	10.6	11.1	14.6	14.9	45.5	14.5	12.2	16.2
30	68	23.5	25.5	27.5	23.4	50.1	27.5	23.9	27.9
30	68	25.4	20.6	29.4	22.9	48	29.4	24.6	12.6

LL: Log-linear model; LLT: Log-linear with tail; BpH: Biphasic model; WB: Weibull Model; MdWB: Mixed Weibull model; Sgm: Sigmoidal model; FFSgm: four factor Sigmoidal model; BRNI: Baranyi Model

Table 4. 3 Akaike weighs (*w*) of primary models for survival curves at different fat content and temperature

Fat (%w/w)	T (ºC)	LL	LLT	WB*	MdWB	SGM	FFSgm	BRNI	BpH
5	55	0.01	0.33	0.23	0.01	0.40	0.03	0.01	0.00
5	60	0.04	0.02	0.69	0.09	0.05	0.09	0.01	0.01
5	65	0.09	0.10	0.63	0.08	0.08	0.00	0.01	0.01
5	68	0.38	0.25	0.19	0.03	0.10	0.00	0.05	0.05
10	55	0.03	0.03	0.28	0.04	0.53	0.04	0.02	0.05
10	60	0.06	0.09	0.42	0.30	0.11	0.00	0.02	0.01
10	65	0.08	0.72	0.14	0.02	0.02	0.00	0.01	0.01
10	68	0.16	0.31	0.28	0.11	0.12	0.00	0.02	0.02
15	55	0.00	0.07	0.60	0.00	0.25	0.08	0.00	0.00
15	60	0.01	0.06	0.52	0.07	0.28	0.07	0.00	0.00
15	65	0.44	0.23	0.20	0.02	0.04	0.00	0.06	0.06
15	68	0.09	0.05	0.74	0.01	0.09	0.00	0.01	0.01
20	55	0.00	0.01	0.84	0.00	0.03	0.11	0.02	0.00
20	60	0.00	0.10	0.11	0.07	0.71	0.00	0.00	0.00
20	65	0.21	0.42	0.25	0.05	0.03	0.00	0.03	0.03
20	68	0.19	0.22	0.43	0.02	0.11	0.00	0.03	0.03
25	55	0.00	0.02	0.79	0.01	0.07	0.11	0.00	0.00
25	60	0.03	0.12	0.17	0.03	0.62	0.02	0.00	0.00
25	65	0.28	0.17	0.16	0.22	0.13	0.00	0.04	0.04
25	68	0.29	0.29	0.18	0.05	0.16	0.00	0.04	0.04

30	55	0.00	0.51	0.06	0.00	0.42	0.01	0.00	0.00
30	60	0.23	0.15	0.34	0.05	0.19	0.00	0.03	0.03
30	65	0.08	0.17	0.37	0.26	0.11	0.00	0.01	0.01
30	68	0.17	0.25	0.14	0.28	0.13	0.00	0.02	0.02
Total		2.90	4.70	**8.77***	1.84	4.78	0.57	0.44	0.44

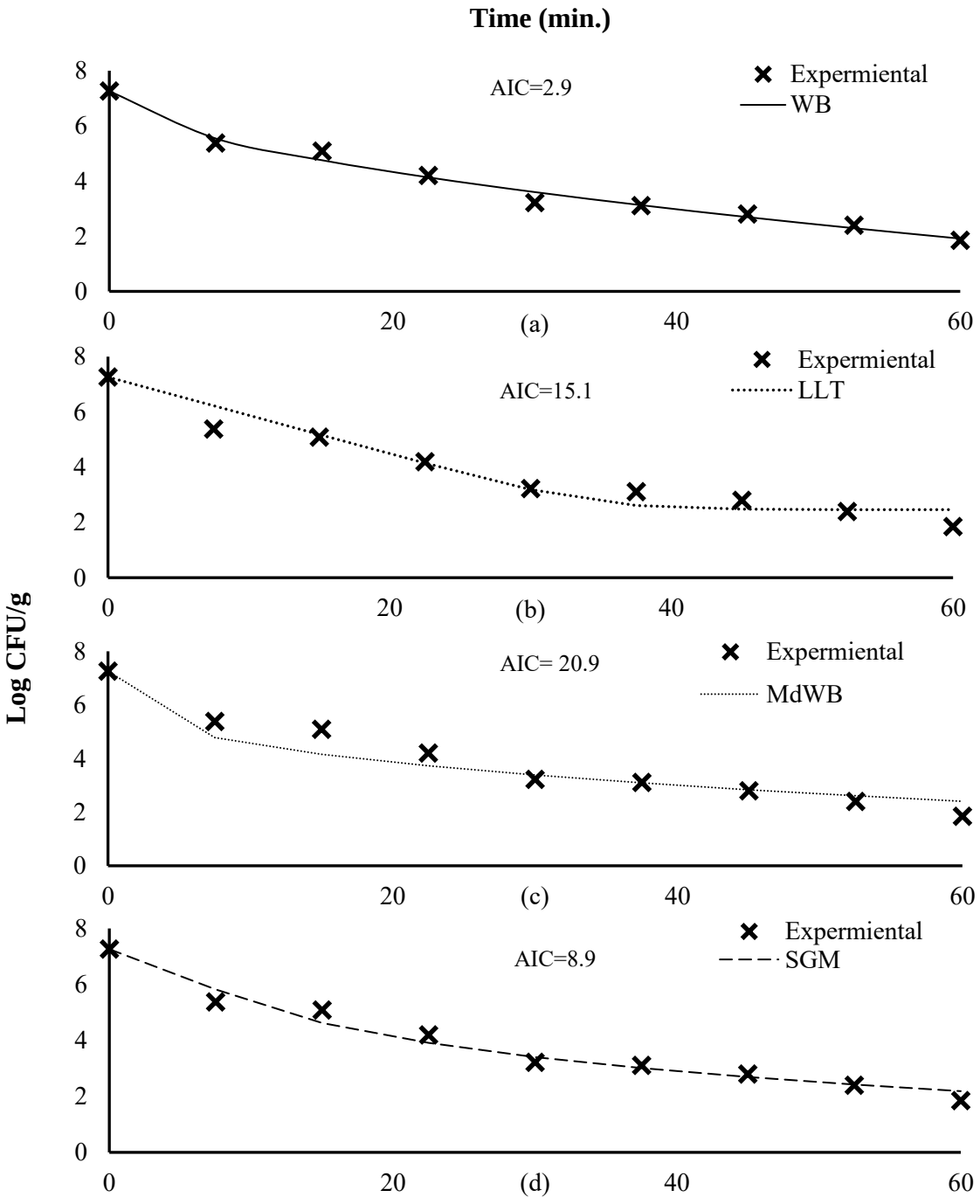
T: Temperature LL: Log-linear model; LLT: Log-linear with tail; WB: Weibull Model; MdWB: Mixed Weibull model; Sgm: Sigmoidal model; FFSgm: four factor Sigmoidal model; BRNI: Baranyi Model: BpH: Biphasic model*Best performing model (Highest combined Akaike weight)

Table 4.4 Weibull model parameters (b and n) estimation, standard error (SE), Root mean square error (RMSE) and coefficient of regression (r^2) calculated from SAS® non-linear mixed program

Fat	Temp	b	SE (b)	n	SE (n)	RMSE	r^2
5	55	0.196	0.086	0.738	0.117	0.417	0.891
5	55	0.388	0.117	0.571	0.081	0.341	0.901
5	55	0.535	0.161	0.538	0.081	0.455	0.888
5	60	1.471	0.186	0.704	0.115	0.383	0.888
5	60	1.683	0.115	0.576	0.064	0.236	0.940
5	60	1.493	0.169	0.652	0.104	0.348	0.888
5	65	10.197	1.163	1.567	0.128	0.172	0.984
5	65	16.395	3.273	1.471	0.220	0.581	0.941
5	65	6.376	0.958	0.675	0.137	0.426	0.869
5	68	20.748	6.778	0.989	0.201	0.718	0.863
5	68	32.486	16.873	1.388	0.336	0.750	0.838
5	68	21.701	10.552	0.877	0.232	0.789	0.829
10	55	0.241	0.087	0.706	0.096	0.394	0.916
10	55	0.673	0.197	0.400	0.080	0.373	0.802
10	55	0.113	0.067	0.869	0.155	0.483	0.871
10	60	1.102	0.238	0.892	0.191	0.464	0.849
10	60	1.558	0.135	0.611	0.079	0.299	0.918
10	60	0.523	0.092	1.469	0.145	0.275	0.966
10	65	6.496	1.071	0.786	0.155	0.431	0.879
10	65	11.687	2.438	1.680	0.239	0.299	0.957
10	65	14.061	3.434	1.295	0.263	0.650	0.909
10	68	49.169	20.819	1.556	0.236	0.403	0.939
10	68	31.211	11.077	1.340	0.229	0.555	0.916
10	68	9.247	4.424	0.575	0.200	0.505	0.743
15	55	0.615	0.071	0.523	0.031	0.184	0.980
15	55	0.018	0.009	1.359	0.126	0.280	0.971
15	55	0.952	0.232	0.386	0.067	0.422	0.842
15	60	1.888	0.156	0.540	0.077	0.341	0.898
15	60	1.212	0.195	0.804	0.143	0.444	0.870
15	60	1.761	0.081	0.541	0.043	0.176	0.966
15	65	8.049	2.053	0.948	0.254	0.581	0.831
15	65	9.984	1.633	0.996	0.163	0.521	0.918
15	65	7.320	1.477	0.756	0.188	0.598	0.820
15	68	46.531	26.740	1.669	0.381	0.786	0.849
15	68	15.919	8.632	0.835	0.302	0.936	0.679
15	68	465.230^	312.59	3.018	0.4318	0.399	0.967
20	55	0.259	0.042	0.709	0.043	0.186	0.982
20	55	0.003	0.001	1.806	0.104	0.190	0.991
20	55	0.386	0.125	0.586	0.087	0.394	0.893
20	60	0.924	0.202	1.037	0.186	0.515	0.882
20	60	1.816	0.143	0.489	0.074	0.321	0.885

20	60	2.099	0.163	0.507	0.073	0.357	0.895
20	65	12.496	4.413	1.596	0.398	0.651	0.872
20	65	8.256	0.638	0.866	0.074	0.238	0.975
20	65	11.901	3.038	1.386	0.278	0.582	0.903
20	68	16.092	6.274	0.968	0.221	0.552	0.846
20	68	36.844	9.585	1.658	0.172	0.277	0.966
20	68	23.469	11.042	1.281	0.300	0.689	0.835
25	55	0.198	0.039	0.787	0.052	0.219	0.979
25	55	0.317	0.091	0.665	0.077	0.360	0.936
25	55	0.570	0.083	0.547	0.039	0.230	0.972
25	60	1.583	0.184	0.656	0.105	0.411	0.881
25	60	2.075	0.190	0.570	0.084	0.420	0.893
25	60	1.210	0.206	0.892	0.148	0.495	0.893
25	65	8.938	1.534	0.996	0.171	0.485	0.914
25	65	17.405	3.363	1.261	0.205	0.735	0.929
25	65	9.236	2.569	1.293	0.293	0.610	0.870
25	68	11.138	2.842	0.770	0.130	0.343	0.903
25	68	20.031	14.689	1.388	0.472	0.856	0.667
25	68	17.882	7.112	0.963	0.244	0.750	0.796
30	55	0.473	0.153	0.573	0.087	0.482	0.888
30	55	0.283	0.108	0.690	0.101	0.470	0.903
30	55	0.690	0.247	0.450	0.098	0.531	0.779
30	60	1.036	0.125	0.783	0.108	0.268	0.919
30	60	0.804	0.164	1.107	0.174	0.399	0.910
30	60	1.758	0.220	0.659	0.113	0.497	0.871
30	65	23.312	7.706	2.355	0.404	0.482	0.949
30	65	7.186	1.204	0.525	0.142	0.734	0.758
30	65	13.477	2.167	1.187	0.169	0.468	0.951
30	68	24.181	6.642	1.100	0.158	0.444	0.933
30	68	10.026	3.688	0.675	0.212	0.851	0.670
30	68	6.957	2.673	0.549	0.213	0.888	0.546

b, n: constants for Weibull Model; SE: Standard Error; RMSE: Root Mean Square Error; r^2: Coefficient of regression^: outlier


Time (min.)
Log CFU/g
AIC=2.9
Expermiental
WB
(a)
AIC=15.1
Expermiental
LLT
(b)
AIC= 20.9
Expermiental
MdWB
(c)
AIC=8.9
Expermiental
SGM
(d)
0
2
4
6
8
0
20
40
60

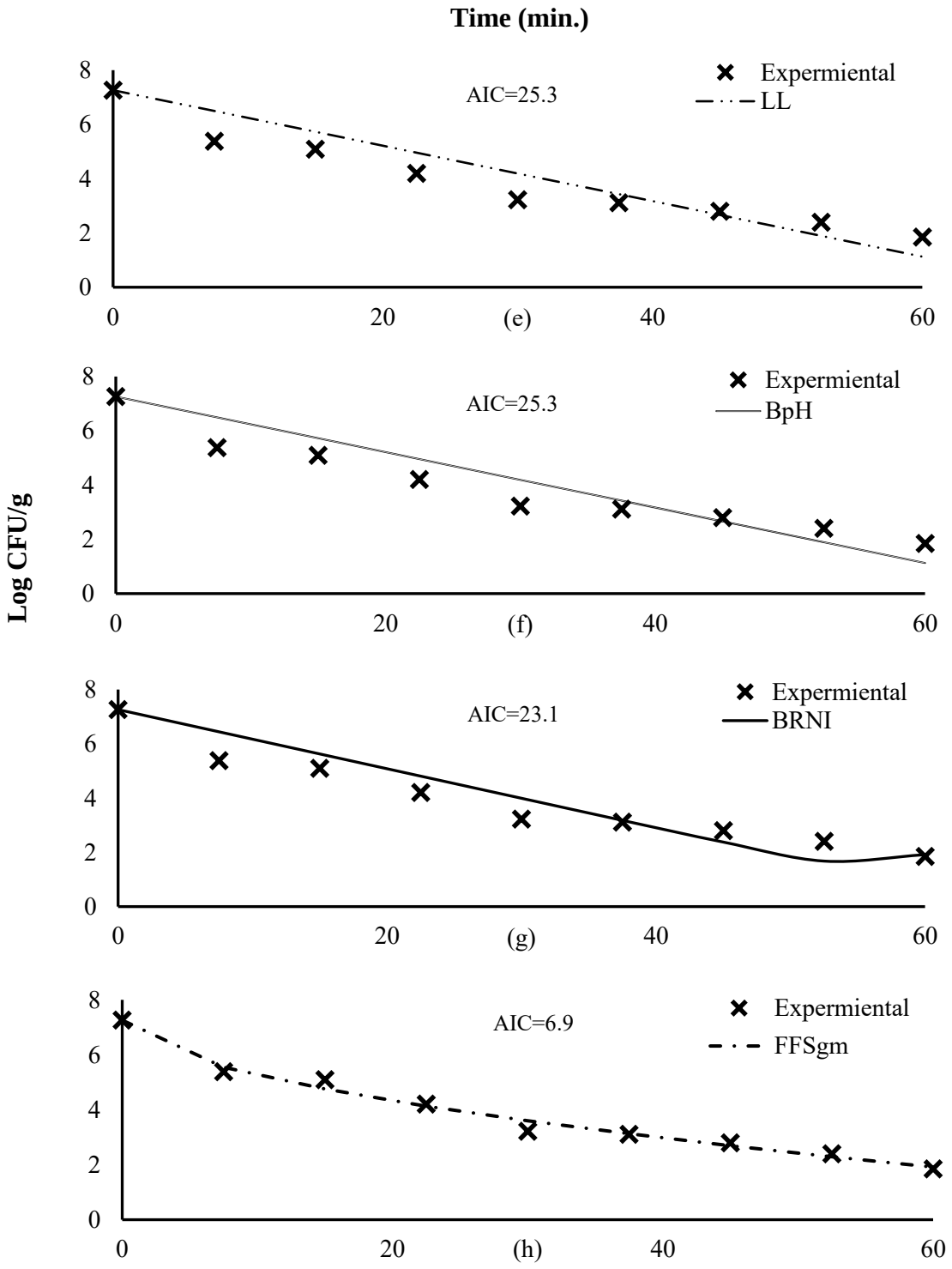


Figure 4.1 Curve fitting of (a) Weibull, (b) Log-linear with Tail, (c) Modified Weibull, (d) Sigmoidal, (e) Log-linear, (f) Biphasic, (g) Baranyi and (h) Four factor Sigmoidal primary models in the survival curve (Log CFU/g vs Time) at 55ºC in 25% (%w/w) of ground beef.

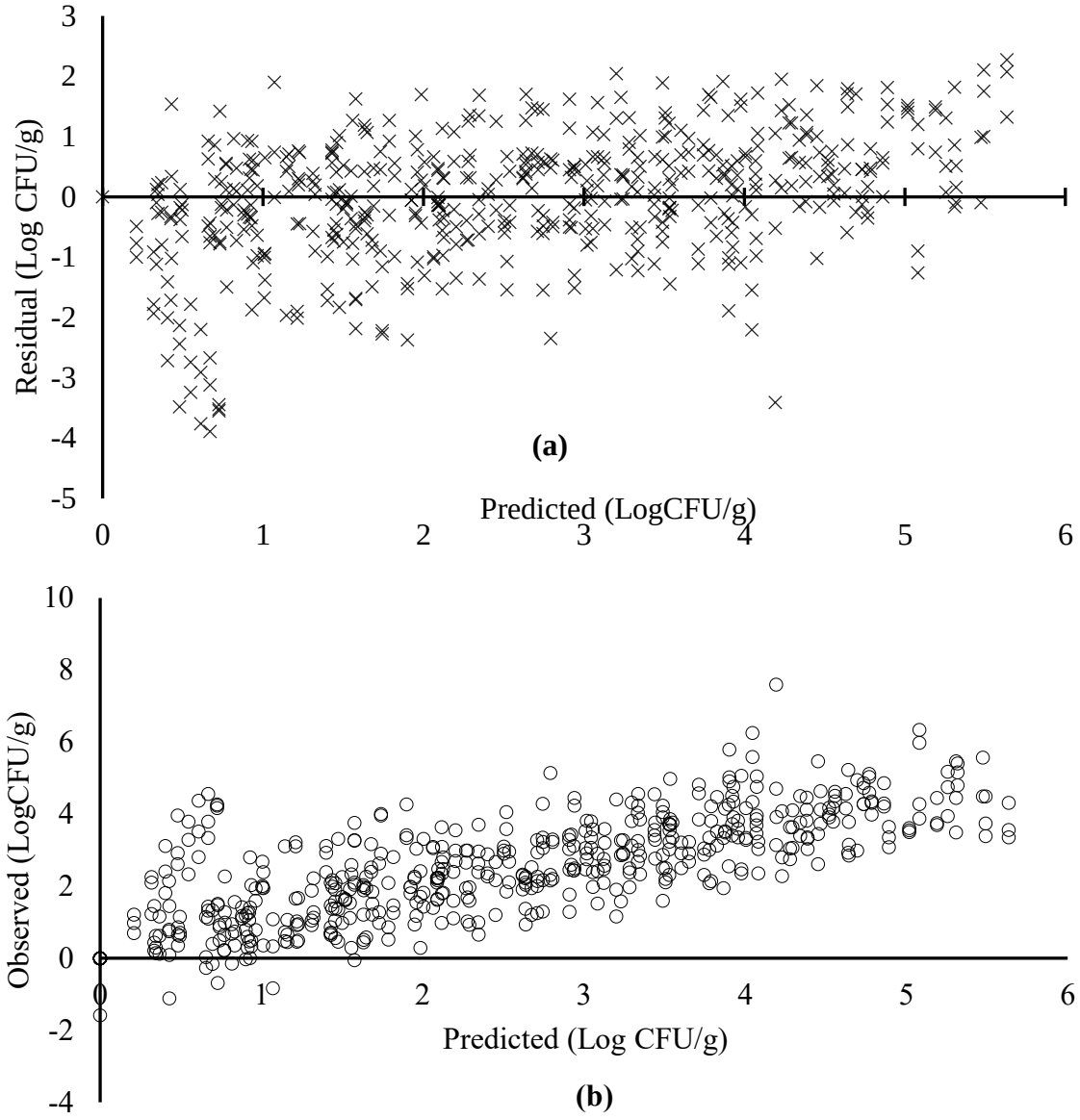


Figure 4.2 (a) Residual plot (Observed value-predicted value) vs predicted Weibull value (b) and comparative plot between observed values v/s predicted values.

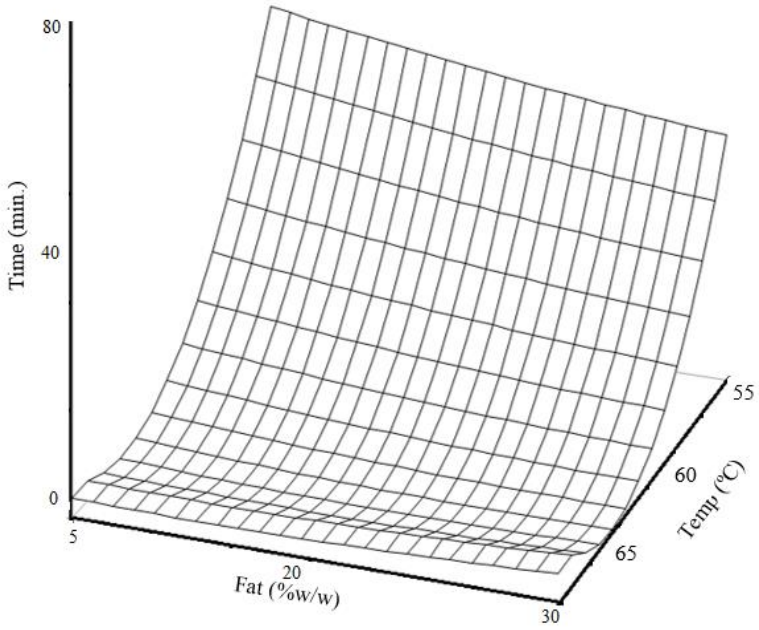


Figure 4.3 Response surface graph for five log reduction time for non-O157 STECs as a function of fat content and temperature of ground beef

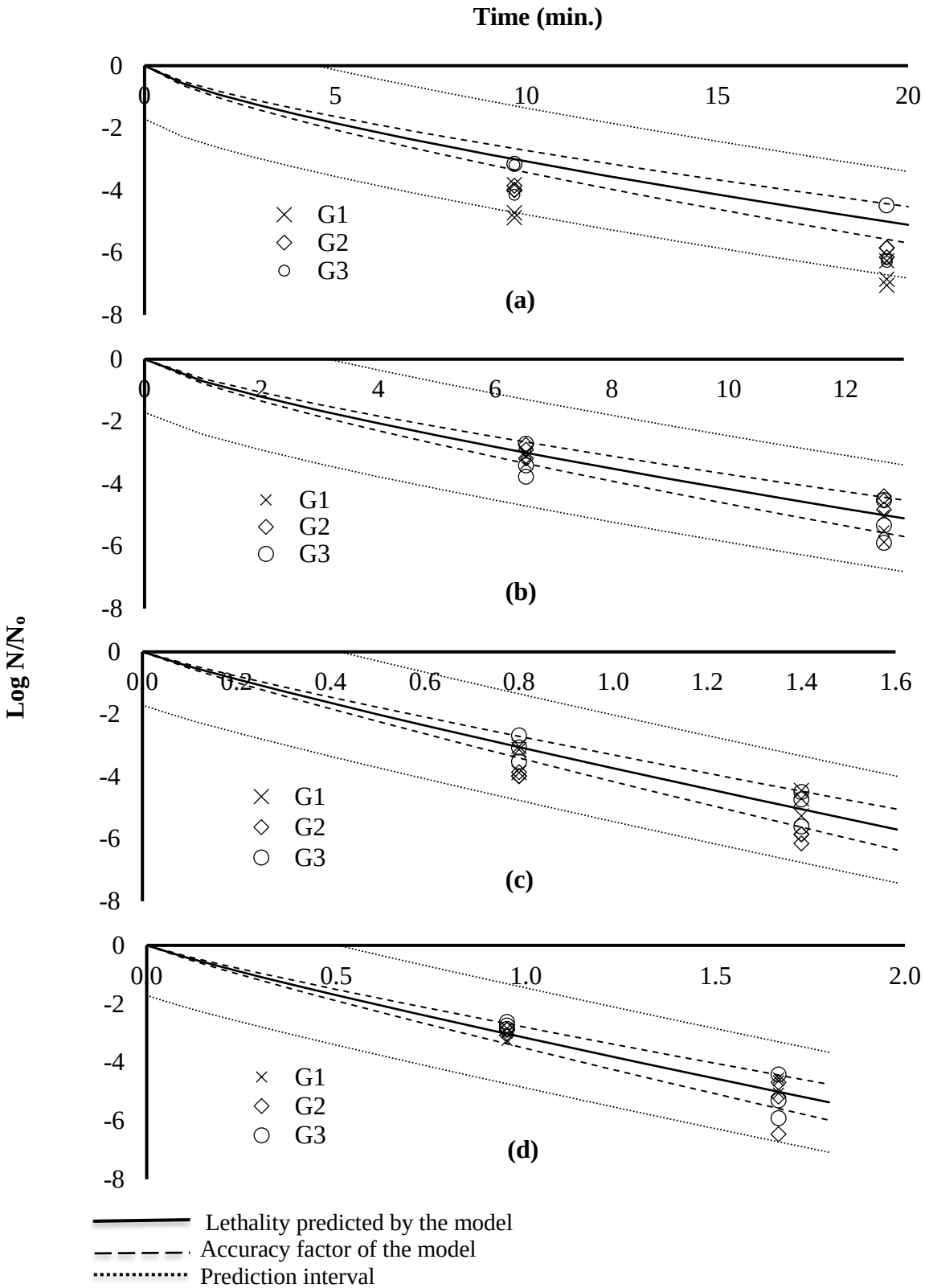


Figure 4. 4 Validation of the predicted lethality (Log N/N_0) with observed lethality in meat from three grocery stores (G1, G2 and G3) at (a) 58ºC, 10%; (b) 58ºC, 27%; (c) 62ºC, 10%; and (d) 62ºC, 27%.

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CHAPTER 5: CONCLUSION

Heat resistance of non-O157 shiga toxin producing *Escherichia coli* (STEC) in laboratory media and ground beef with varying fat content was studied. Six non-O157 shiga toxin producing *Escherichia coli* (STEC), *E. coli* O26:H1 ATCC BAA 2196, *E. coli* O45:H2 SJ9, *E. coli* O103:H2 87.1368, *E. coli* O111:H8 ATCC BAA 179, *E. coli* O121:H9 ATCC BAA 2221, and *E. coli* O145: Non-motile ATCC BAA 2192 were used. In the first phase of the study, heat resistance of individual grown strains was measured in tryptic soy broth (TSB) media. There was no statistical difference ($p>0.05$) in the heat response among the strains, therefore, cocktail of these strains was used to study the heat resistance in ground beef. Ground beef with six fat levels of 5, 10, 15, 20, 25, and 30% (%w/w) was used. The ground beef was inoculated with a cocktail of non-O157 STEC strains and their inactivation rate was studied at 55, 60, 65, 68 and 71.1°C. Survival curves, surviving population versus time, were generated at each temperature and fat level with three replicates. A significant decrease ($p<0.05$) in heat resistance was observed with an increase of fat content at 55°C. However, no significant impact of fat content was observed at higher temperature.

In the second phase of the experiment, data generated in the first phase was used to develop a predictive inactivation model. Rate of inactivation of non-O157 STEC was modeled as a function of fat content of ground beef and temperature. Nine primary models were used to determine the distribution of data in survival curves. Three models were based on log-linear decline of pathogens, Log-linear, log-linear with tail and Biphasic model. Six models were used for non-linear decline; Sigmoidal, Gompertz, four

factor sigmoidal, Baranyi, Weibull and mixed Weibull models. Primary modeling analysis showed Weibull model has the highest accuracy factor and Akaike's weight, making it the best fitting model. The parameters of Weibull model were expressed as a function of fat content and temperature by using response surface modeling. The equations of the developed predicted model are given below. The percentage discrepancy factor of the model was 11.43%. The model was successfully validated in ground beef obtained from three grocery stores.

$$log\ N_t = \quad log\ N_0 - b * (t)^n$$

$$\sqrt{b} = 57.309488 + 0.239032f - 2.020609T - 7.85\ X\ 10^{-4}f^2 - 3.532\ X\ 10^{-3}fT + 0.201106T^2 - 0.06911$$

$$\sqrt{n} = -0.543841 + 3.2695\ X\ 10^{-2}f + 1.895\ X\ 10^{-2}T - 3.6\ X\ 10^{-4}f^2 - 3.12\ X\ 10^{-3}fT + 5.6864\ X\ 10^{-5}T^2$$

Where Log N_t = population at time t;
Log N_o= initial population
t= time (min.)
T= Temperature (^oC)
f= fat content of ground beef (%w/w)

APPENDIX A: SAS CODES

1. SAS® Code for curve fitting, AIC values, and parameters estimation

```
data mod;
input Sample $ time logCFU @@;
datalines;
F05T55r1    0     7.32
F05T55r1    7.5   6.62
F05T55r1    15    6.09
F05T55r1    22.5  4.91
F05T55r1    30    4.40
F05T55r1    37.5  4.99
F05T55r1    45    4.51
F05T55r1    52.5  3.53
F05T55r1    60    3.15
F05T55r2    0     7.10
.
.
.
.
.
.
F30T68r3    0.23  2.41
F30T68r3    0.27  1.78
;

libname fit clear;
data beef;
        set mod;
        Fat=substr(Sample,2,2);
        Temp=substr(Sample,5,2);
        Rep=substr(Sample,8);
        N=10**logCFU;
        y=logCFU;
        if time=0 then N0=N;
run;

libname fit clear;
```

```
data beef1;
        set beef;
        retain N01;
        if not missing(N0) then N01=N0;
        else N0=N01;
        drop N01;
run;

proc sort data=beef1;
        by fat temp rep;
run;

proc univariate data=beef1 normal plots;
        by fat temp rep;
        var y;
run;

ods output ConvergenceStatus=Convergence
ParameterEstimates=Parameters FitStatistics=FitStat;
ods output clear;
ods output ConvergenceStatus=Convergence
ParameterEstimates=Parameters FitStatistics=FitStat;

proc nlmixed data=beef1;
where time ^=0;
by fat temp rep;
parms b=0.5 n=0.5 var=0.5;
model = log10(N0) - b*time**n;
model y ~ normal(model,var);
predict model out=p_beef;
run;

proc reg data=p_beef noprint outest=r2;
by fat temp rep;
model y=pred/rsquare;
run;

proc print data=r2;
var fat temp rep _rsq_;
run;
```

```
ods output clear;

proc print data=convergence;
        where status=3;
run;
```

Replace the bold part with the following for the specific model

I. Log-linear model:

```
        proc nlmixed data=beef1;
        where time ^=0;
        by fat temp rep;
        parms b=0.5  var=0.5;
        model = log10(N0) - b*time;
        model y ~ normal(model,var);
        predict model out=p_beef;
        run;
```

II. Log-linear with a Tail

```
        proc nlmixed data=beef1;
        where time ^=0;
        by fat temp rep;
        parms k=0.5 Nres=10 var=0.5;
        model = log10((N0-Nres)*exp (-k*time)+Nres);
        model y ~ normal(model,var);
        predict model out=p_beef;
        run;
```

III. Sigmoidal Model

```
        proc nlmixed data=beef1;
        where time ^=0;
        by fat temp rep;
        parms a=0.5 b=0.5 var=0.5;
        model =log10(N0)-log10(1+exp(a+b*log(time)));
        model y ~ normal(model,var);
        predict model out=p_beef;
        run;
```

IV. Baranyi Model

```
        proc nlmixed data=beef1;
        where time ^=0;
```

```
by fat temp rep;
parms umax=0.1 Nmin=10 h0=0 var=0.2;
a = log(exp(umax*time) + exp(-h0) - exp((umax*time)-h0));
b= (exp(-umax*time)-a/umax)-1;
c= exp(log(N0)-log(Nmin));
model = log(N0)- umax*time - a/umax - log(1 + b/c);
model y ~ normal(model,var);
predict model out=p_beef;
run;
```

V. Biphasic Model

```
proc nlmixed data=beef1;
where time ^=0;
by fat temp rep;
parms f=0.5 k1=0.5 k2=0.5 var=0.5;
model =log10(N0)+ log10(f*exp(k1*time)+(1-f)*exp(k2*time));
model y ~ normal(model,var);
predict model out=p_beef;
run;
```

VI. Four Factor Sigmoidal

```
proc nlmixed data=beef1;
where time ^=0;
by fat temp rep;
parms n1=1 n2=1 k1=0.5 k2=0.5 var=0.5;
y= Log10(N0)-k1*(time**n1)*k2*(time**n2);
model y ~ normal(model,var);
predict model out=p_beef;
run;
```

VII. Gompertz Model

```
proc nlmixed data=beef1;
where time ^=0;
by fat temp rep;
parms C=1.00 A=1.00 B=2.00 M=0.05 var=0.05;
alpha=exp(B*M-B*time);
model=log10(N0) + A-C*exp(-alpha);
model y ~ normal(model,var);
predict model out=p_beef;
run;
```

VIII. Mixed Weibull

```
proc nlmixed data=beef1;
where time ^=0;
by fat temp rep;
parms alpha=2 delta1=0.1 delta2=0.1 p=0.5 var=0.5;
a= 1+10**alpha;
b= (time/delta1)**p;
c= (time/delta2)**p;
model = Log10(N0)-Log10(a)+ Log10(10**(-b+alpha)+10**(-c));
model y ~ normal(model,var);
model y ~ normal(model,var);
predict model out=p_beef;
run;
```

IX. Weibull Model

```
proc nlmixed data=beef1;
where time ^=0;
by fat temp rep;
parms b=0.5 n=0.5 var=0.5;
model = log10(N0) - b*time**n;
model y ~ normal(model,var);
predict model out=p_beef;
run;
```

2. SAS® Code for Response surface modeling

```
data mod;
input fat temp Sqrtb Sqrtn;
datalines;
8.03    55      0.44260592    0.858836422
8.03    55      0.622655603   0.755843899
8.03    55      0.731436942   0.733143915
8.03    60      1.212806662   0.839166253
8.03    60      1.297420518   0.758814865
.
.
.
.
.
.
.
.
31.04   65      2.68069021    0.724361788
31.04   65      3.671089756   1.089541188
31.04   68      4.91737735    1.048999523
31.04   68      3.166401743   0.821827232
31.04   68      2.637517772   0.740675367

;

proc univariate;
histogram/midpoints= -0.6 to 0.6 by 0.05
                    normal;
probplots;
run;
proc rsreg data=mod;
model Sqrtb=temp fat/lackfit;
run;
ods graphics on;

proc rsreg data=mod plots=all;
model Sqrtb=temp fat/lackfit;
run;
ods graphics off;
```

3. SAS® code for Calculating Error Term

```
data model;
input fat temp time ocfu cfu0;
datalines;
8.03    55      0       7.32    7.32
8.03    55      7.5     6.62    7.32
8.03    55      15      6.09    7.32
8.03    55      22.5    4.91    7.32
8.03    55      30      4.4     7.32
8.03    55      37.5    4.99    7.32
8.03    55      45      4.51    7.32
8.03    55      52.5    3.53    7.32
8.03    55      60      3.15    7.32
8.03    55      0       7.1     7.10
8.03    55      7.5     5.89    7.10
.
.
.
.
.
31.04   68      0.17    1.74    4.77
31.04   68      0.2     1.48    4.77
31.04   68      0.23    2.41    4.77
31.04   68      0.27    1.78    4.77
;
run;
proc print data=model (obs=10);
run;

data model1;
set model;
sqrb = 57.309488 + (0.239032*fat) - (2.202609*temp) -
(0.000785*fat*fat) - (0.003532*fat*temp)
+ (0.021106*temp*temp);
b=sqrb*sqrb;
sqrn = -0.543841 + 0.032695*fat + 0.01895*temp - 0.00036*fat*fat -
0.000312*fat*temp
+ 0.000056864*temp*temp;
n=sqrn*sqrn;
ont_n0 = ocfu-cfu0;
```

```
do x=-0.0692 to 0.0691 by 0.00001;
pnt_n0 = -((sqrb+x)**2)*(time**n);
output;
end;

*residual = pnt_n0 - ont_n0;
drop sqrb sqrn;
run;

data model2;
set model1;
residual = pnt_n0 - ont_n0;
run;

proc sort data=model2;
by x;
run;
proc means data=model2 noprint;
by x;
var residual;
output out=s_resid sum=sum_residual;
run;

proc sort data=s_resid;
by sum_residual;
run;

proc print data=s_resid;
where -0.1<= sum_residual <=0.1;
run;
title;

title 'using random term = -0.06911';
data model3;
set model;
sqrb = 57.309488 + (0.239032*fat) - (2.202609*temp) -
(0.000785*fat*fat) - (0.003532*fat*temp)
+ (0.021106*temp*temp);
b=sqrb*sqrb;
```

```
        sqrn = -0.543841 + 0.032695*fat + 0.01895*temp - 0.00036*fat*fat -
0.000312*fat*temp
        + 0.000056864*temp*temp;
        n=sqrn*sqrn;
        ont_n0 = ocfu-cfu0;
        pnt_n0 = -((sqrb-0.06911)**2)*(time**n);
        *residual = pnt_n0 - ont_n0;
        drop sqrb sqrn;
        run;

        proc reg data=model3;
        model ont_n0 = pnt_n0;
        run;
        title;
        ods pdf close;
```

4. SAS® code for Surface plot

```
data mod;
input fat temp b n logf;
datalines;
8.03    55      0.443   0.859   80.8073022
8.03    55      0.623   0.756   87.85514572
8.03    55      0.731   0.733   63.94367946
8.03    60      1.213   0.839   5.68324661
8.03    60      1.297   0.759   6.624195981
.
.
.
.
.
31.04   65      3.671   1.090   0.43376094
31.04   68      4.917   1.049   0.238757012
31.04   68      3.166   0.822   0.35695845
31.04   68      2.638   0.741   0.547733718

;
run;

proc rsreg data=mod;
  model logf = fat temp/ predict;
 run;

data surf;
        do Fat=8 to 31;
                do Temperature=55 to 67;
                Logf = 3656.906443 - 5.312920*fat -
111.883774*temperature + 0.009781*fat*fat + 0.075168*fat*temperature +
0.855170*temperature*temperature;
                output;
                end;
                output;
        end;
run;

 data surf1;
        set surf;
```

```
            label logf='Time (min.)';
            label fat ='Fat (%w/w)';
            label Temperature= 'Temp (C)';
        run;

        title "5-LogCFU/g reduction time";
        proc g3d data=surf1;
          plot fat*temperature=logf /cbottom=black ctop=black zaxis=axis3
rotate=320 tilt=60
                                                    xticknum=7 yticknum=7;
        run;
        quit;
```

APPENDIX B: PARAMETER ESTIMATIONS

Table 1. Akaike's information criterion (AIC), parameter estimates, Root mean square error (RMSE) and coefficient of regression (RSQ) of log-linear model obtained from nlmixed model in SAS®

Fat (%w/w)	Temp (ºC)	AIC	b	D-value (min.)	RMSE	RSQ
5	55	13.8	0.07	13.85	0.44	0.88
5	55	17.3	0.08	13.11	0.34	0.90
5	55	23	0.09	10.82	0.60	0.80
5	60	13.2	1.07	0.94	0.35	0.91
5	60	14.5	1.06	0.94	0.17	0.97
5	60	13.5	1.02	0.98	0.31	0.91
5	65	7.9	6.05	0.17	0.29	0.96
5	65	16.1	10.60	0.09	0.66	0.92
5	65	11.9	8.74	0.11	0.35	0.91
5	68	19.2	21.10	0.05	0.72	0.86
5	68	21.9	17.65	0.06	0.85	0.79
5	68	13.7	27.85	0.04	0.82	0.81
10	55	15	0.08	12.68	0.46	0.89
10	55	22.3	0.07	14.41	0.45	0.71
10	55	13.4	0.07	14.58	0.49	0.87
10	60	12.9	0.98	1.02	0.44	0.86
10	60	14.6	1.02	0.98	0.35	0.89
10	60	11.6	0.89	1.12	0.32	0.95
10	65	10	7.98	0.13	0.41	0.89
10	65	11	6.28	0.16	0.44	0.91
10	65	15.1	10.68	0.09	0.73	0.88
10	68	12.4	17.70	0.06	0.49	0.91
10	68	17.6	18.28	0.05	0.62	0.90
10	68	9.5	24.31	0.04	0.51	0.74
15	55	22.1	0.10	9.93	0.32	0.94
15	55	10.9	0.07	14.10	0.36	0.95
15	55	27	0.09	10.74	0.54	0.74
15	60	18.6	1.15	0.87	0.38	0.87
15	60	12.9	0.98	1.02	0.46	0.86
15	60	16.1	1.07	0.93	0.23	0.94
15	65	12.6	8.46	0.12	0.57	0.84
15	65	10.8	10.03	0.10	0.52	0.92
15	65	13.8	9.27	0.11	0.56	0.84
15	68	24.2	16.70	0.06	0.96	0.81
15	68	24	16.38	0.06	0.88	0.81
15	68	21	21.23	0.05	0.91	0.70
20	55	11.4	0.09	11.67	0.23	0.97

20	55	18.6	0.07	13.79	0.47	0.94
20	55	18.5	0.08	12.48	0.45	0.86
20	60	14.3	0.96	1.04	0.51	0.89
20	60	19.5	1.04	0.96	0.42	0.80
20	60	20.8	1.23	0.81	0.41	0.86
20	65	16	7.22	0.14	0.64	0.88
20	65	3.9	9.39	0.11	0.23	0.98
20	65	14.1	8.31	0.12	0.60	0.90
20	68	13.2	17.01	0.06	0.55	0.85
20	68	14.5	13.20	0.08	0.45	0.91
20	68	19.4	15.07	0.07	0.69	0.83
25	55	8.3	0.09	11.35	0.25	0.97
25	55	16.5	0.09	11.28	0.41	0.92
25	55	21.3	0.10	9.78	0.28	0.96
25	60	15.9	1.09	0.92	0.45	0.85
25	60	20.3	1.30	0.77	0.49	0.85
25	60	14.1	1.07	0.93	0.52	0.88
25	65	9.9	8.97	0.11	0.49	0.91
25	65	16.6	13.63	0.07	0.80	0.92
25	65	14.2	7.02	0.14	0.55	0.89
25	68	8	17.14	0.06	0.37	0.89
25	68	22.7	10.85	0.09	0.86	0.67
25	68	20.3	18.96	0.05	0.74	0.80
30	55	22.3	0.09	10.70	0.62	0.82
30	55	17.9	0.09	11.46	0.56	0.86
30	55	24.7	0.09	11.66	0.59	0.73
30	60	6.3	0.82	1.22	0.26	0.93
30	60	10.1	0.91	1.10	0.40	0.91
30	60	18.5	1.21	0.83	0.57	0.83
30	65	19.7	6.89	0.15	0.49	0.95
30	65	20	11.43	0.09	0.78	0.73
30	65	11.4	11.31	0.09	0.55	0.93
30	68	10.6	20.34	0.05	0.45	0.93
30	68	23.5	16.92	0.06	0.88	0.65
30	68	25.4	14.39	0.07	0.97	0.46

Table 2. Akaike's information criterion (AIC), parameter estimates, Root mean square error (RMSE) and coefficient of regression (RSQ) of log-linear with tail model obtained from nlmixed model in SAS®

Fat (%w/w)	Temp (ºC)	AIC	k	Nres	RMSE	RSQ
5	55	15.8	0.17	0.90	0.44	0.88
5	55	15.4	0.22	2227.96	0.42	0.85
5	55	2.2	0.32	773.42	0.22	0.97
5	60	15.2	2.49	30.59	0.36	0.90
5	60	14.7	2.78	208.95	0.30	0.90
5	60	15.1	2.47	152.36	0.35	0.89
5	65	3.5	11.84	-7885.68	0.22	0.97
5	65	17.9	23.52	-1.45	0.69	0.91
5	65	13.9	20.00	-0.16	0.34	0.92
5	68	20.5	46.74	-2.93	0.67	0.88
5	68	21.8	36.85	-84.09	0.75	0.84
5	68	15.1	69.52	0.90	0.76	0.84
10	55	12.8	0.22	1878.30	0.43	0.90
10	55	24.3	0.16	0.89	0.45	0.71
10	55	13.1	0.17	1039.70	0.44	0.89
10	60	12.9	2.08	-294.87	0.31	0.93
10	60	11.6	2.98	1222.48	0.37	0.87
10	60	12.1	1.92	-1018.47	0.34	0.95
10	65	11.5	17.49	-1.91	0.34	0.92
10	65	2.7	11.52	-694.64	0.22	0.98
10	65	9	21.36	-49.41	0.36	0.97
10	68	13.6	37.52	-1138.03	0.51	0.90
10	68	11.5	37.38	-38.55	0.39	0.96
10	68	10.3	88.94	3.50	0.58	0.66
15	55	11.6	0.31	138.38	0.28	0.96
15	55	8.7	0.15	-227.20	0.32	0.96
15	55	11.3	0.35	790.90	0.30	0.92
15	60	14.7	4.28	1902.69	0.48	0.80
15	60	12.9	2.47	512.24	0.43	0.88
15	60	7	3.29	2581.77	0.22	0.95
15	65	13.4	18.08	-3.73	0.46	0.90
15	65	12.7	22.85	-0.34	0.52	0.92
15	65	15.1	20.05	-185.26	0.44	0.90
15	68	25.5	27.15	-1198.85	0.89	0.81
15	68	23	35.54	-170.65	0.89	0.71
20	55	8.8	47.86	-0.76	0.28	0.96
20	55	16.1	0.22	384.40	0.43	0.95
20	55	14	0.15	-1005.36	0.40	0.89
20	60	15.8	0.23	1831.11	0.45	0.91
20	60	4.4	2.36	328.45	0.25	0.93

20	60	14.8	4.04	1202.00	0.47	0.82
20	65	12.3	4.54	564.86	0.49	0.93
20	65	2.5	13.04	-60.41	0.21	0.98
20	65	15.1	22.83	95.45	0.60	0.90
20	68	13.2	17.66	-38.00	0.44	0.90
20	68	12.7	35.51	-7.52	0.40	0.93
20	68	20.3	27.00	-1148.66	0.67	0.84
25	55	8.8	32.07	-377.60	0.28	0.97
25	55	14	0.21	125.89	0.42	0.91
25	55	15.1	0.24	156.25	0.41	0.91
25	60	11.2	0.32	290.24	0.37	0.90
25	60	16.6	3.06	365.33	0.52	0.83
25	60	14.6	4.15	119.18	0.44	0.91
25	65	11.4	2.77	314.11	0.46	0.92
25	65	16.1	19.84	-1.51	0.69	0.94
25	65	16.1	28.92	-19.87	0.51	0.91
25	68	4.2	16.60	35.74	0.26	0.95
25	68	24.7	46.19	630.78	0.86	0.66
25	68	22.2	24.42	-7.97	0.73	0.81
30	55	2.6	43.12	-7.28	0.23	0.98
30	55	13.1	0.30	259.25	0.43	0.92
30	55	17.5	0.26	252.36	0.50	0.80
30	60	8.2	0.29	319.44	0.27	0.92
30	60	12	1.93	233.41	0.40	0.91
30	60	17.1	2.04	-71.70	0.56	0.84
30	65	19.1	3.70	95.79	0.70	0.89
30	65	18.3	12.31	-455.28	0.82	0.70
30	65	9.3	41.45	17.56	0.37	0.97
30	68	11.1	24.06	-9.59	0.41	0.94
30	68	25.5	44.33	-3.35	0.86	0.66
30	68	20.6	37.93	-1.72	0.64	0.76

Table 3. Akaike's information criterion (AIC), parameter estimates, Root mean square error (RMSE) and coefficient of regression (RSQ) of Sigmoidal model obtained from nlmixed model in SAS®

Fat (%w/w)	Temp (ºC)	AIC	a	b	RMSE	RSQ
5	55	13.2	-6.87	3.79	0.44	0.88
5	55	11.8	-3.97	3.10	0.40	0.86
5	55	7.2	-5.71	4.04	0.30	0.95
5	60	15.6	3.75	3.23	0.51	0.80
5	60	10.2	4.19	2.78	0.36	0.86
5	60	13.9	3.76	2.93	0.46	0.81
5	65	7.3	11.94	6.59	0.30	0.95
5	65	15.5	20.63	11.18	0.59	0.94
5	65	14.2	10.66	3.19	0.58	0.75
5	68	25	18.99	5.69	0.91	0.78
5	68	25.1	22.55	8.72	0.83	0.80
5	68	12.5	19.82	5.47	0.59	0.90
10	55	8.3	-7.58	4.17	0.32	0.94
10	55	8.8	-1.77	2.35	0.33	0.84
10	55	16.4	-9.59	4.43	0.53	0.85
10	60	19.9	3.00	3.36	0.66	0.69
10	60	5.6	3.66	3.11	0.27	0.93
10	60	9.8	-1.51	7.27	0.33	0.95
10	65	13	10.45	3.53	0.53	0.81
10	65	11.3	12.88	7.36	0.42	0.92
10	65	20.6	17.54	8.15	0.95	0.80
10	68	7.7	23.03	8.74	0.26	0.97
10	68	24	19.10	6.50	0.86	0.80
10	68	9.2	11.63	2.56	0.51	0.74
15	55	-0.3	-4.73	3.98	0.19	0.98
15	55	13.1	-21.33	7.63	0.38	0.95
15	55	10.9	-1.85	3.00	0.38	0.87
15	60	8.9	4.48	3.12	0.33	0.90
15	60	14	2.72	3.76	0.46	0.86
15	60	1.9	4.19	2.88	0.22	0.95
15	65	18.4	10.89	3.67	0.83	0.65
15	65	15.6	16.50	7.62	0.54	0.91
15	65	16.9	11.72	3.75	0.74	0.73
15	68	19.7	33.85	15.48	0.73	0.89
15	68	24.9	28.80	13.07	0.78	0.85
15	68	25	15.00	3.77	1.11	0.55
20	55	6.6	-7.25	4.25	0.29	0.96
20	55	13.3	-33.02	10.76	0.39	0.96
20	55	11.2	-4.98	3.49	0.39	0.90
20	60	11.2	1.07	5.39	0.39	0.93

20	60	3	4.16	2.81	0.23	0.94
20	60	9.2	4.96	3.20	0.34	0.90
20	65	17	13.00	6.55	0.73	0.84
20	65	11.3	12.78	4.61	0.46	0.91
20	65	16.9	13.90	6.53	0.74	0.84
20	68	17.2	14.15	4.12	0.64	0.79
20	68	11.4	20.63	8.84	0.29	0.96
20	68	21.8	17.01	6.06	0.75	0.80
25	55	9.9	-8.95	4.77	0.35	0.95
25	55	11	-6.86	4.23	0.38	0.93
25	55	8.9	-4.87	4.04	0.33	0.94
25	60	11.7	3.71	3.51	0.40	0.89
25	60	10.7	4.89	3.75	0.38	0.91
25	60	10.3	2.44	4.77	0.36	0.94
25	65	13.7	12.97	5.08	0.56	0.88
25	65	20	24.51	12.40	0.82	0.91
25	65	11.7	12.87	6.52	0.47	0.92
25	68	8.2	12.51	3.40	0.35	0.90
25	68	21.3	15.65	6.33	0.72	0.76
25	68	25	21.61	7.78	0.73	0.81
30	55	8.9	-6.52	4.29	0.34	0.95
30	55	9.5	-8.15	4.56	0.35	0.95
30	55	16	-2.79	3.05	0.52	0.79
30	60	9.8	2.44	2.93	0.35	0.86
30	60	15.2	1.26	4.72	0.49	0.86
30	60	10.9	4.04	4.06	0.38	0.93
30	65	14.2	18.75	12.86	0.58	0.93
30	65	16.4	13.95	4.03	0.71	0.78
30	65	18.9	19.74	9.78	0.68	0.90
30	68	14.9	21.33	7.33	0.47	0.92
30	68	23.4	14.01	3.80	0.83	0.69
30	68	22.9	11.91	3.16	0.80	0.63

Table 4. Akaike's information criterion (AIC), parameter estimates, Root mean square error (RMSE) and coefficient of regression (RSQ) of Baranyi model obtained from nlmixed model in SAS®

Fat (%w/w)	Temp (ºC)	AIC	h0	Nmin	Umax	RMSE	RSQ
5	55	17.4	0.00	10.00	0.17	0.45	0.87
5	55	20.1	0.00	10.00	0.19	0.38	0.88
5	55	20.4	0.00	10.00	0.24	0.42	0.90
5	60	17.2	18.22	10.00	1.45	0.35	0.91
5	60	18.4	17.11	10.00	1.45	0.17	0.97
5	60	17.5	17.56	10.00	1.35	0.31	0.91
5	65	11.3	0.04	10.00	13.52	0.27	0.96
5	65	19.9	0.01	10.00	23.97	0.65	0.93
5	65	15.9	6985.50	0.00	19.13	0.35	0.91
5	68	23.2	1.49	10.00	47.60	0.72	0.86
5	68	25.8	0.00	10.00	40.31	0.84	0.80
5	68	17.7	8.16	9.99	63.12	0.82	0.81
10	55	16.9	0.00	10.00	0.19	0.44	0.89
10	55	23	0.00	10.00	0.18	0.42	0.75
10	55	13.4	0.00	10.00	0.17	0.39	0.92
10	60	16.9	16.98	10.00	1.25	0.44	0.86
10	60	18.5	19.20	10.00	1.35	0.35	0.89
10	60	11.2	0.05	10.00	1.63	0.28	0.96
10	65	14	5126.11	0.00	17.37	0.41	0.89
10	65	14.6	0.03	10.00	14.07	0.43	0.91
10	65	18.9	0.00	10.00	24.36	0.72	0.89
10	68	16.5	8.48	10.00	39.75	0.49	0.91
10	68	21.4	0.00	10.00	41.81	0.61	0.90
10	68	13.6	19.68	9.80	54.99	0.51	0.74
15	55	22.2	0.00	10.00	0.25	0.26	0.96
15	55	9.5	0.00	10.00	0.14	0.30	0.97
15	55	25.2	0.00	10.00	0.25	0.30	0.92
15	60	22.6	17.16	10.00	1.64	0.38	0.87
15	60	16.9	17.48	10.00	1.25	0.46	0.86
15	60	20	17.97	10.00	1.46	0.23	0.94
15	65	16.5	17.68	9.98	18.48	0.57	0.84
15	65	14.8	1.26	10.00	22.13	0.52	0.92
15	65	17.8	14.53	10.00	20.34	0.56	0.84
15	68	28.2	4.44	10.00	37.44	0.96	0.81
15	68	27.9	0.01	10.00	37.34	0.88	0.81
15	68	25	1.76	10.00	47.92	0.91	0.70
20	55	12.9	0.00	10.00	0.21	0.25	0.97
20	55	4.2	0.03	10.00	0.05	0.22	0.99
20	55	17.8	0.00	10.00	0.20	0.38	0.90
20	60	18.2	1.08	10.00	1.32	0.52	0.88

20	60	23.4	18.87	10.00	1.40	0.42	0.80
20	60	24.8	17.70	10.00	1.84	0.41	0.86
20	65	19.9	0.02	10.00	16.23	0.64	0.88
20	65	7.8	19.42	10.00	20.61	0.23	0.98
20	65	17.9	0.01	10.00	18.77	0.59	0.90
20	68	17.2	4.98	10.00	38.17	0.55	0.85
20	68	18.5	4.50	10.00	29.39	0.45	0.91
20	68	23.4	2.70	10.00	33.70	0.69	0.83
25	55	11.5	0.00	10.00	0.21	0.26	0.97
25	55	18.2	0.00	10.00	0.22	0.41	0.92
25	55	23.1	0.00	10.00	0.25	0.32	0.95
25	60	19.9	19.35	10.00	1.50	0.45	0.85
25	60	24.3	16.92	10.00	1.99	0.49	0.85
25	60	18.1	16.20	10.00	1.47	0.52	0.88
25	65	13.9	16.22	9.99	19.66	0.49	0.91
25	65	20.6	2.54	10.00	30.39	0.80	0.92
25	65	18	0.08	10.00	15.57	0.56	0.89
25	68	12	8.66	10.00	38.46	0.37	0.89
25	68	26.7	5.20	10.00	23.99	0.86	0.67
25	68	24.3	1.39	10.00	42.69	0.74	0.80
30	55	18.2	0.00	10.00	0.24	0.39	0.93
30	55	20.4	0.00	10.00	0.21	0.55	0.87
30	55	26.1	0.00	10.00	0.22	0.57	0.75
30	60	10.3	19.11	10.00	0.88	0.26	0.93
30	60	13.7	0.33	10.00	1.38	0.40	0.91
30	60	22.5	17.27	10.00	1.79	0.57	0.83
30	65	23.5	0.03	10.00	15.45	0.48	0.95
30	65	24	13.54	10.00	25.32	0.78	0.73
30	65	15.1	0.00	10.00	25.72	0.53	0.94
30	68	14.5	0.02	10.00	46.32	0.45	0.93
30	68	27.5	15.79	9.97	37.95	0.88	0.65
30	68	29.4	3.07	10.00	32.13	0.97	0.46

Table 5. Akaike's information criterion (AIC), parameter estimates, Root mean square error (RMSE) and coefficient of regression (RSQ) of Biphasic model obtained from nlmixed model in SAS®

Fat (%w/w)	Temp (ºC)	AIC	f	k_1	k_2	RMSE	RSQ
5	55	17.8	0.5	-0.2	-0.2	0.44	0.88
5	55	21.3	0.5	-0.2	-0.2	0.34	0.90
5	55	27	0.5	-0.2	-0.2	0.60	0.80
5	60	17.2	0.5	-2.5	-2.5	0.35	0.91
5	60	18.5	0.5	-2.4	-2.4	0.17	0.97
5	60	17.5	0.5	-2.4	-2.4	0.31	0.91
5	65	11.9	0.5	-13.9	-13.9	0.29	0.96
5	65	20.1	0.5	-24.4	-24.4	0.66	0.92
5	65	15.9	0.5	-20.1	-20.1	0.35	0.91
5	68	23.2	0.5	-48.6	-48.6	0.72	0.86
5	68	25.9	0.5	-40.6	-40.6	0.85	0.79
5	68	17.7	0.5	-64.1	-64.1	0.82	0.81
10	55	19	0.5	-0.2	-0.2	0.46	0.89
10	55	11.2	1.0	-0.4	0.0	0.34	0.84
10	55	17.4	0.5	-0.2	-0.2	0.49	0.87
10	60	16.9	0.5	-2.3	-2.3	0.44	0.86
10	60	18.6	0.5	-2.3	-2.3	0.35	0.89
10	60	15.6	0.5	-2.0	-2.0	0.32	0.95
10	65	14	0.5	-18.4	-18.4	0.41	0.89
10	65	15	0.5	-14.5	-14.5	0.44	0.91
10	65	19.1	0.5	-24.6	-24.6	0.73	0.88
10	68	16.4	0.5	-40.8	-40.8	0.49	0.91
10	68	21.6	0.5	-42.1	-42.1	0.62	0.90
10	68	13.5	0.5	-56.0	-56.0	0.51	0.74
15	55	26.1	0.5	-0.2	-0.2	0.32	0.94
15	55	14.9	0.5	-0.2	-0.2	0.36	0.95
15	55	31	0.5	-0.2	-0.2	0.54	0.74
15	60	22.6	0.5	-2.6	-2.6	0.38	0.87
15	60	16.9	0.5	-2.2	-2.2	0.46	0.86
15	60	20.1	0.5	-2.5	-2.5	0.23	0.94
15	65	16.6	0.5	-19.5	-19.5	0.57	0.84
15	65	14.8	0.5	-23.1	-23.1	0.52	0.92
15	65	17.8	0.5	-21.3	-21.3	0.56	0.84
15	68	28.2	0.5	-38.4	-38.4	0.96	0.81
15	68	28	0.5	-37.7	-37.7	0.88	0.81
15	68	25	0.5	-48.9	-48.9	0.91	0.70
20	55	15.4	0.5	-0.2	-0.2	0.23	0.97
20	55	22.6	0.5	-0.2	-0.2	0.47	0.94
20	55	22.5	0.5	-0.2	-0.2	0.45	0.86
20	60	18.3	0.5	-2.2	-2.2	0.51	0.89

20	60	23.5	0.5	-2.4	-2.4	0.42	0.80
20	60	24.8	0.5	-2.8	-2.8	0.41	0.86
20	65	20	0.5	-16.6	-16.6	0.64	0.88
20	65	7.9	0.5	-21.6	-21.6	0.23	0.98
20	65	18.1	0.5	-19.1	-19.1	0.60	0.90
20	68	17.2	0.5	-39.2	-39.2	0.55	0.85
20	68	18.5	0.5	-30.4	-30.4	0.45	0.91
20	68	23.4	0.5	-34.7	-34.7	0.69	0.83
25	55	12.1	0.5	-0.2	-0.2	0.24	0.97
25	55	20.5	0.5	-0.2	-0.2	0.41	0.92
25	55	25.3	0.5	-0.2	-0.2	0.28	0.96
25	60	19.9	0.5	-2.5	-2.5	0.45	0.85
25	60	24.3	0.5	-3.0	-3.0	0.49	0.85
25	60	18.1	0.5	-2.5	-2.5	0.52	0.88
25	65	13.9	0.5	-20.7	-20.7	0.49	0.91
25	65	20.6	0.5	-31.4	-31.4	0.80	0.92
25	65	18.2	0.5	-16.2	-16.2	0.55	0.89
25	68	12	0.5	-39.5	-39.5	0.37	0.89
25	68	26.7	0.5	-25.0	-25.0	0.86	0.67
25	68	24.3	0.5	-43.7	-43.7	0.74	0.80
30	55	26.2	0.5	-0.2	-0.2	0.61	0.82
30	55	21.9	0.5	-0.2	-0.2	0.56	0.86
30	55	28.7	0.5	-0.2	-0.2	0.59	0.73
30	60	10.3	0.5	-1.9	-1.9	0.26	0.93
30	60	14.1	0.5	-2.1	-2.1	0.40	0.91
30	60	22.5	0.5	-2.8	-2.8	0.57	0.83
30	65	23.7	0.5	-15.9	-15.9	0.49	0.95
30	65	24	0.5	-26.3	-26.3	0.78	0.73
30	65	15.4	0.5	-26.0	-26.0	0.55	0.93
30	68	14.6	0.5	-46.8	-46.8	0.45	0.93
30	68	27.5	0.5	-38.9	-38.9	0.88	0.65
30	68	29.4	0.5	-33.1	-33.1	0.97	0.46

Table 6. Akaike's information criterion (AIC), parameter estimates, Root mean square error (RMSE) and coefficient of regression (RSQ) of Four Factors Sigmoidal model obtained from nlmixed model in SAS®

Fat (%w/w)	Temp (ºC)	AIC	k_1	k_2	n_1	n_2	RMSE	RSQ
5	55	16.4	0.28	-0.71	0.37	0.37	0.42	0.89
5	55	13.2	0.31	-1.24	0.29	0.29	0.34	0.90
5	55	18	-0.78	0.69	0.27	0.27	0.46	0.89
5	60	15.2	-1.16	1.27	0.35	0.35	0.38	0.89
5	60	7.5	1.28	-1.32	0.29	0.29	0.24	0.94
5	60	13.6	0.32	-4.60	0.33	0.33	0.35	0.89
5	65	35.3	0.00	0.00	1.56	1.56	1.21	0.00
5	65	42.1	-0.39	-0.39	10.20	10.20	2.13	0.00
5	65	39.7	-0.01	-0.01	5.83	5.83	1.06	0.00
5	68	53.3	-1.20	-1.20	5.78	5.78	1.80	0.00
5	68	50.6	-0.34	-0.34	5.49	5.49	1.73	0.00
5	68	35.7	-0.26	-0.26	3.81	3.81	1.65	0.00
10	55	15.7	0.85	-0.28	0.35	0.35	0.39	0.92
10	55	14.7	0.69	-0.98	0.20	0.20	0.37	0.80
10	55	18.8	-0.78	0.14	0.43	0.43	0.48	0.87
10	60	47.4	0.00	0.00	2.19	2.19	1.11	0.00
10	60	11.1	-1.41	1.10	0.31	0.31	0.30	0.92
10	60	9.7	-0.70	0.75	0.73	0.73	0.27	0.97
10	65	38.6	0.00	0.00	4.13	4.13	1.11	0.00
10	65	35.8	0.00	0.00	1.77	1.77	1.29	0.00
10	65	42.1	-0.29	-0.29	10.61	10.61	1.92	0.00
10	68	37.3	0.00	0.00	2.73	2.73	1.46	0.00
10	68	51	-0.40	-0.40	5.73	5.73	1.77	0.00
10	68	27.8	0.00	0.00	1.80	1.80	0.81	0.00
15	55	3.4	1.33	-0.46	0.26	0.26	0.18	0.98
15	55	10.4	0.03	-0.54	0.68	0.68	0.28	0.97
15	55	16.6	0.86	-1.10	0.19	0.19	0.42	0.84
15	60	13.2	-0.56	3.35	0.27	0.27	0.34	0.90
15	60	17.4	1.28	-0.95	0.40	0.40	0.44	0.87
15	60	2.6	1.39	-1.27	0.27	0.27	0.18	0.97
15	65	39.3	0.02	0.02	5.11	5.11	1.26	0.00
15	65	41.3	0.19	0.19	9.67	9.67	1.62	0.00
15	65	40.4	-0.02	-0.02	7.13	7.13	1.26	0.00
15	68	43.7	0.00	0.00	3.84	3.84	2.00	0.00
15	68	49.7	-0.15	-0.15	5.07	5.07	1.87	0.00
15	68	46.4	-0.58	-0.58	4.95	4.95	1.51	0.00
20	55	3.5	0.57	-0.45	0.35	0.35	0.19	0.98
20	55	4.4	0.03	-0.11	0.90	0.90	0.19	0.99
20	55	15.5	-0.56	0.69	0.29	0.29	0.39	0.89
20	60	20.2	1.63	-0.57	0.52	0.52	0.52	0.88
20	60	48.7	0.00	0.00	2.51	2.51	0.88	0.00

20	60	13.9	1.43	-1.47	0.25	0.25	0.36	0.90
20	65	37.7	0.00	0.00	2.95	2.95	1.63	0.00
20	65	40.4	-0.09	-0.09	7.13	7.13	1.34	0.00
20	65	39.2	0.00	0.00	4.73	4.73	1.67	0.00
20	68	43.1	0.00	0.00	3.75	3.75	1.28	0.00
20	68	45.9	-0.01	-0.01	3.10	3.10	1.39	0.00
20	68	48.1	0.05	0.05	4.40	4.40	1.57	0.00
25	55	6.1	0.26	-0.76	0.39	0.39	0.22	0.98
25	55	14.1	0.42	-0.75	0.33	0.33	0.36	0.94
25	55	6.9	-1.08	0.53	0.27	0.27	0.23	0.97
25	60	16.2	0.78	-2.04	0.33	0.33	0.41	0.88
25	60	16.6	0.88	-2.36	0.28	0.28	0.42	0.89
25	60	19.6	1.21	-1.00	0.45	0.45	0.49	0.89
25	65	40	0.05	0.05	6.30	6.30	1.48	0.00
25	65	45	-2.76	-2.76	12.89	12.89	2.47	0.00
25	65	37.3	0.00	0.00	2.70	2.70	1.52	0.00
25	68	36.8	-0.03	-0.03	2.50	2.50	0.98	0.00
25	68	43.7	0.00	0.00	2.21	2.21	1.37	0.00
25	68	51.6	-0.53	-0.53	6.04	6.04	1.54	0.00
30	55	18.9	-1.36	0.35	0.29	0.29	0.48	0.89
30	55	18.6	-0.39	0.72	0.35	0.35	0.47	0.90
30	55	20.3	-0.88	0.78	0.22	0.22	0.53	0.78
30	60	40.1	-0.08	0.08	2.36	2.36	0.48	0.75
30	60	15.7	0.72	-1.11	0.55	0.55	0.40	0.91
30	60	19.4	0.97	-1.81	0.33	0.33	0.50	0.87
30	65	37.6	0.00	0.00	2.68	2.68	1.92	0.00
30	65	43.1	-1.73	-1.73	9.70	9.70	1.34	0.00
30	65	42.7	-1.23	-1.23	9.89	9.89	1.88	0.00
30	68	45.5	-0.36	-0.36	4.82	4.82	1.57	0.00
30	68	50.1	-0.23	-0.23	5.20	5.20	1.37	0.00
30	68	48	0.04	0.04	4.16	4.16	1.22	0.00

Table 7. Akaike's information criterion (AIC), parameter estimates, Root mean square error (RMSE) and coefficient of regression (RSQ) of Mixed Weibull model obtained from nlmixed model in SAS®

Fat (%w/w)	Temp (ºC)	AIC	Alpha	$Delta_1$	$Delta_2$	p	RMSE	RSQ
5	55	16.4	20.53	9.12	23.59	0.74	0.42	0.89
5	55	13.2	15.90	5.25	8.46	0.57	0.34	0.90
5	55	24.4	2.40	0.54	0.00	0.32	0.39	0.92
5	60	15.1	1.53	0.58	0.00	0.70	0.38	0.89
5	60	7.5	1.95	0.41	0.00	0.58	0.24	0.94
5	60	13.6	1.83	0.55	0.00	0.66	0.35	0.89
5	65	3.8	1.97	0.23	0.23	1.57	0.17	0.98
5	65	18.1	1.98	0.15	0.00	1.47	0.58	0.94
5	65	14.6	1.88	0.06	0.00	0.68	0.43	0.87
5	68	32.9	2.01	0.02	0.00	0.56	0.79	0.83
5	68	27.4	1.97	0.07	0.00	1.25	0.78	0.82
5	68	10.4	2.88	0.05	0.15	4.10	0.32	0.97
10	55	15.7	17.87	7.49	13.01	0.71	0.39	0.92
10	55	14.7	9.37	2.69	6.76	0.40	0.37	0.80
10	55	18.8	18.88	12.28	31.41	0.87	0.48	0.87
10	60	16.9	-0.16	1.28	0.00	1.12	0.42	0.88
10	60	2.8	-1.92	3.00	0.61	2.05	0.18	0.97
10	60	9.7	2.06	1.56	0.01	1.47	0.27	0.97
10	65	14.5	2.00	0.09	0.09	0.79	0.43	0.88
10	65	10.6	1.88	0.23	0.00	1.69	0.30	0.96
10	65	19.8	1.93	0.13	0.00	1.30	0.65	0.91
10	68	23.1	2.01	0.03	0.00	0.74	0.57	0.88
10	68	4.9	-1.65	0.18	0.05	3.55	0.20	0.99
10	68	13.9	2.01	0.02	0.00	0.63	0.50	0.74
15	55	19.3	2.12	0.47	0.00	0.32	0.16	0.99
15	55	10.4	4.62	19.32	7.62	1.36	0.28	0.97
15	55	17.8	2.28	0.52	0.00	0.32	0.41	0.85
15	60	13.2	1.99	0.31	0.20	0.54	0.34	0.90
15	60	17.4	7.79	0.79	0.20	0.80	0.44	0.87
15	60	2.6	2.03	0.35	0.27	0.54	0.18	0.97
15	65	19.7	2.01	0.08	0.00	0.75	0.63	0.80
15	65	16.4	2.27	0.13	0.29	1.78	0.48	0.93
15	65	18.8	2.00	0.09	0.00	0.85	0.58	0.83
15	68	27.7	2.04	0.10	0.00	1.53	0.74	0.89
15	68	26.6	1.86	0.10	0.00	1.67	0.79	0.85
15	68	26.7	1.99	0.04	0.00	0.84	0.94	0.68
20	55	3.5	17.54	6.73	3.38	0.71	0.19	0.98
20	55	39.5	1.98	0.12	0.00	0.17	0.89	0.79
20	55	15.5	14.65	5.08	5.74	0.59	0.39	0.89
20	60	20.2	7.69	1.08	0.05	1.04	0.52	0.88

20	60	2.8	-2.28	3.60	0.57	1.25	0.18	0.96
20	60	13.9	1.99	0.23	0.16	0.51	0.36	0.90
20	65	11.1	-2.01	0.47	0.17	16.38	0.31	0.97
20	65	16	2.00	0.09	0.00	0.93	0.23	0.98
20	65	16	-1.38	0.34	0.12	3.03	0.48	0.93
20	68	19.2	2.00	0.06	0.00	0.97	0.55	0.85
20	68	21.4	2.00	0.10	0.00	1.30	0.35	0.94
20	68	20.6	-1.92	0.21	0.08	4.48	0.54	0.90
25	55	6.1	17.79	7.82	3.97	0.79	0.22	0.98
25	55	14.1	14.64	5.63	0.26	0.66	0.36	0.94
25	55	20.9	2.11	0.46	0.00	0.32	0.25	0.97
25	60	14.6	3.73	0.64	62503494	0.84	0.37	0.90
25	60	16.6	2.00	0.28	0.21	0.57	0.42	0.89
25	60	19.6	7.33	0.81	0.08	0.89	0.49	0.89
25	65	7	-2.05	0.38	0.10	3.65	0.23	0.98
25	65	21	1.93	0.10	0.10	1.26	0.74	0.93
25	65	14	3.49	0.21	1907.29	2.09	0.41	0.94
25	68	7.7	3.06	0.05	878430	1.15	0.24	0.95
25	68	27.9	2.00	0.12	0.10	1.39	0.86	0.67
25	68	26.3	1.95	0.05	0.00	0.97	0.75	0.80
30	55	25.9	2.38	0.55	0.00	0.32	0.40	0.92
30	55	18.6	14.46	6.22	4.04	0.69	0.47	0.90
30	55	20.3	7.19	2.28	0.04	0.45	0.53	0.78
30	60	8.9	0.06	1.29	0.00	0.93	0.26	0.92
30	60	15.7	2.05	1.22	0.03	1.11	0.40	0.91
30	60	19.4	2.09	0.43	0.32	0.66	0.50	0.87
30	65	14.8	-4.36	40.08	0.28	3.18	0.43	0.96
30	65	13	3.66	0.08	0.50	94.92	0.38	0.93
30	65	16.2	1.99	0.11	0.11	1.19	0.47	0.95
30	68	16.2	1.94	0.06	0.00	1.10	0.44	0.93
30	68	27.9	2.01	0.03	0.00	0.68	0.85	0.67
30	68	12.6	2.97	0.08	7.53	7.32	0.31	0.94

Table 8. Akaike's information criterion (AIC), parameter estimates, Root mean square error (RMSE) and coefficient of regression (RSQ) of Gompertz model obtained from nlmixed model in SAS®

Fat (%w/w)	Temp (ºC)	AIC	A	B	C	M	RMSE	RSQ
5	55	-	-	-	-	-	-	-
5	55	3.7	41.47	0.01	51.67	-	0.34	0.90
5	55	2.5	-0.34	0.11	4.07	13.26	0.19	0.98
5	60	-	-	-	-	-	-	-
5	60	-	-	-	-	-	-	-
5	60	-	-	-	-	-	-	-
5	65	11.8	0.25	1.38	40.67	1.13	0.16	0.99
5	65	17.5	-0.76	9.55	5.81	0.31	0.43	0.97
5	65	-	-	-	-	-	-	-
5	68	-2.7	0.09	1.08	1243.93	1.81	0.65	0.89
5	68	-1.4	-0.93	5.56	19.63	0.34	0.66	0.87
5	68	-	-	-	-	-	-	-
10	55	-	-	-	-	-	-	-
10	55	4.3	7.59	0.07	10.90	-14.88	0.31	0.86
10	55	31.7	-0.72	0.07	3.55	28.57	0.45	0.89
10	60	-	-	-	-	-	-	-
10	60	-	-	-	-	-	-	-
10	60	41.7	-0.24	0.71	5.32	2.60	0.25	0.97
10	65	-	-	-	-	-	-	-
10	65	-	-	-	-	-	-	-
10	65	-	-	-	-	-	-	-
10	68	0.4	-0.49	34.97	3.44	0.12	0.10	1.00
10	68	-	-	-	-	-	-	-
10	68	-	-	-	-	-	-	-
15	55	30.6	1.24	0.05	6.83	4.23	0.14	0.99
15	55	-	-	-	-	-	-	-
15	55	14.6	-1.99	0.17	2.31	19.81	0.20	0.96
15	60	-	-	-	-	-	-	-
15	60	-	-	-	-	-	-	-
15	60	22	-1.02	1.02	2.76	1.40	0.14	0.98
15	65	5.8	-1.69	15.97	3.42	0.39	0.18	0.98
15	65	18.5	-0.92	11.14	3.96	0.24	1.62	0.00
15	65	15.7	-1.55	0.69	19368.00	3.62	1.26	0.00
15	68	8.7	-0.22	1.96	33937.00	1.34	2.00	0.00
15	68	27.6	-0.92	71.92	3.95	0.18	1.87	0.00
15	68	10.8	-1.77	21.77	3.92	0.16	0.79	0.77
20	55	-	-	-	-	-	-	-
20	55	-	-	-	-	-	-	-
20	55	-	-	-	-	-	-	-
20	60	14.4	-0.07	1.82	3.34	1.50	0.34	0.95

20	60	-	-	-	-	-	-	-
20	60	-	-	-	-	-	-	-
20	65	-	-	-	-	-	-	-
20	65	17.5	-1.11	8.13	3.87	0.26	0.13	0.99
20	65	-	-	-	-	-	-	-
20	68	-	-	-	-	-	-	-
20	68	-1.4	-0.55	16.24	4.41	0.18	0.15	0.99
20	68	-	-	-	-	-	-	-
25	55	-	-	-	-	-	-	-
25	55	-	-	-	-	-	-	-
25	55	31.7	138.32	0.02	144.76	-	0.21	0.98
25	60	28.7	-1.00	0.05	4.80	19.19	0.39	0.89
25	60	-	-	-	-	-	-	-
25	60	-	-	-	-	-	-	-
25	65	24.2	316.72	0.59	321.12	-6.72	1.48	0.00
25	65	13.1	24.96	0.23	114.16	1.82	0.70	0.94
25	65	0	-0.93	5.14	9.87	0.34	0.43	0.93
25	68	0.4	0.23	9.88	3.94	0.22	0.18	0.97
25	68	5.8	-0.75	36.95	2.31	0.09	0.52	0.88
25	68	15.3	0.03	134.21	2.53	0.11	0.59	0.87
30	55	30.6	-1.40	15.26	4.48	0.16	0.22	0.98
30	55	-	-	-	-	-	-	-
30	55	14.6	486.93	0.04	491.77	-97.92	0.48	0.82
30	60	-	-	-	-	-	-	-
30	60	19.9	3.78	0.05	64.07	19.97	0.40	0.91
30	60	-	-	-	-	-	-	-
30	65	-	-	-	-	-	-	-
30	65	-	-	-	-	-	-	-
30	65	15.7	543.37	4.75	548.63	-0.98	0.27	0.98
30	68	-	-	-	-	-	-	-
30	68	27.6	3.27	1.29	96.93	0.94	0.84	0.68
30	68	10.8	378.63	8.33	383.03	-0.52	0.29	0.95

APPENDIX C: SOXHLET FAT ANALYSIS PROTOCOL

The following protocol for measuring fat content of the ground beef samples was used: -

1. Measure and record the weight of a filter paper
2. Add 2-3 g of meat to the filter paper (Mark meat weight as A)
3. Fold the filter paper to make a pouch
4. Dry samples at 105ºC for 18-24 hours in an oven
5. Measure the weight of dried samples
6. Subtract the weight of filter paper from the dried sample (Mark weight as B)
7. Load the dried samples in extraction unit of Soxhlet apparatus with Petroleum either in the flat bottom flask
8. Set up the temperature to obtain condensation rate of 4-5 drops/sec
9. Let the extraction run for 4-6 hours
10. Switch off heating and collect petroleum either in the flat bottom flask
11. Take the samples out from the extraction unit
12. Let the samples cool down for 20-30 min at a room temperature in a biosafety hood
13. Dry the cooled samples overnight at 105ºC
14. Measure weight of the dried extracted samples and subtract the weight of filter paper from it (Mark weight as C)

The following equations can be used to measure moisture content and fat content of the samples

$$Moisture\ (\%w/w) = \frac{A-B}{A}\ X\ 100$$

$$Fat\ content\ (\%w/w) = \frac{B-C}{A} X\ 100$$