Development of Predictive Microbial Models for Food Safety using Alternate Approaches

Project Number: 8072-42000-083-00D

Dr. Lihan Huang
Dr. Cheng-An (Andy) Hwang

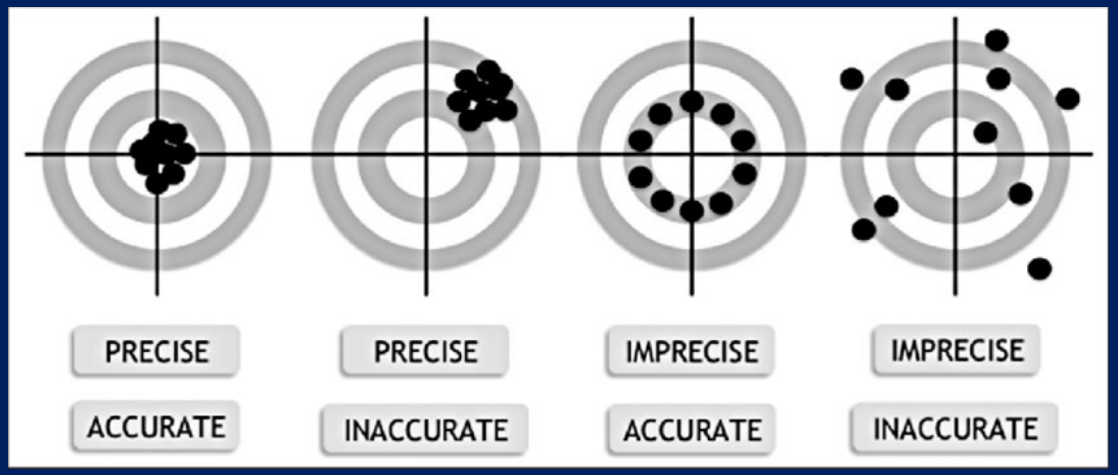
Residue Chemistry & Predictive Microbiology Research Unit
Eastern Regional Research Center
USDA ARS
Wyndmoor, PA

Objectives of This Talk

- What are the Alternate Approaches?
- What are the advantages?
- How have they changed the way the we do modeling?
- How useful?
- How can they benefit you?

Thrusts

- More accurate models
- Faster model development
- More efficient development
- More useful models
- All validated



Main Research Objectives

1. Development and validation of predictive models for growth of high priority pathogens in processed foods
2. Dynamic simulation and probabilistic modeling of growth of foodborne pathogens in foods
3. Development of an advanced decision support system and software for predictive microbiology and food safety regulations
4. Expand where necessary the ARS curve-fitting (modeling) program also known as the “Integrated Pathogen Modeling Program (IPMP)”

Predictive modeling – different perspectives

- End users – the persons who use models
  - A model with necessary parameters to make a meaningful prediction
  - Forward problem or forward analysis
- Developers – the persons who develop models
  - Performing kinetic analysis (data collection)
  - Identifying a model and determining kinetic parameters
  - Inverse problem or inverse analysis – digging information from laboratory data
  - In-house capacity – experimental design, data analysis, and computer programming

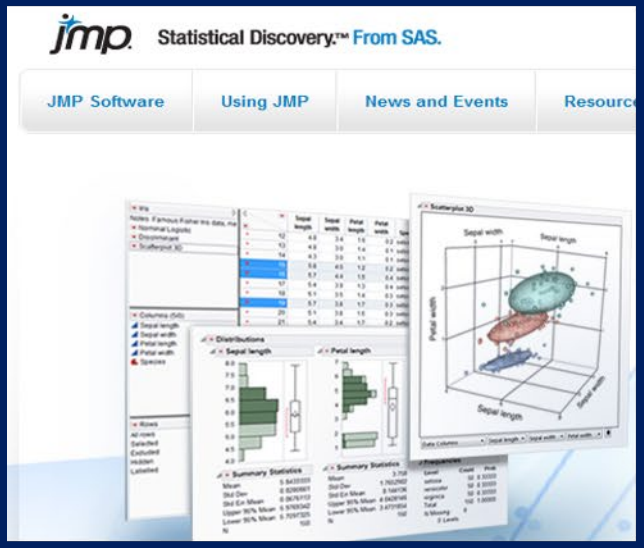
Mysteries of Predictive Modeling

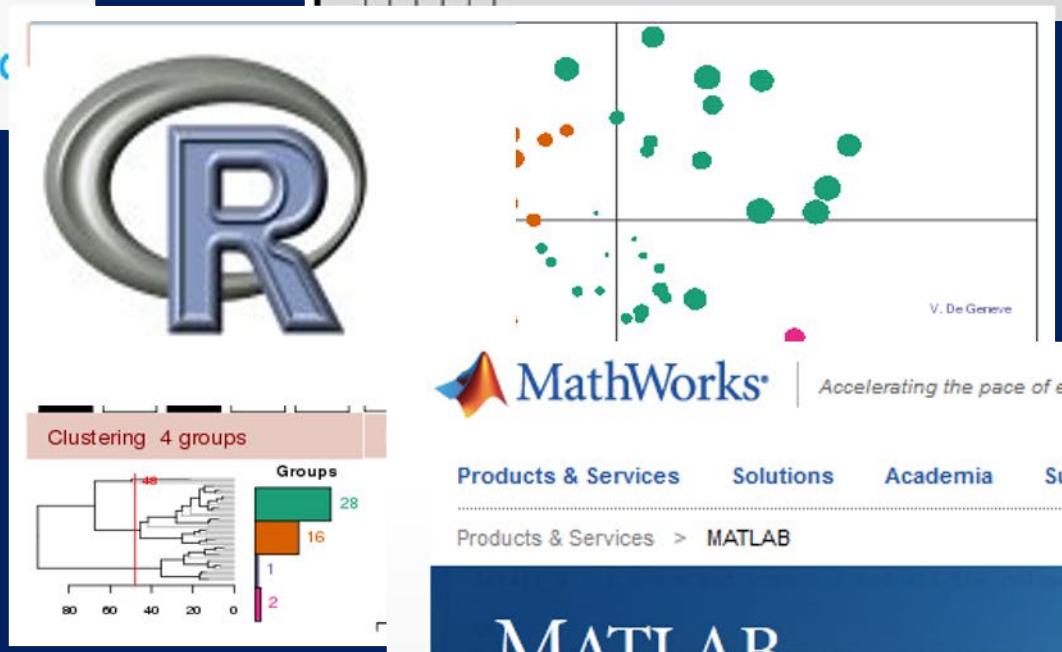
- Math is too hard
- I don’t know which model to choose
- I don’t know which model is better
- I don’t have a program to do it
- I don’t know how to program

Interpretation and application of models

- There are so many different variations of models
- Model parameters may not be interchangeable, leading to inaccuracy
- Proper interpretation and application of models may be challenging
- Standardized and unified models are needed (structure)
- Everyone will use the same language when talking to each other

Commercial Data Analysis and Statistical/Math Tools





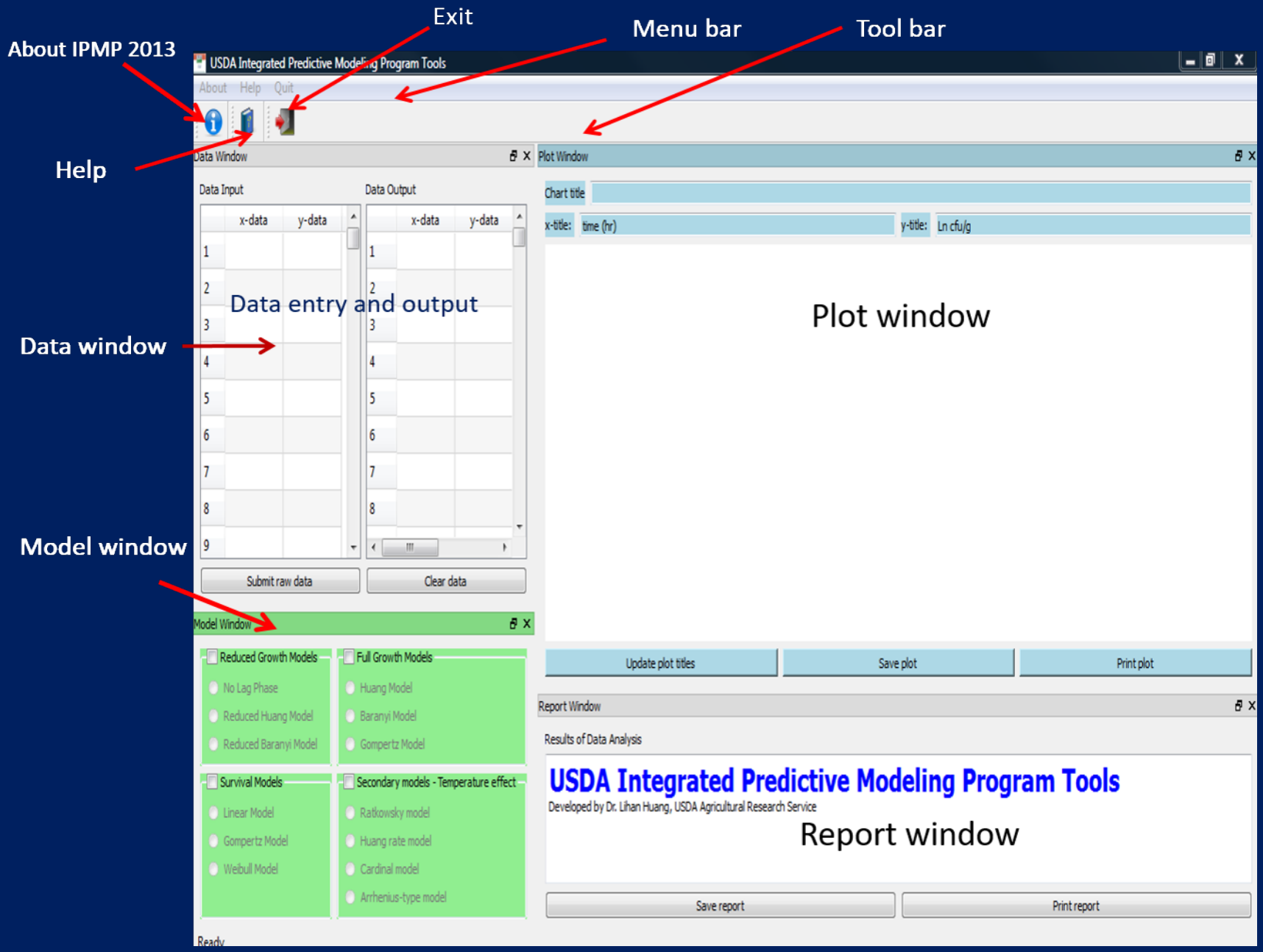
Commercial Data Analysis and Statistical/Math Tools (2)

- Very powerful
- Product-specific programming
- Not so user-friendly
- Training and learning
- Most are very expensive
- Major contribution of variations in models

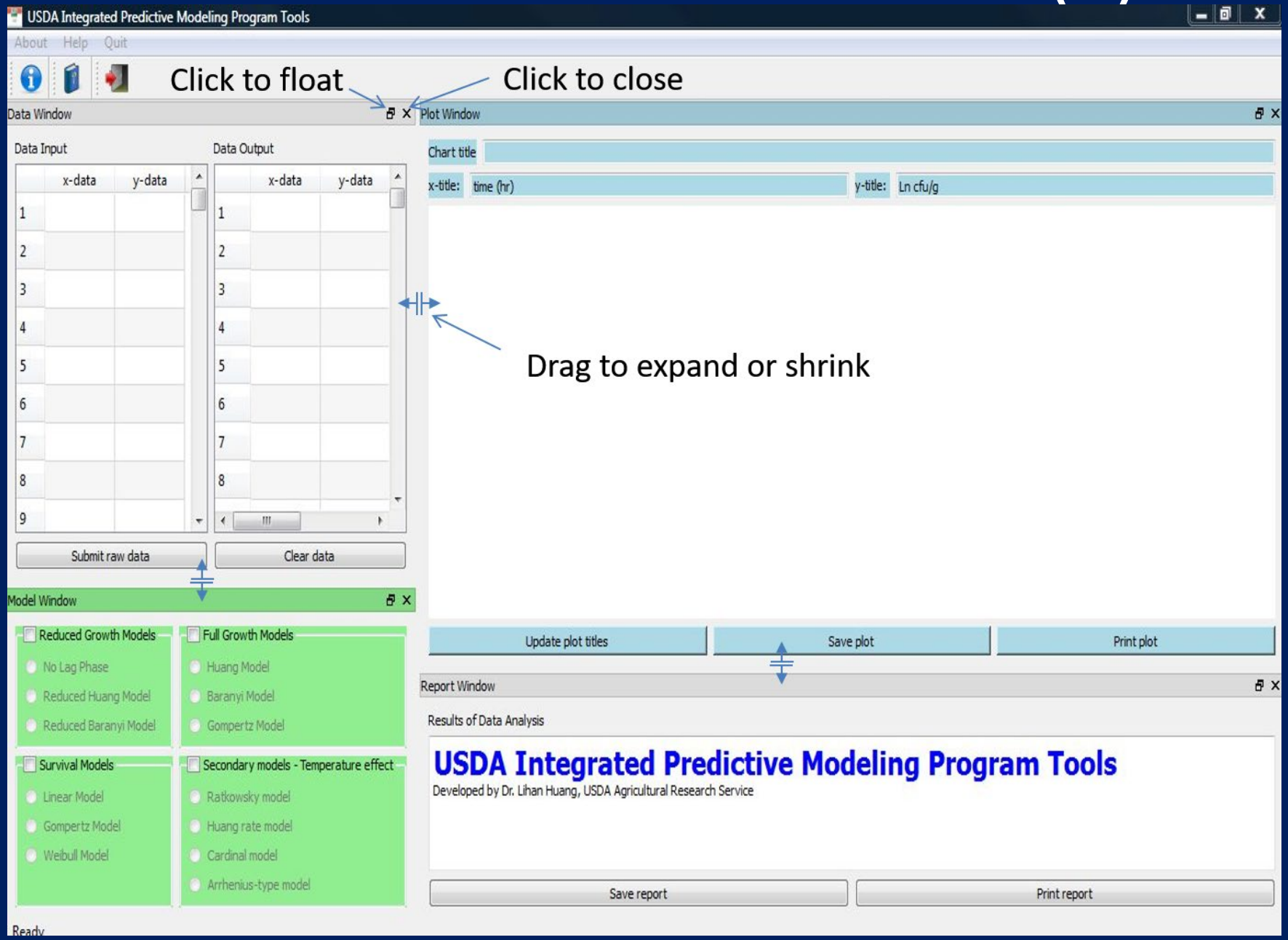
Integrated Pathogen Modeling Program

- Based on a modern object-oriented computing language
- Advanced scientific computing libraries
- Standardized and unified model structures
- Modern graphical-user interfaces
- 20+ mathematical models
- Directly comparable and interchangeable model parameters

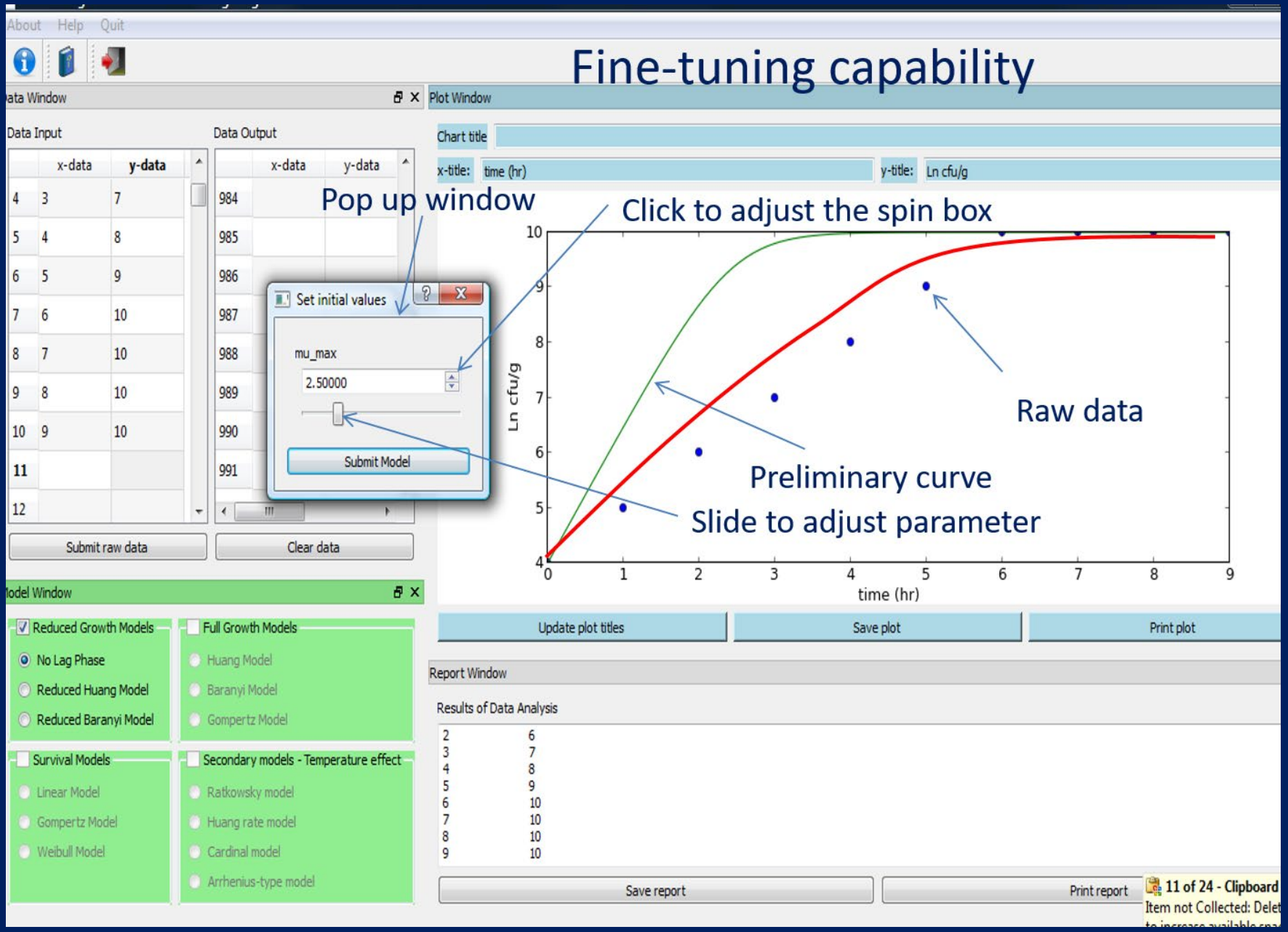
IPMP 2013 User Interface



IPMP 2013 User Interface (2)



IPMP 2013 Data Analysis



Report Generation

Error analysis

Parameters

Raw data

Report of Data Analysis - No Lag Phase Growth Model
Data regression successful

degree of freedom	7
SSE	0.216
MSE	0.031
RMSE	0.176
residual stdev	0.147
AIC (the smaller the better)	-22.361
critical t-value	2.36462

Sum of squared errors

Mean squared errors

Root mean squared errors

Akaike criterion

Lower and upper 95% confidence intervals

Parameters	Value	Std-Error	t-value	p-value	L95CI	U95CI
Y0	2.901	0.123	23.671	6.102E-08	2.611	3.191
Ymax	10.295	0.160	64.518	5.646E-11	9.917	10.672
mumax	1.064	0.038	28.062	1.875E-08	0.975	1.154

Predicted value

data output report

x	y	Pred	L95MCI	U95MCI	L95PCI	U95PCI
0.00	3.00	2.90	2.61	3.19	2.39	3.41
1.00	4.00	3.96	3.74	4.19	3.49	4.44
2.00	5.00	5.03	4.85	5.20	4.57	5.48
3.00	6.00	6.08	5.91	6.25	5.63	6.53
4.00	7.00	7.12	6.92	7.31	6.66	7.57
5.00	8.00	8.10	7.88	8.33	7.63	8.58
6.00	9.00	8.98	8.76	9.19	8.51	9.44
7.00	10.00	9.63	9.44	9.82	9.17	10.09
8.00	10.00	10.01	9.77	10.25	9.53	10.49
9.00	10.00	10.19	9.88	10.50	9.67	10.71

Lower and upper 95% confidence intervals for the expected value (mean)

Lower and upper 95% confidence intervals for individual prediction

Mendeley report

IPMP 2013 - A comprehensive data analysis tool for predictive microbiology

Huang L

International Journal of Food Microbiology, vol. 171 (2014)

View on ScienceDirect

Citations	Views	Readers
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Metrics

Citations

CrossRef - Citation Indexes: 41
PubMed - Citation Indexes: 7
Scopus - Citation Indexes: 58

Google Scholar

IPMP 2013--a comprehensive data analysis tool for predictive ... - NCBI
https://www.ncbi.nlm.nih.gov/pubmed/24334095
by L Huang - 2014 - Cited by 71 - Related articles
Int J Food Microbiol. 2014 Feb 3;171:100-7. doi: 10.1016/j.ijfoodmicro.2013.11.019. Epub 2013 Nov 23.
IPMP 2013--a comprehensive data analysis tool for ...

IPMP 2013 — A comprehensive data analysis tool for predictive ...
https://www.sciencedirect.com/science/article/pii/S0168160513005400
by L Huang - 2014 - Cited by 71 - Related articles
Feb 3, 2014 - Data curves previously published in literature were used to test the models in IPMP 2013. The accuracies of the data analysis and models ...

IPMP in Action

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Growth modeling of Uropathogenic *Escherichia coli* in ground chicken meat☆

Christopher Sommers [a,*], Chi-Yun Huang [b], Lee-Yan Sheen [b], Shiowshuh Sheen [a], Lihan Huang [a]

[a] U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038, USA
[b] Institute of Food Science and Technology, The National Taiwan University, Taipei, Taiwan, ROC

ARTICLE INFO

Article history:
Received 25 September 2017
Received in revised form
3 December 2017
Accepted 4 December 2017
Available online 6 December 2017

Keywords:
Uropathogenic *E. coli*
Extraintestinal pathogenic E.coli
Growth model
Ground chicken

ABSTRACT

Extraintestinal Pathogenic *Escherichia coli* (ExPEC), including Uropathogenic *E. coli* (UPEC), are common contaminants in poultry meat, and are a major pathogen associated with inflammatory bowel disease, ulcerative colitis, sepsis, and urinary tract infections. The purpose of this study was to determine the growth potential of UPEC in ground chicken meat. A multi-isolate cocktail of UPEC was inoculated into ground chicken meat 10^{3-4} log CFU/g and incubated at 4, 10, 15, 22, and 30 °C. The USDA Integrated Pathogen Modeling Program (IPMP) was used to conduct mathematical modeling and validation of UPEC growth using the Huang Primary Model and the Huang Square Root Secondary Model. No growth occurred at 4 °C, while the lag phases were ca. 23.6, 11.5, 5.2, and 0.36 h at 10, 15, 22, and 30 °C. According to the model, the T_{min}, the minimum temperature for UPEC growth in ground chicken, was 5.1 °C. The growth rates (μ_{max}, ln CFU/g h^{-1}) were ca. 0.06, 0.27, 0.48, and 0.90. Approximately 83.9% of the residual errors are between ±0.5 log CFU/g, suggesting that the predictive models and the associated kinetic parameters are sufficiently accurate in predicting the growth of UPEC in ground chicken. These models have been validated and can be used in risk assessment of ExPEC in poultry meat.

IPMP in Action (1)

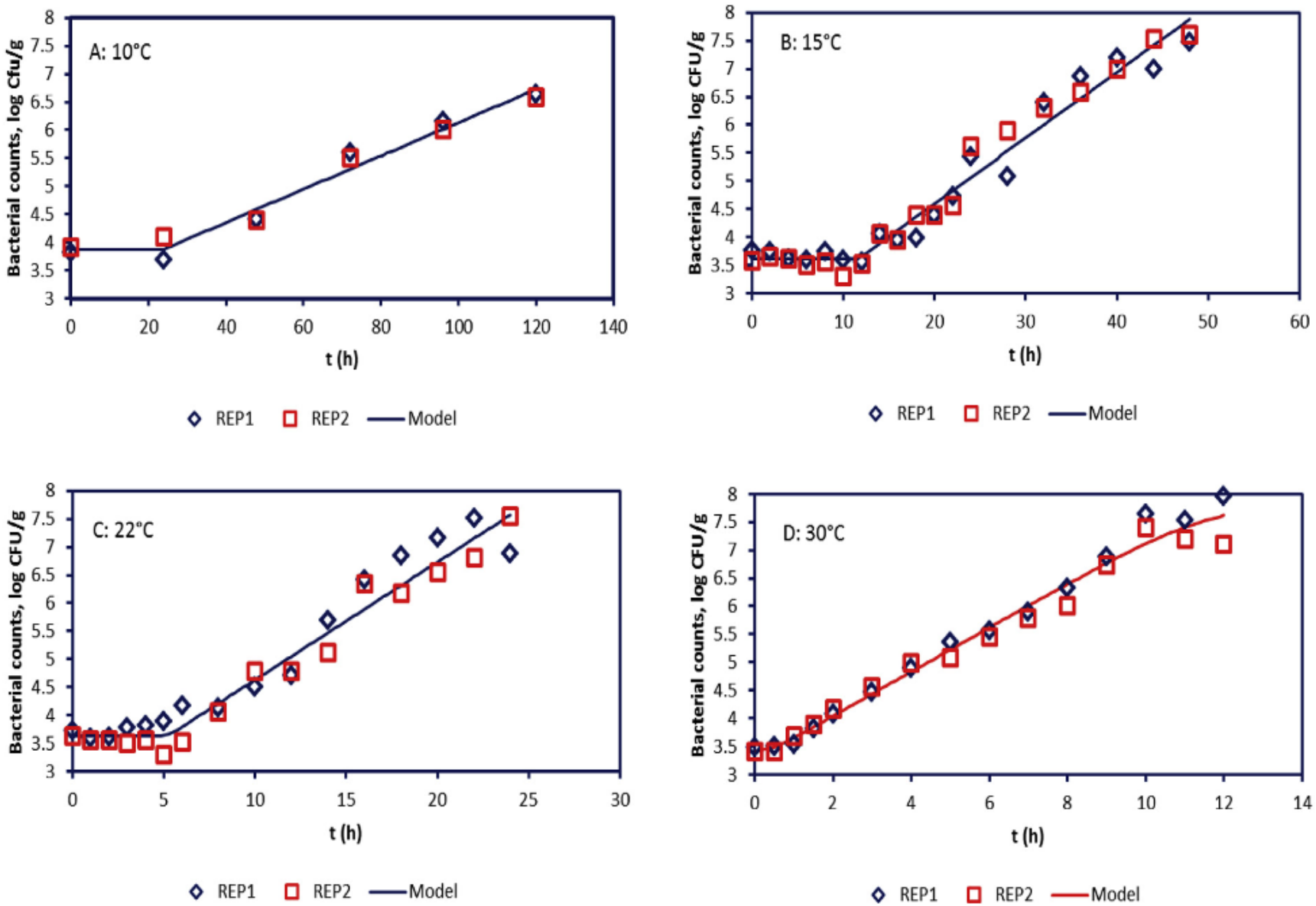


Fig. 2. Growth curves of ExPEC in ground chicken at 10, 15, 22, and 30 °C. Empty diamonds (REP1) and squares (REP2) are raw data. Solid curves are model values.

IPMP in Action (2)

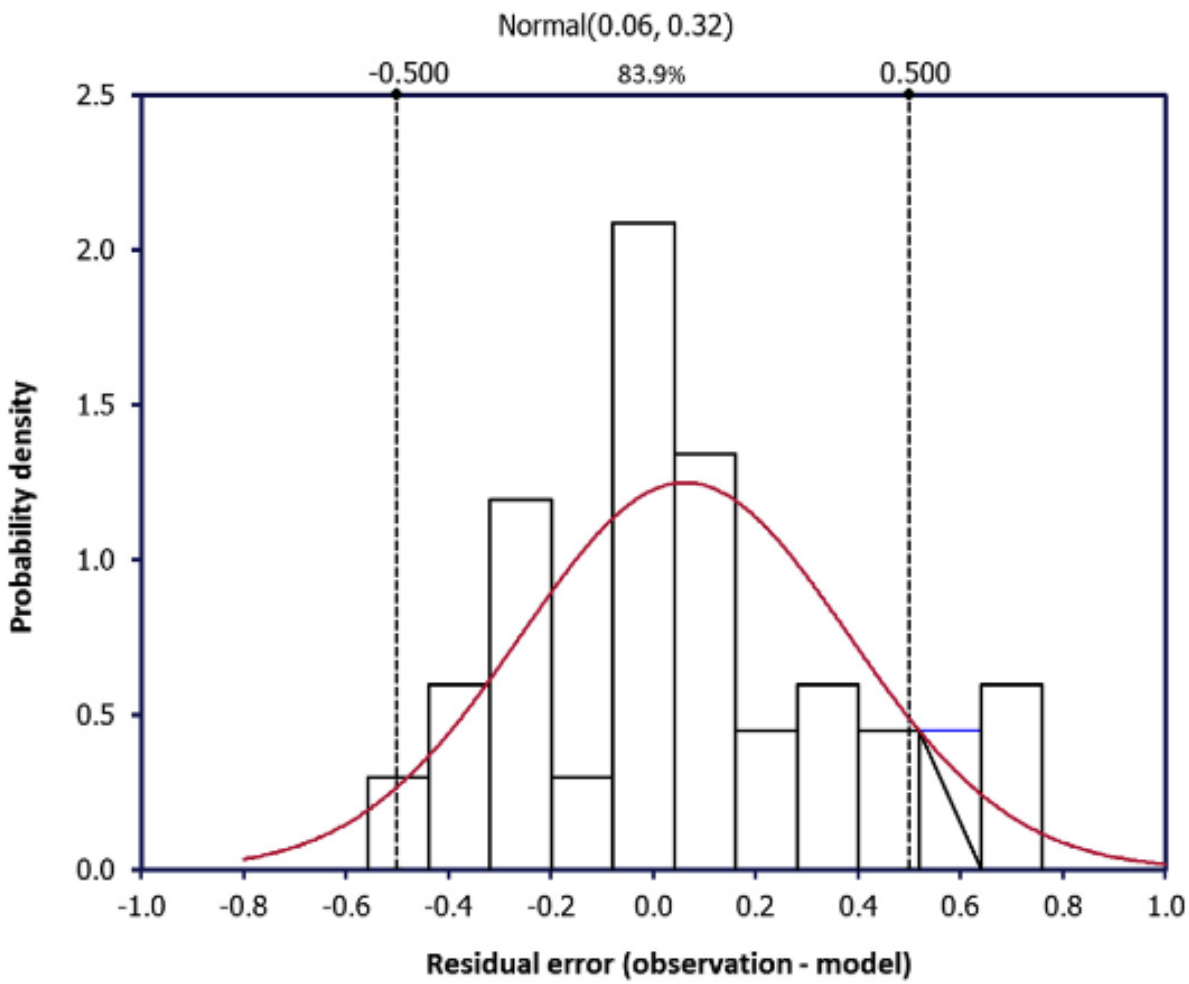
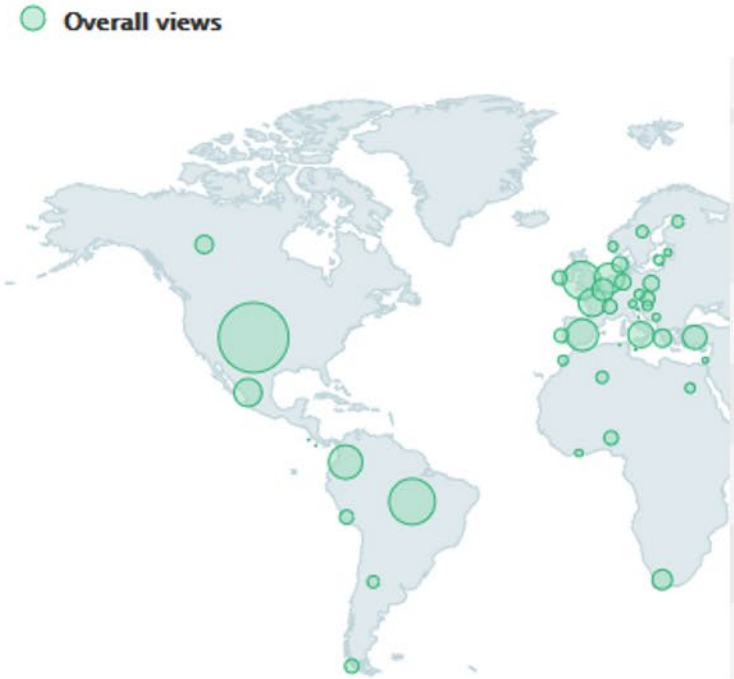


Fig. 5. Distribution of residual errors of validation.

Mendeley Report - IPMP Viewers

Where are your viewers coming from?



Top countries

Netherlands	86
France	79
Mexico	76
Italy	65
Turkey	59
India	58
Belgium	47
South Africa	36
Malaysia	35
Indonesia	33

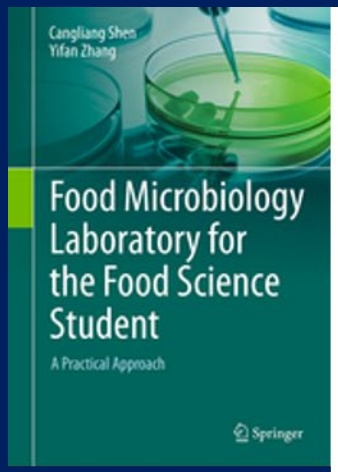
Top countries

United States	553
China	393
Brazil	221
United Kingdom	149
Thailand	129
South Korea	125
Colombia	122
Taiwan	107
Spain	100
Australia	89

Who uses USDA IPMP?

- It has been used in research and education around the world
  - University of Maryland
  - West Virginia University
  - Athens Agricultural University (Greece)
  - Konkut University (South Korea)
  - National Taiwan University
- Scientific publications

Who uses USDA IPMP? (1)



USDA-Integrated-Predictive-Modeling-Program Software

IPMP 2013 is a new-generation predictive microbiology tool. It is designed to analyze experimental data commonly encountered in predictive microbiology and for the development of predictive models. In this chapter, we will use survival models to analyze the results.

Brief procedures:

Step 1. Download and install "USDA-IPMP 2013 software" into your computer:
https://www.ars.usda.gov/northeast-area/wyndmoor-pa/eastern-regional-research-center/docs/integrated-pathogen-modeling-program-ipmp-2013/
Then, click "*Link to download IPMP2013*."

Step 2. Enter cooking time into "x-data" column, and enter microbial raw data into "y-data" column. Click "submit raw data"; all raw microbial data will transfer to "log CFU/unit."

Step 3. Select the "Survival Models," and click the radio button of "Linear Model," "Gompertz Model," "Weibull Model," and "Two/Three-Phase Linear Model." For "Linear Model," choose both "with" and "without" tails.

Step 4. Your results should be fit to one of the following curves:

1. Linear curve
2. Linear curve with tail
3. Reparametrized Gompertz survival model
4. Weibull model
5. Linear curve with shoulder, two-phase model
6. Linear and tail three-phase model

Step 5. Find your RMSE and AIC number of each model equation; the best model that fits your results should have the smallest value of RMSE and AIC.

References (After Class Reading)

Huang, L. 2013. USDA Integrated Pathogen Modeling Program (http://www.ars.usda.gov/Main/docs.htm?docid=23355). USDA Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA.

Huang, L. 2014. IPMP 2013 – A comprehensive data analysis tool for predictive microbiology. International Journal of Food Microbiology, 171: 100–107.

Who uses USDA IPMP? (2)

Meat Science 107 (2015) 20–25

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Predictive model for the growth kinetics of *Staphylococcus aureus* in raw pork developed using Integrated Pathogen Modeling Program (IPMP) 2013

Yong Ju Lee [a], Byeong Su Jung [a], Kee-Tae Kim [b], Hyun-Dong Paik [a,b,*]

[a] Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 143-701, Republic of Korea
[b] Bio/Molecular Informatics Center, Konkuk University, Seoul 143-701, Republic of Korea

IPMP for Data Analysis

- Simple data entry
- Choose models based on your data
- Use graphical interfaces to adjust parameters for faster (almost guaranteed) convergence
- All data analysis and calculation are done behind the scene - fast and accurate
- Compare different models for the same set of data
- It is a game-changer ----- IPMP has significantly lowered the bar for those who want to work on predictive modeling

The Traditional Approach in Predictive Modeling: A 3-step process

Step 1 – Primary model

Data collection

Isothermal 1
Isothermal 2
Isothermal 3
Isothermal i
Isothermal n

$y = f(t)$

Inverse problem

Step 2 – Secondary model

Data analysis

μ_1
μ_2
μ_3
μ_i
μ_n

$\mu = g(T)$

μ

Step 3 - Predictions

Predictions $y = f(t)$

Forward problem

Limitations of the Traditional Approach

- Each step accumulates and propagates errors
- The errors of the predictions can be difficult or impossible to estimate

Is there a better way to extract information from isothermal experiments?

Global Analysis

One-step Direct Kinetic Analysis of Isothermal Curves

1. Data for primary model

x_1,	y_1	x_1,	y_1	x_1,	y_1	x_1,	y_1
x_2,	y_2	x_2,	y_2	x_2,	y_2	x_2,	y_2
:	:	:	:	:	:	:	:
:	:	:	:	:	:	:	:
x_i,	y_i	x_i,	y_i	x_i,	y_i	x_i,	y_i
:	:	:	:	:	:	:	:
:	:	:	:	:	:	:	:
x_n	y_n	x_n	y_n	x_n	y_n	x_n	y_n

2. A secondary model

3. Search parameters of the secondary model so that the errors of growth curves are minimized

4. Direct construction of tertiary models

Global Analysis (2)

- It tries to minimize the global residual error for the entire data set
- It directly constructs a tertiary model
- It is validated with experimental data
- The model error can be easily estimated and interpreted
- The model is directly usable

One-Step Global Analysis

Food Control 68 (2016) 69–76

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Mathematical modeling and validation of growth of *Salmonella* Enteritidis and background microorganisms in potato salad – One-step kinetic analysis and model development☆

Lihan Huang

US. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

ARTICLE INFO

Article history:
Received 28 December 2015
Received in revised form
17 March 2016
Accepted 22 March 2016
Available online 24 March 2016

ABSTRACT

This study was conducted to examine the growth of *Salmonella* Enteritidis (SE) in potato salad caused by cross-contamination and temperature abuse, and develop mathematical models to predict its growth. The growth of SE was investigated under constant temperature conditions (8, 10, 15, 20, 25, 30, and 37 °C) to evaluate the effect of temperature on growth rates and lag times. Duplicated experiments were conducted. The data set from one replicate was used to develop kinetic models and determine kinetic

Salmonella Enteritidis and background microorganisms in potato salad

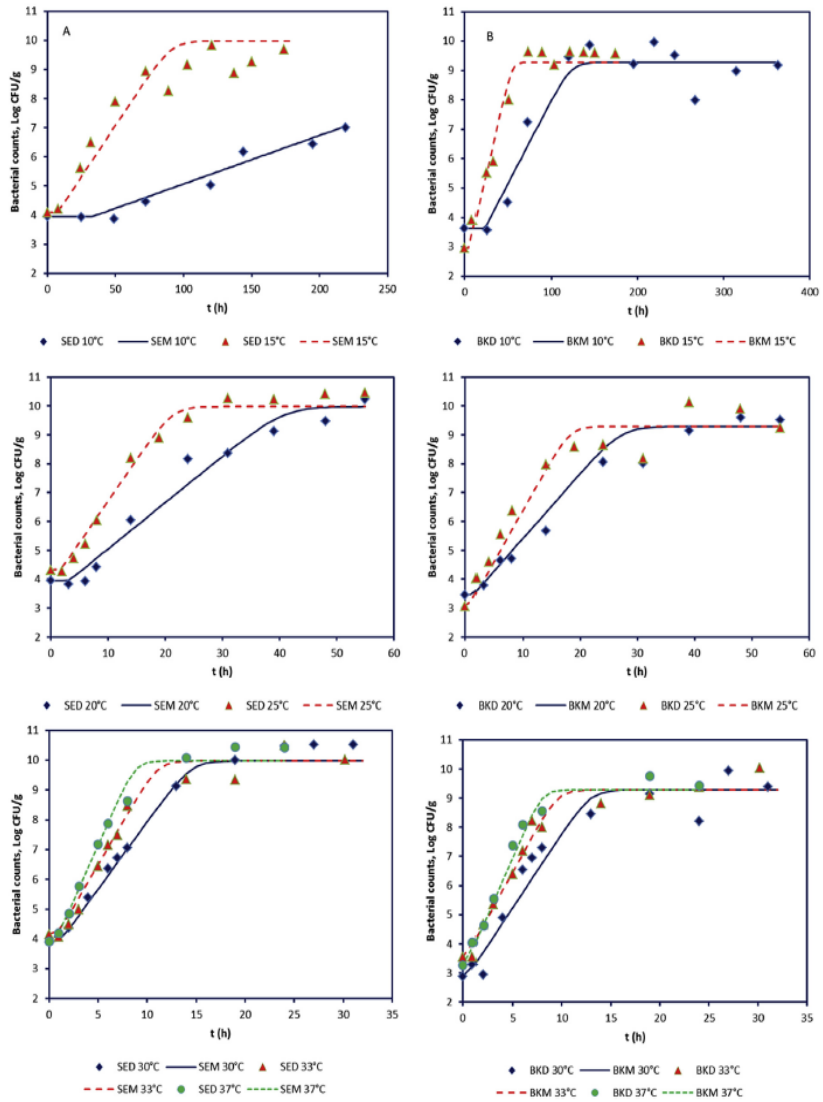


Fig. 1. One-step curve fitting of multiple growth curves. Column A: SE; Column B: BK. SED: SE growth data; SEM: SE growth model. BKD: BK growth data; BKM: BK growth model.

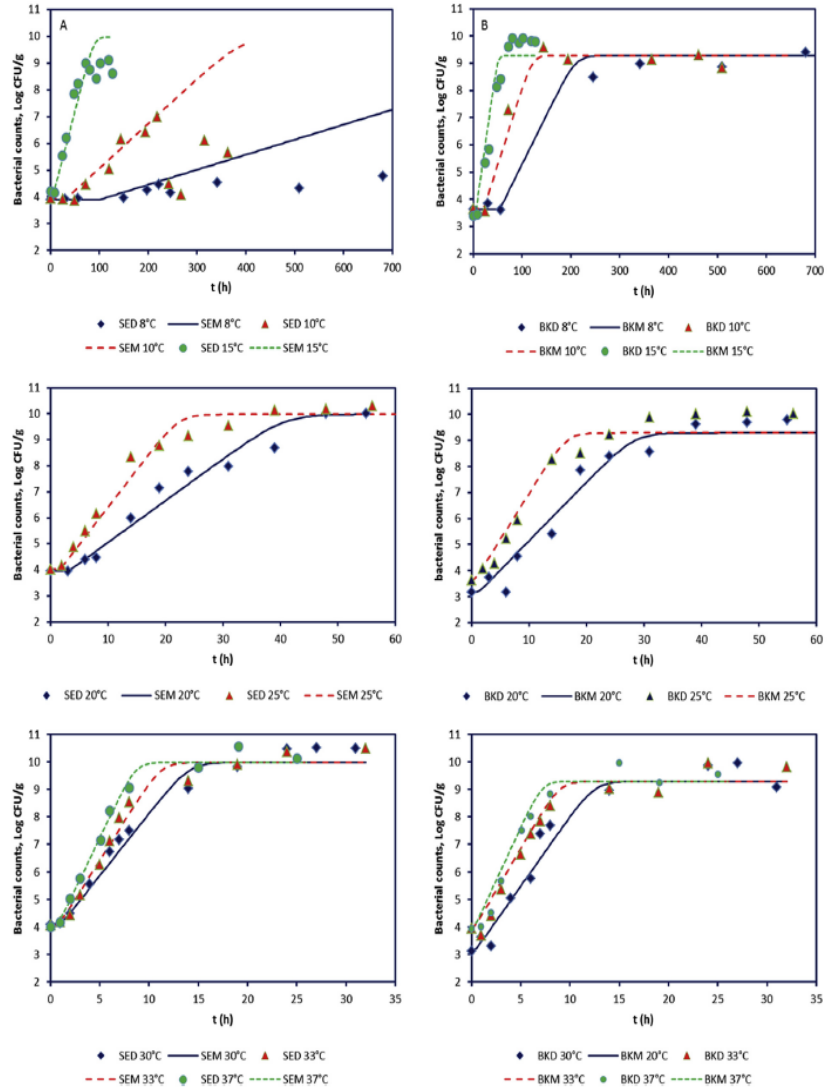
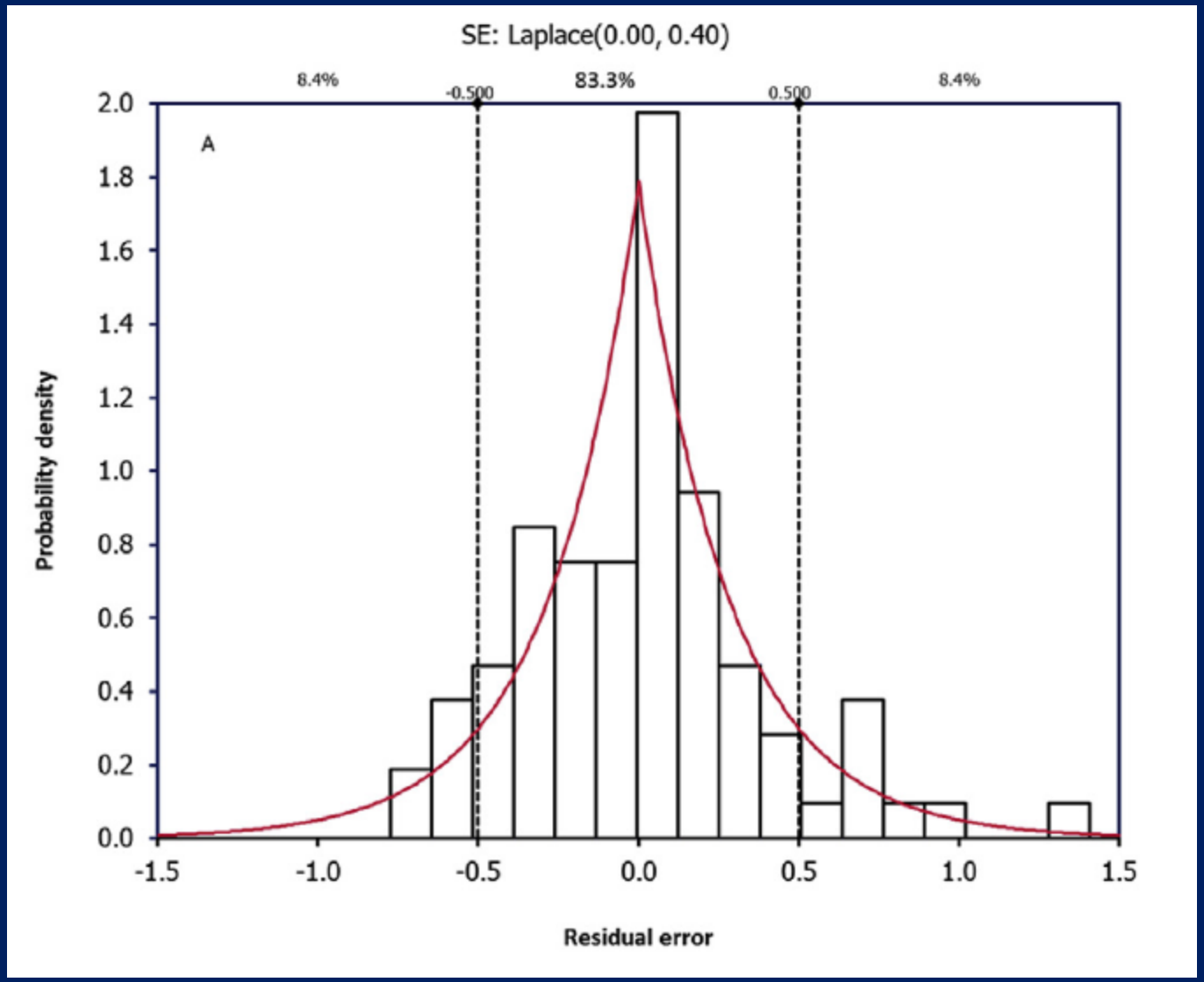


Fig. 3. Validation of mathematical models. Column A: SE; Column B: BK. SED: SE growth data; SEM: SE growth model. BKD: BK growth data; BKM: BK growth model. SEM: SE growth model. BKD: BK growth data; BKM: BK growth model.

Accuracy of prediction for *S.* Enteritidis



Accuracy of prediction for background microbiota

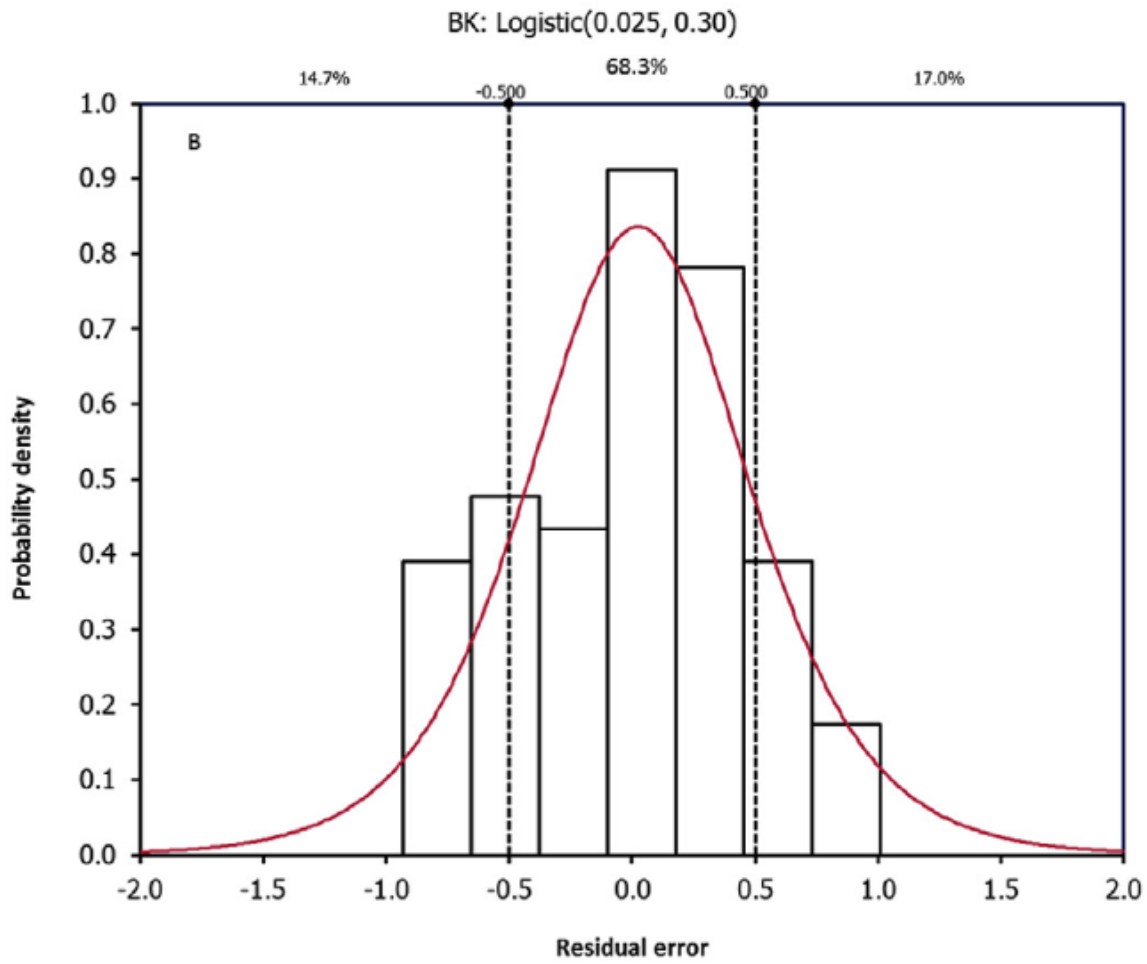


Fig. 4. The distribution of residual errors (in Log CFU/g) of prediction. A: SE; B: BK.

One-Step Global Analysis (2)

Journal of Food Science
A Publication of the Institute of Food Technologists

M: Food Microbiology & Safety | Full Access

Growth of *Staphylococcus aureus* in Cooked Potato and Potato Salad—A One-Step Kinetic Analysis*

Lihan Huang

First published: 05 November 2015 | https://doi.org/10.1111/1750-3841.13110 | Cited by: 3

† Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Dept. of Agriculture. USDA is an equal opportunity employer and provider.

ELSEVIER

Food Control
Volume 57, November 2015, Pages 76-81

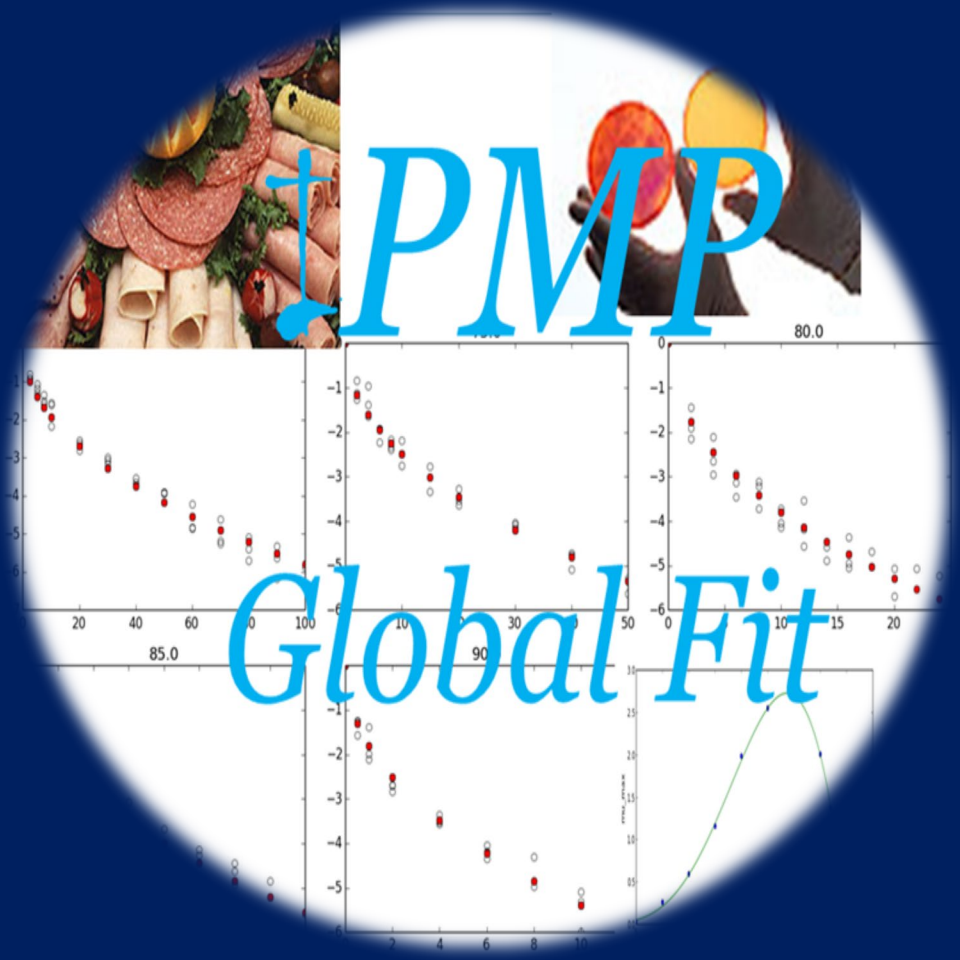
Direct construction of predictive models for describing growth of *Salmonella* Enteritidis in liquid eggs – A one-step approach ☆

Lihan Huang

Algorithm development

- We started testing in 2014
- By 2016, the methodology was firmly established
- Computational algorithm was implemented in SAS
- Not many can benefit from this method

IPMP - Global Fit (1)



Expanded version available online since Jan. 12, 2017

IPMP - Global Fit (2)

ELSEVIER

International Journal of Food Microbiology

Volume 262, 4 December 2017, Pages 38-48

IPMP Global Fit – A one-step direct data analysis tool for predictive microbiology ☆

Lihan Huang [1]

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https://doi.org/10.1016/j.ijfoodmicro.2017.09.010

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Highlights

- A new integrated one-step kinetic analysis tool has been developed.
- This tool allows estimation of kinetic parameters with minimized global error.
- It is an easy-to-use tool for analyzing growth and survival curves.

IPMP - Global Fit (3)

IPMP Global Fit

About Help Quit

Data Window

	Treatment	x	y
1	20	0	3.27
2	20	24	3.23
3	20	48	3.37
4	20	72	3.64
5	20	96	3.70
6	20	192	7.35
7	20	216	7.61
8	20	264	7.78
9	20	336	8.74
10	20	360	7.85
11	20	0	3.45
12	20	24	3.49
13	20	48	3.68
14	20	72	3.30
15	20	96	3.61
16	20	168	7.58
17	20	192	8.00
18	20	216	8.34
19	20	240	7.94
20	20	264	8.67
21	20	336	7.18
22	20	360	8.10
23	20	0	3.59
24	20	24	3.71
25	20	48	3.68

Submit or Clear Data

Submit raw data | Clear data

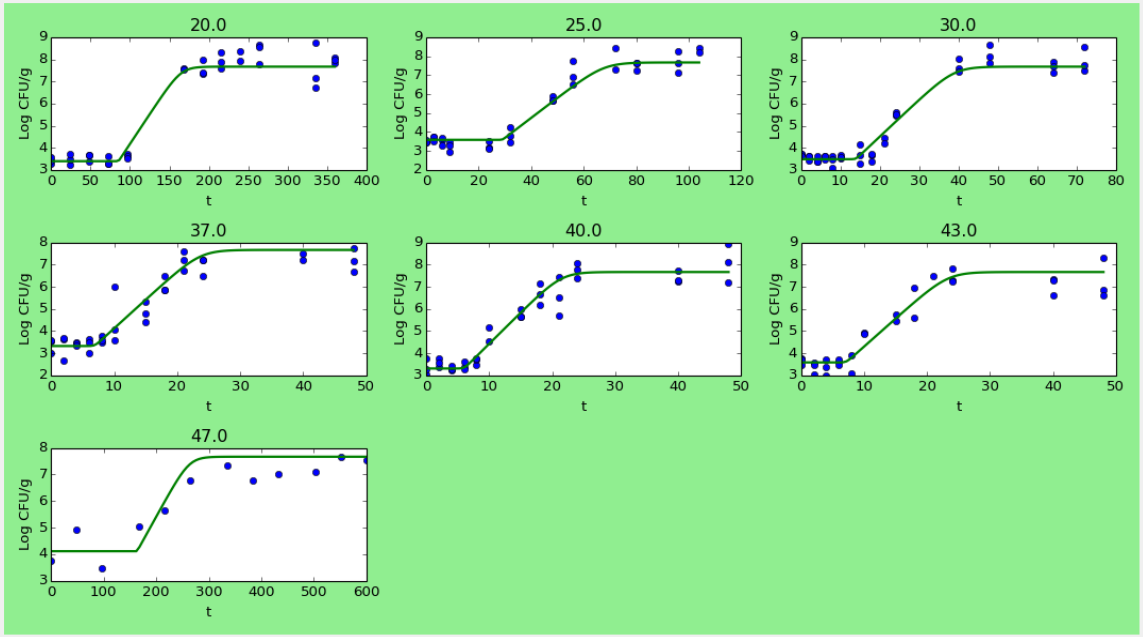
Model Selection Window

Choose a model from List ---

Huang Full Growth Model

Commit model

Plot Window



Save plot | Print plot

Report Window

Results of Data Analysis

Primary Model	Huang Full Growth Model
Secondary Model	Huang full range
Degree of freedom	207
SSE	4.656e+01
MSE	2.249e-01
RMSE	4.743e-01
Residual Error Std	4.590e-01
AIC	-311.860

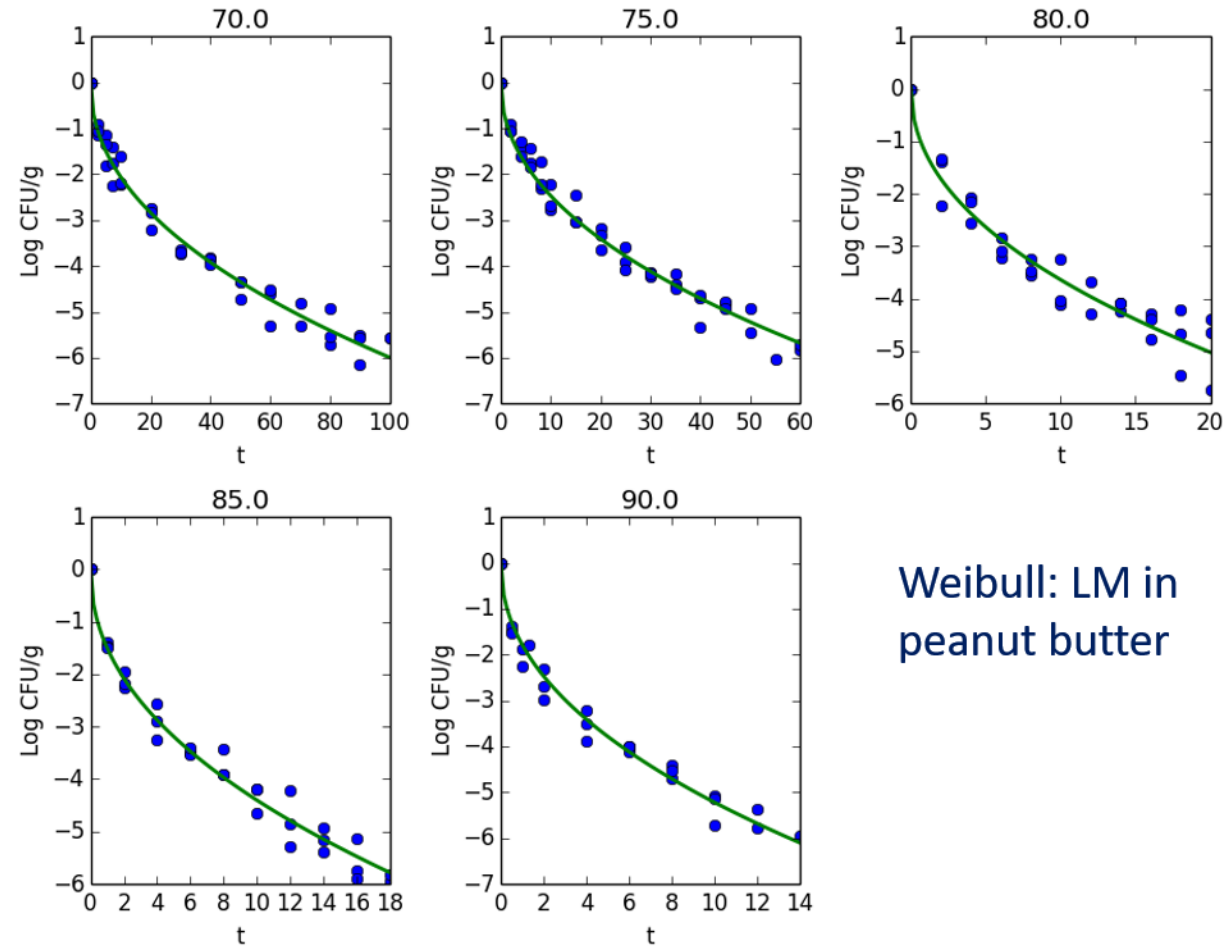
Estimated Parameters

Parameters	Value	Std-Error	t-value	p-value	L95CI	U95CI
a	6.89E-02	9.12E-03	7.56	1.30E-12	5.10E-02	8.69E-02
b	3.56E-01	7.36E-02	4.83	2.62E-06	2.11E-01	5.01E-01
Tmin	1.12E+01	2.98E+00	3.76	2.24E-04	5.33E+00	1.71E+01
Tmax	4.79E+01	3.17E-01	151.26	6.96E-214	4.73E+01	4.86E+01
A	1.11E+00	4.93E-01	2.24	2.58E-02	1.35E-01	2.08E+00
m	1.60E+00	4.19E-01	3.81	1.82E-04	7.72E-01	2.43E+00
y0, T20.0	3.40E+00	2.84E-01	11.96	2.11E-25	2.84E+00	3.95E+00
y0, T25.0	3.59E+00	2.26E-01	15.91	9.15E-38	3.14E+00	4.03E+00
y0, T30.0	3.49E+00	2.08E-01	16.79	1.70E-40	3.08E+00	3.90E+00
y0, T37.0	3.32E+00	2.29E-01	14.48	2.74E-33	2.87E+00	3.78E+00

Clear report | Save report | Print report

IPMP – Global Fit examples (1)

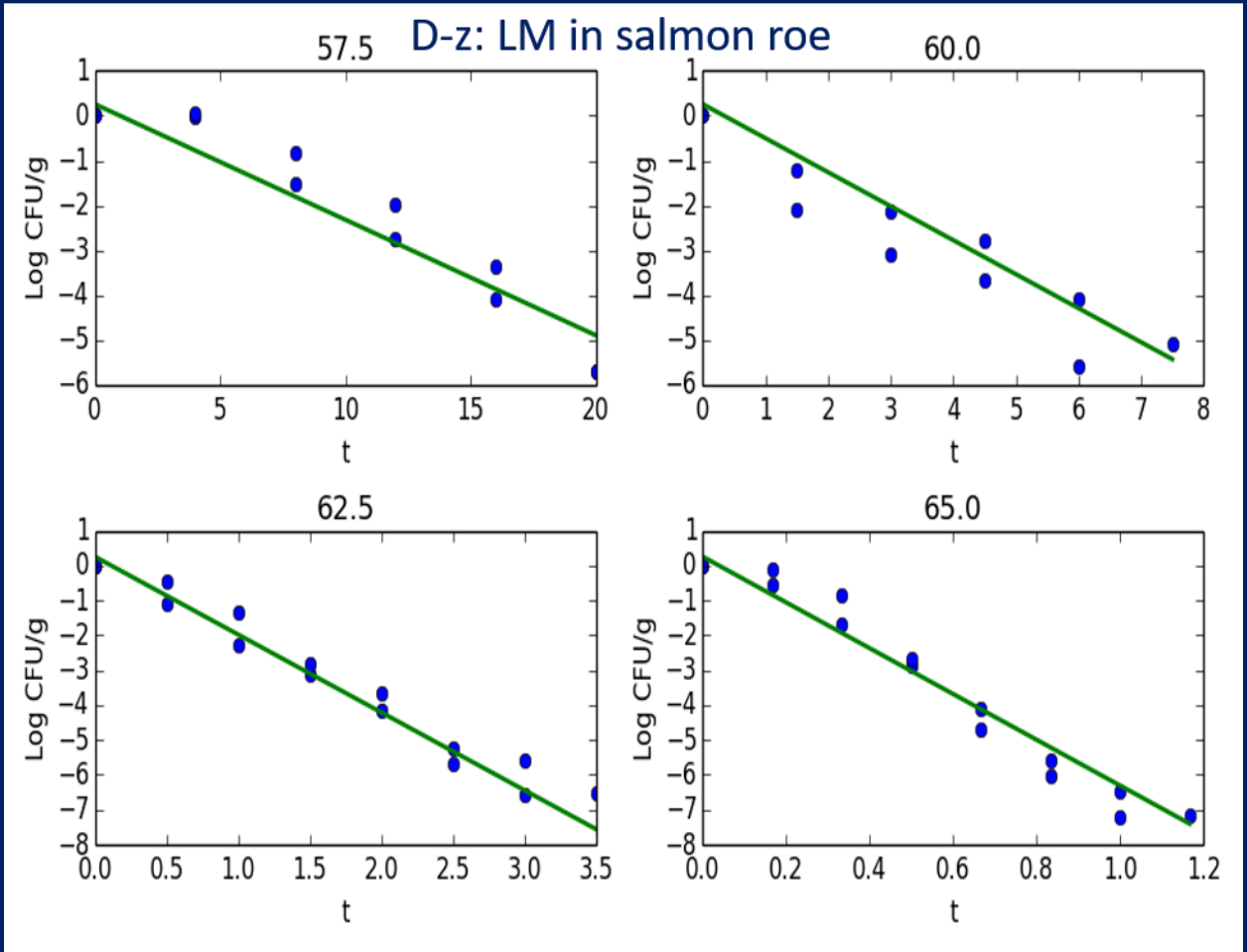
Survival curves



Weibull: LM in peanut butter

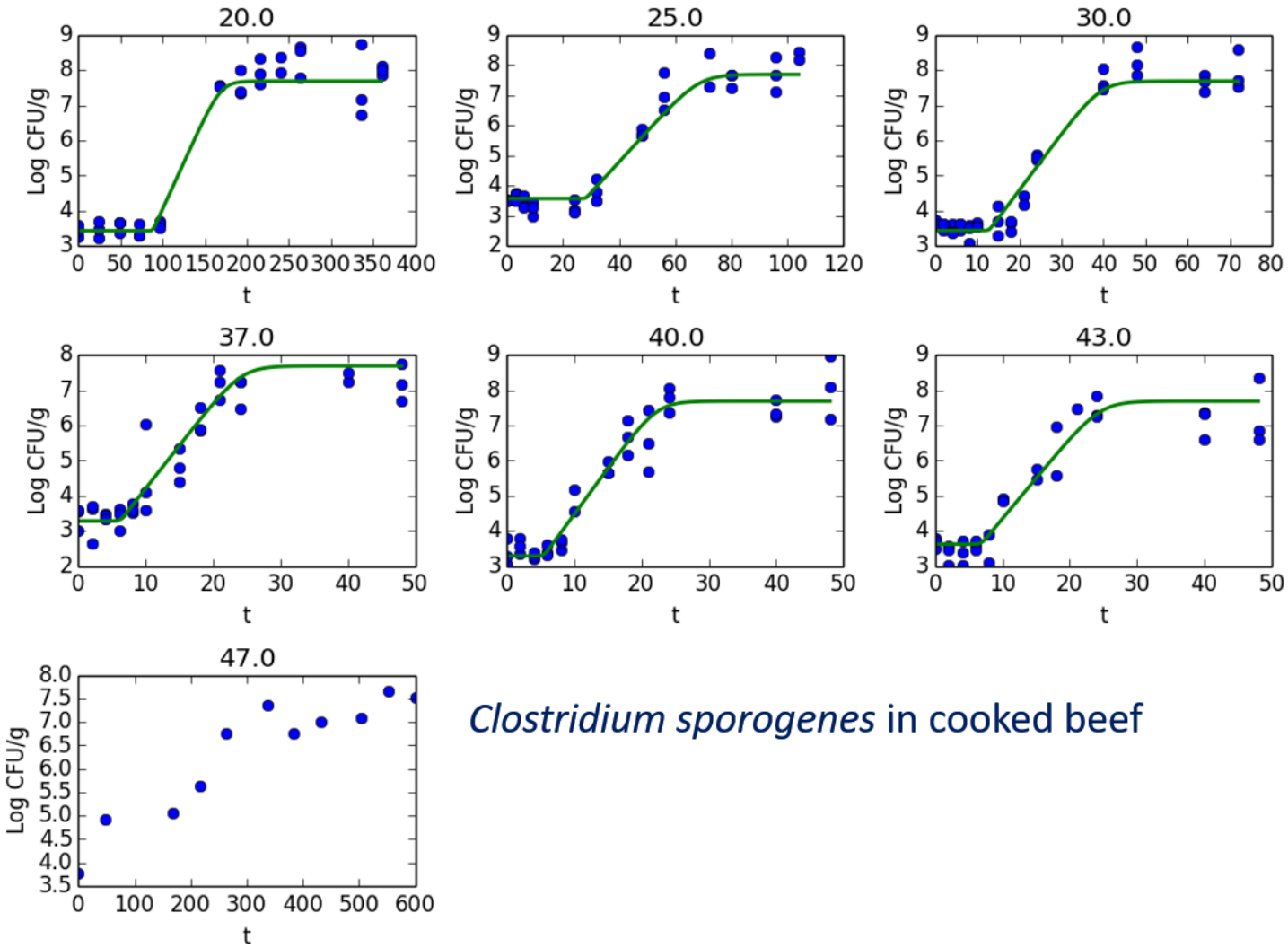
IPMP – Global Fit examples (2)

Survival curves



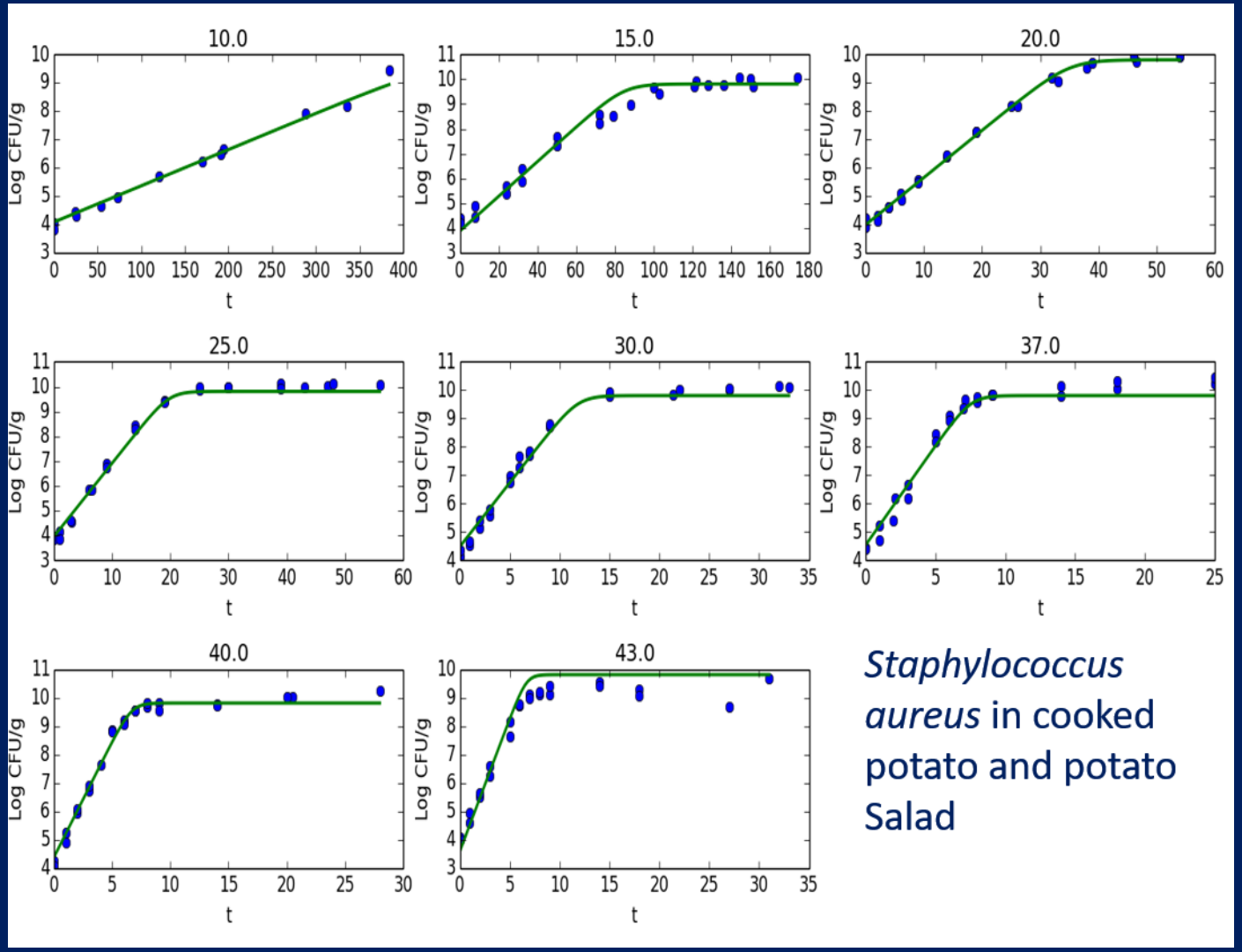
IPMP – Global Fit examples (3)

Growth curves



Clostridium sporogenes in cooked beef

IPMP – Global Fit examples (4)



Staphylococcus aureus in cooked potato and potato Salad

IPMP - Global Fit (4)

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

IPMP Global Fit – A one-step direct data analysis tool for predictive microbiology☆

Lihan Huang[1]

Residue Chemistry and Predictive Microbiology Research Unit, Eastern Regional Research Center, USDA Agricultural Research Service, 600 E. Mermaid Lane, Wyndmoor, PA 19038, United States of America

ARTICLE INFO

Keywords:
One-step kinetic analysis
Inverse analysis
Predictive modeling

ABSTRACT

The objective of this work is to develop and validate a unified optimization algorithm for performing one-step global regression analysis of isothermal growth and survival curves for determination of kinetic parameters in predictive microbiology. The algorithm is incorporated with user-friendly graphical interfaces (GUIs) to develop a data analysis tool, the USDA IPMP-Global Fit. The GUIs are designed to guide the users to easily navigate through the data analysis process and properly select the initial parameters for different combinations of mathematical models. The software is developed for one-step kinetic analysis to directly construct tertiary models by minimizing the global error between the experimental observations and mathematical models.

The current version of the software is specifically designed for constructing tertiary models with time and temperature as the independent model parameters in the package. The software is tested with a total of 9 different combinations of primary and secondary models for growth and survival of various microorganisms. The results of data analysis show that this software provides accurate estimates of kinetic parameters. In addition, it can be used to improve the experimental design and data collection for more accurate estimation of kinetic parameters. IPMP-Global Fit can be used in combination with the regular USDA-IPMP for solving the inverse problems and developing tertiary models in predictive microbiology.

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Global Fit in Action

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Growth of non-toxigenic *Clostridium botulinum* mutant LNT01 in cooked beef: One-step kinetic analysis and comparison with *C. sporogenes* and *C. perfringens*☆

Lihan Huang

Residue Chemistry and Predictive Microbiology Research Unit, Eastern Regional Research Center, Agricultural Research Service, The United States Department of Agriculture, 600 E. Mermaid Lane, Wyndmoor, PA 19038, United States

ARTICLE INFO

Keywords:
Clostridium botulinum
Non-toxigenic *C. botulinum* mutant
Growth kinetics
One-step kinetic analysis
Predictive microbiology

ABSTRACT

The objective of this study was to investigate the growth kinetics of *Clostridium botulinum* LNT01, a non-toxigenic mutant of *C. botulinum* 62A, in cooked ground beef. The spores of *C. botulinum* LNT01 were inoculated to ground beef and incubated anaerobically under different temperature conditions to observe growth and develop growth curves. A one-step kinetic analysis method was used to analyze the growth curves simultaneously to minimize the global residual error. The data analysis was performed using the USDA IPMP-Global Fit, with the Huang model as the primary model and the cardinal parameters model as the secondary model.

The results of data analysis showed that the minimum, optimum, and maximum growth temperatures of this mutant are 11.5, 36.4, and 44.3 °C, and the estimated optimum specific growth rate is 0.633 ln CFU/g per h, or 0.275 log CFU/g per h. The maximum cell density is 7.84 log CFU/g. The models and kinetic parameters were validated using additional isothermal and dynamic growth curves. The resulting residual errors of validation

Data analysis

Food Research International 107 (2018) 248–256

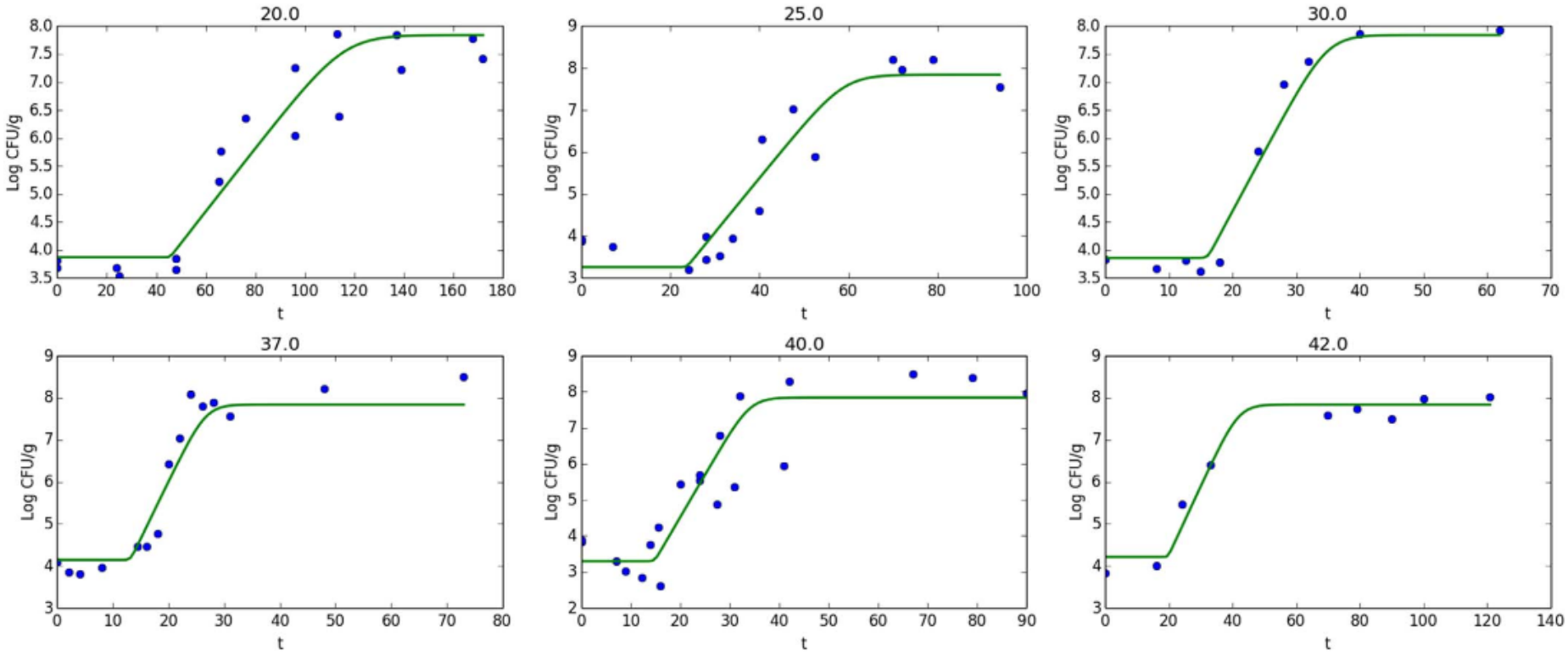


Fig. 1. Growth of *C. botulinum* LNT01 in cooked beef. The data points in each plot represent the compilation of two replicated observations.

Accuracy of prediction

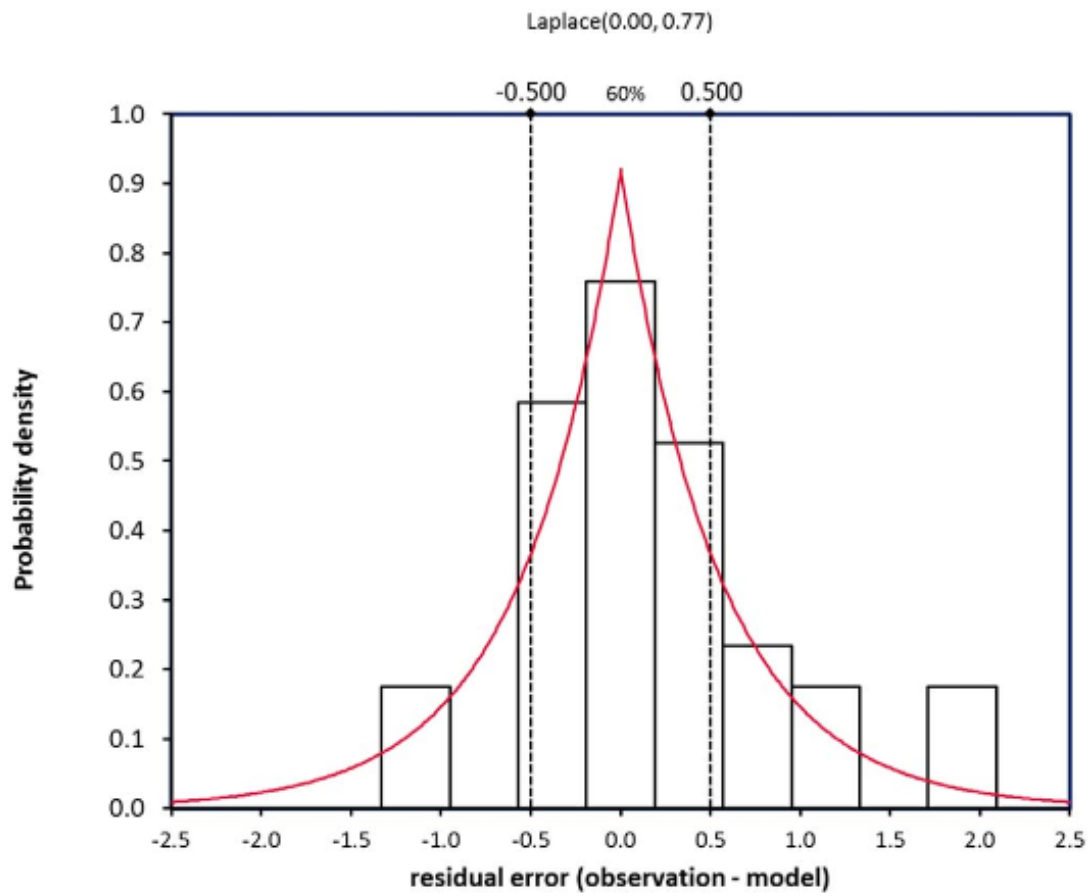


Fig. 4. Distribution of residual errors of validation.

Comparison with *C. sporogenes* and *C. perfringens*

Growth rate

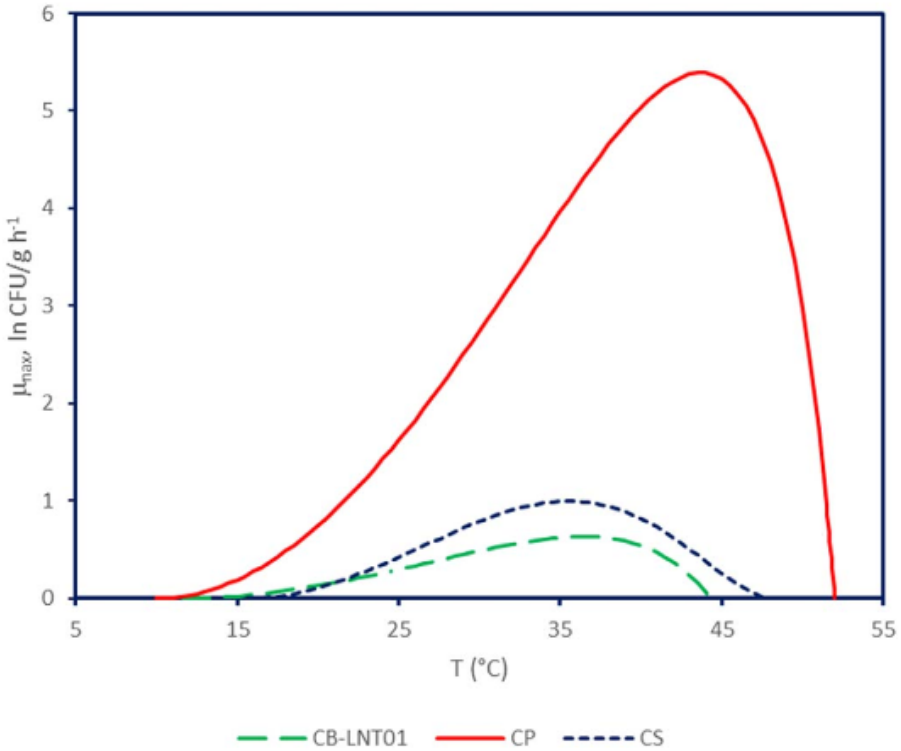


Fig. 5. Comparison of specific growth rates of *C. botulinum* LNT01, *C. perfringens*, and *C. sporogenes* in cooked beef.

Lag time

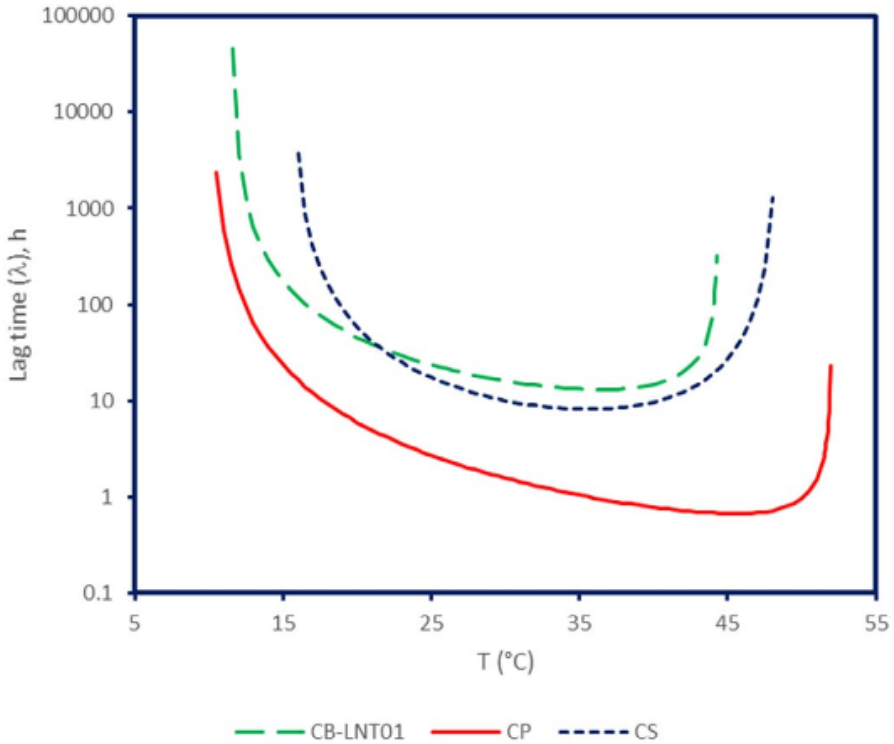
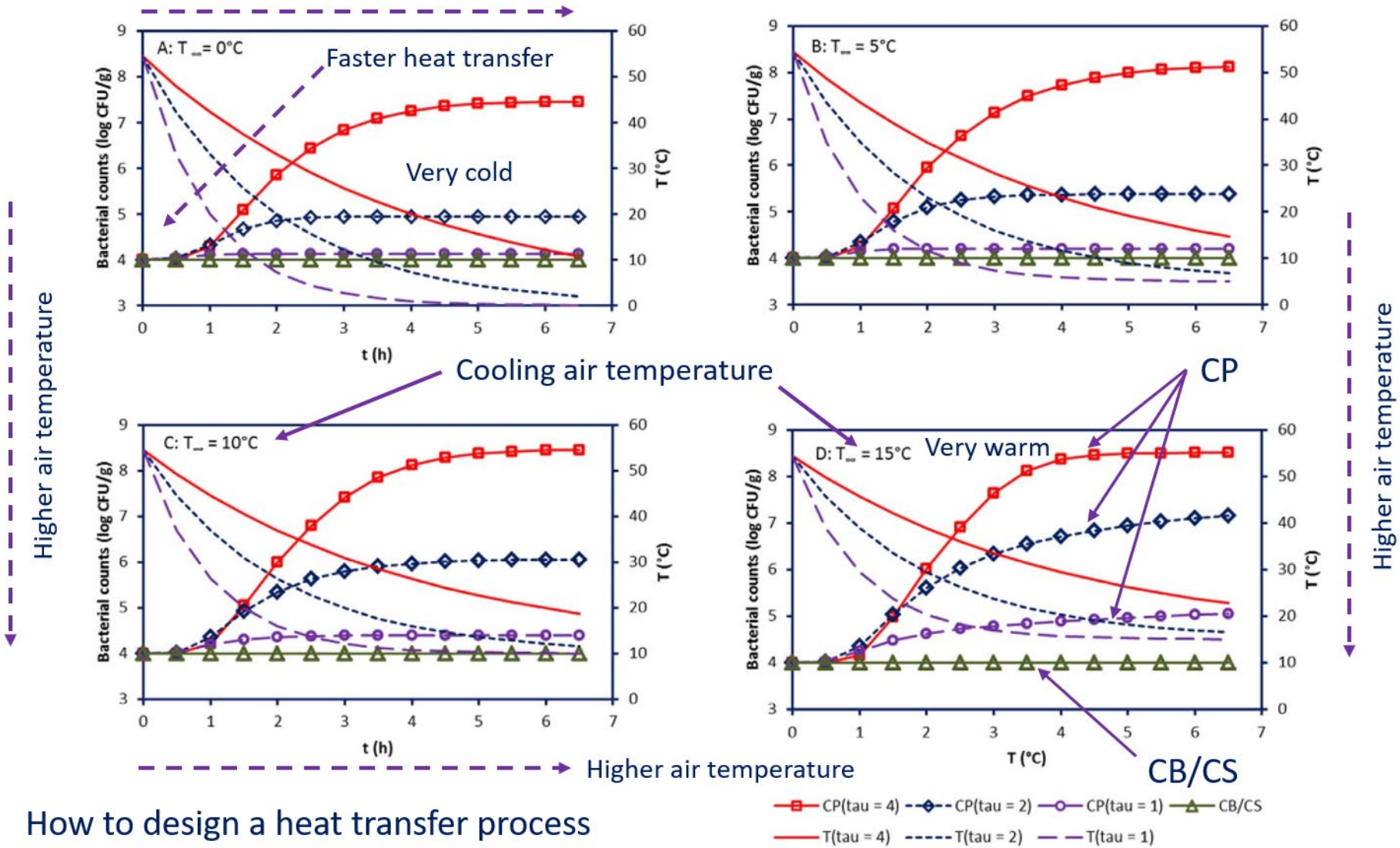


Fig. 6. Comparison of lag times of *C. botulinum* LNT01, *C. perfringens*, and *C. sporogenes* in cooked beef.

Computer simulation: Dynamic cooling and comparison with *C. sporogenes* and *C. perfringens*

Designing a cooling process - Effect of cooling air temperature and heat transfer rate on bacterial growth



How to design a heat transfer process

Fig. 7. Dynamic simulation of growth of *C. botulinum* LNT01 and comparison with *C. perfringens* and *C. sporogenes* (Legends: tau = τ; CP = *C. perfringens*; CB = *C. botulinum*; CS = *C. sporogenes*; T = temperature).

Kinetic analysis and dynamic prediction of growth of *vibrio parahaemolyticus* in raw white shrimp at refrigerated and abuse temperatures

ELSEVIER

Food Control

Volume 100, June 2019, Pages 204-211

Kinetic analysis and dynamic prediction of growth of *vibrio parahaemolyticus* in raw white shrimp at refrigerated and abuse temperatures ☆

Ying-Rong Chen [a], Cheng-An Hwang [b], Lihan Huang [b], Vivian C.H. Wu [c], Hsin-I Hsiao [a]

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Highlights

- The growth of *Vibrio parahaemolyticus* in raw white shrimps at refrigerated and abuse temperatures (8–35 °C) were determined.
- Growth kinetic models of *V. parahaemolyticus* in white shrimps were developed and validated.
- The models may be used for conducting risk assessment for *V. parahaemolyticus* in white shrimps.

IPMP Global Fit in Action

The most accurate available

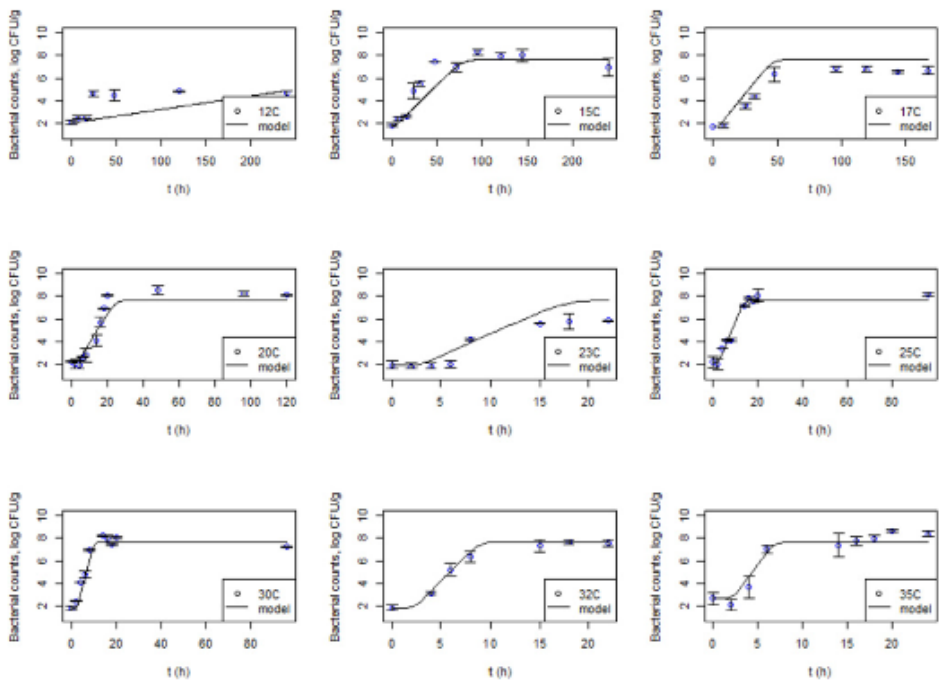


Fig. 1. One-step kinetic analysis using the combination of the Huang primary model and the suboptimal Huang square-root model. The time (t) is in hour.

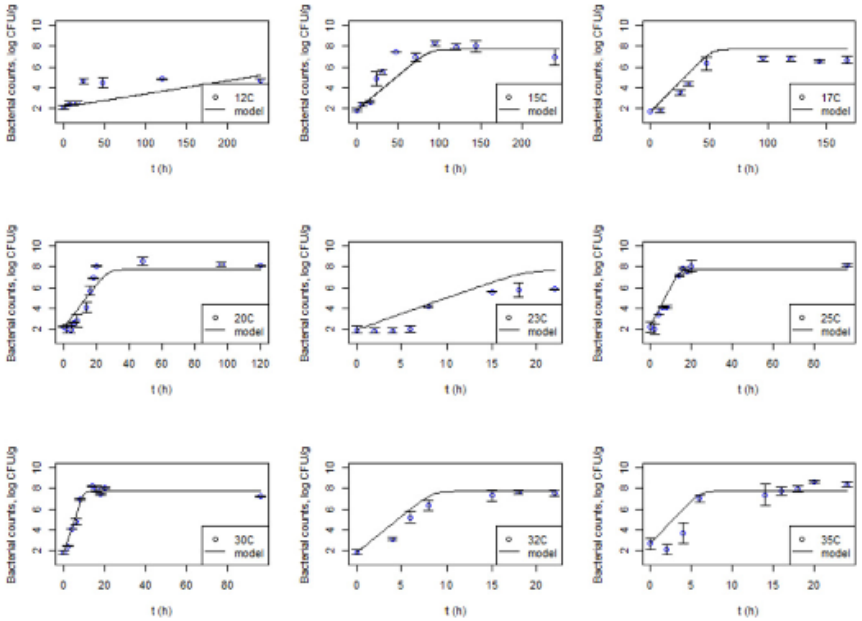


Fig. 2. One-step kinetic analysis using the combination of the No-lag phase model and the suboptimal Huang square-root model. The time (t) is in hour.

IPMP Global Fit in Action (2)

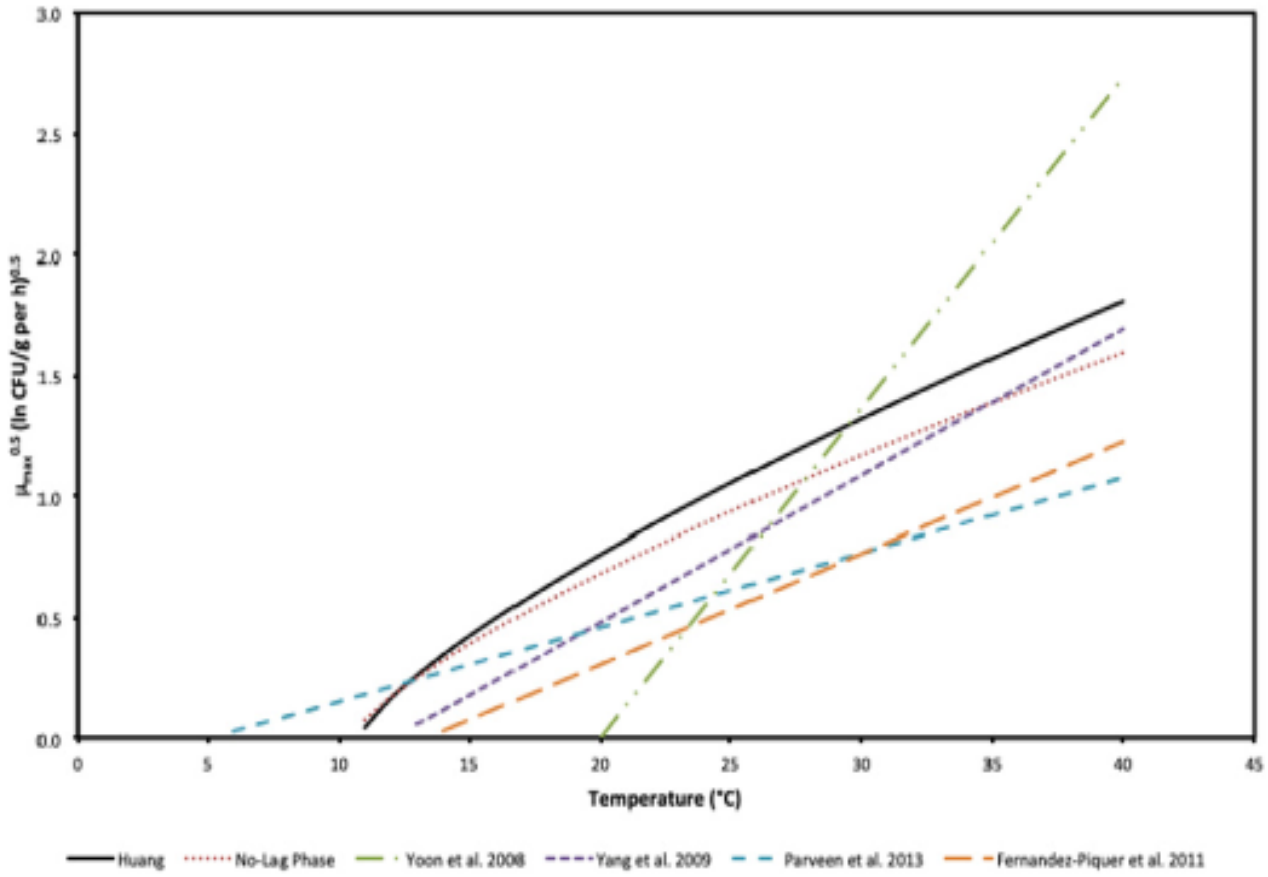


Fig. 3. Effect of temperature on the specific growth rates of *V. parahaemolyticus* in various seafood.

What do we work on?

- Major foodborne pathogens
  - *Salmonellas*
  - *Listeria monocytogenes*
  - STEC
  - *Clostridium perfringens*
- Meat, poultry, and egg products

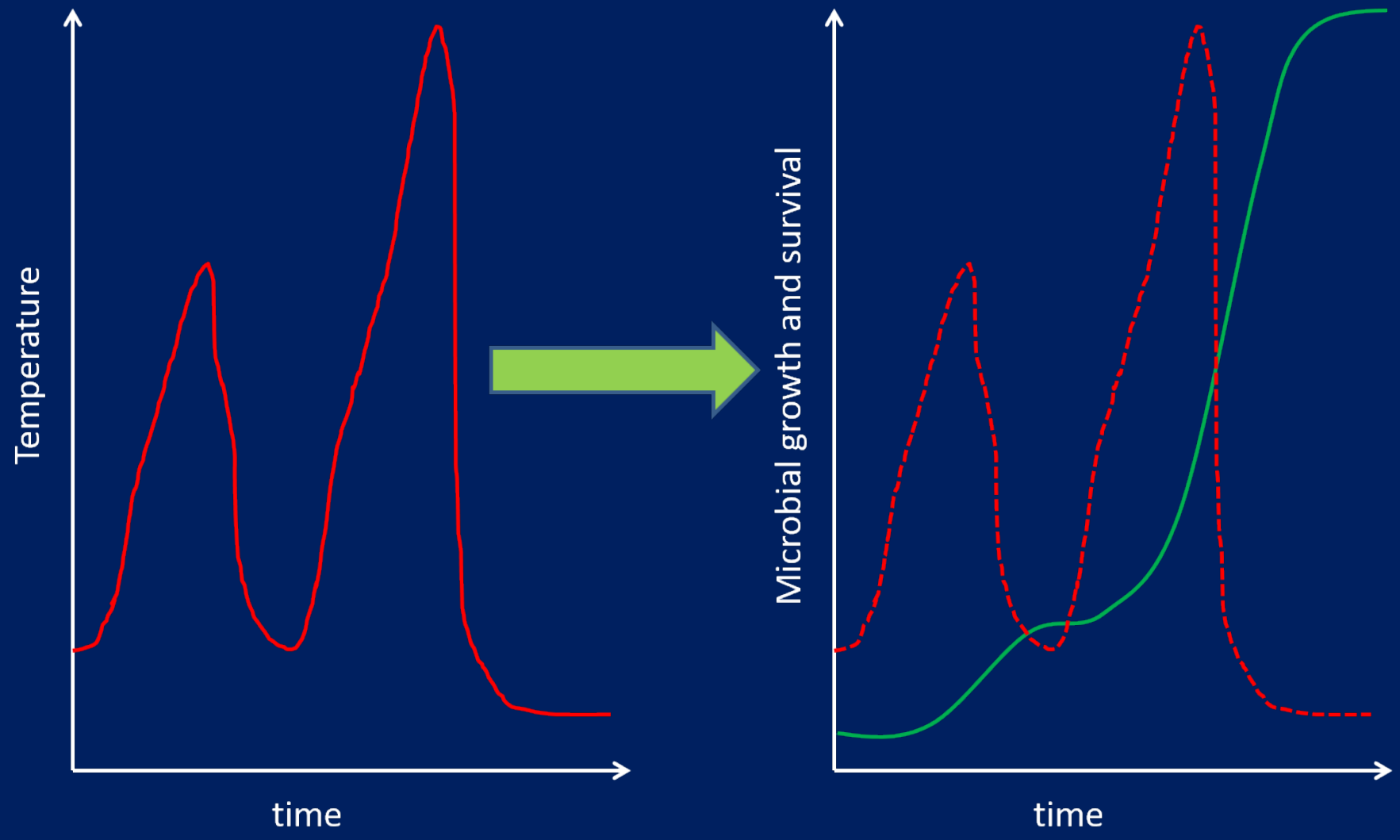
A Bigger Question -

- Food is rarely stored under isothermal conditions
- Can we construct a model directly from dynamic curves and use it for dynamic prediction?
- Yes!
- But it is more sophisticated and needs more advanced mathematical methods (numerical analysis and optimization)

One-Step Dynamic Analysis

- Dynamic conditions to expose the bacteria to a wide range of temperature conditions
- Dynamic analysis – solving differential equations numerically
- Numerical optimization

One-Step Direct Dynamic Analysis



One-step dynamic analysis of growth of *C. perfringens* in cooked beef

International Journal of Food Microbiology 195 (2015) 20–29

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Dynamic determination of kinetic parameters, computer simulation, and probabilistic analysis of growth of *Clostridium perfringens* in cooked beef during cooling ☆

Lihan Huang

U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

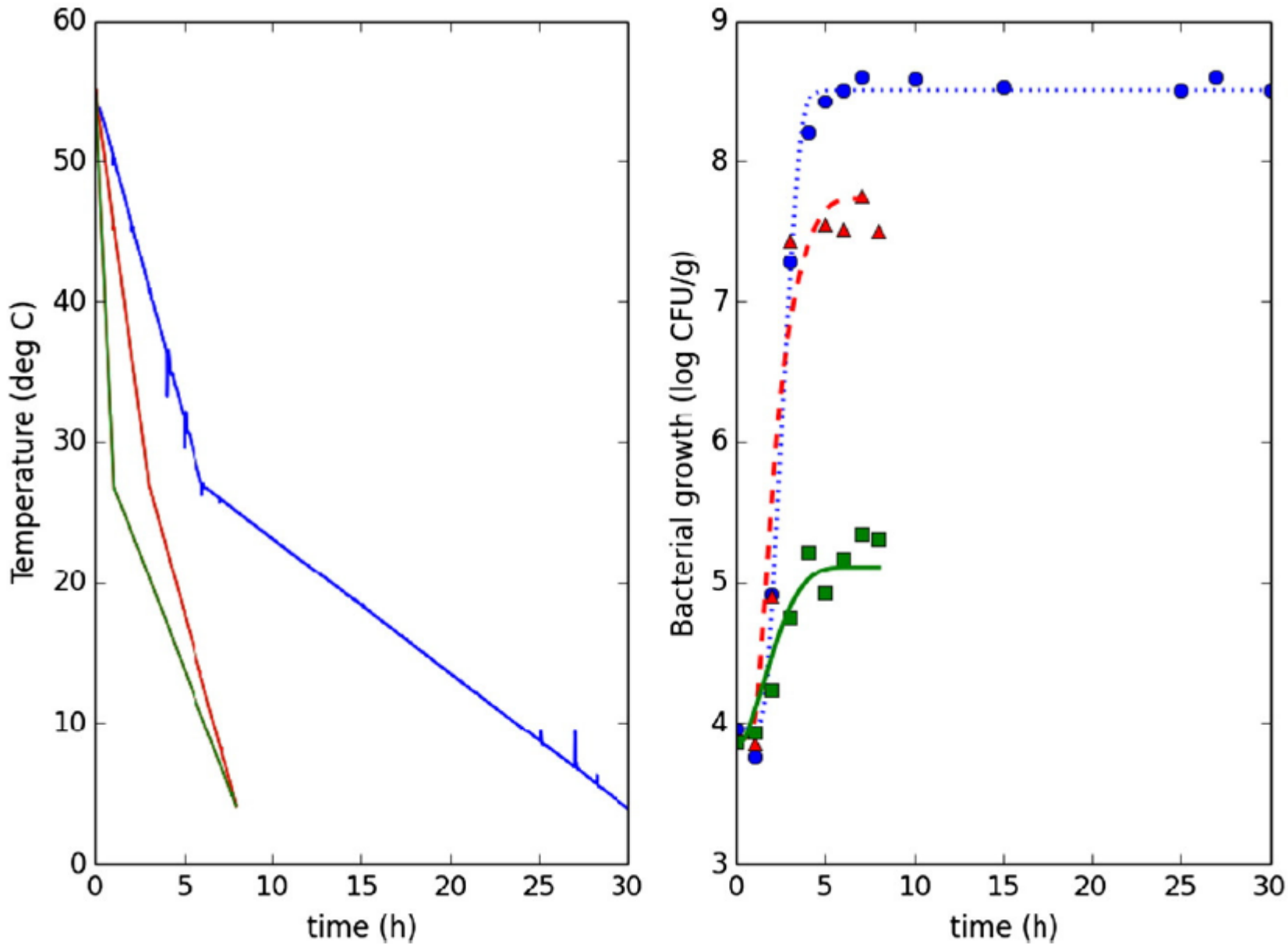
ARTICLE INFO

Article history:
Received 11 March 2014
Received in revised form 1 October 2014
Accepted 22 November 2014
Available online 2 December 2014

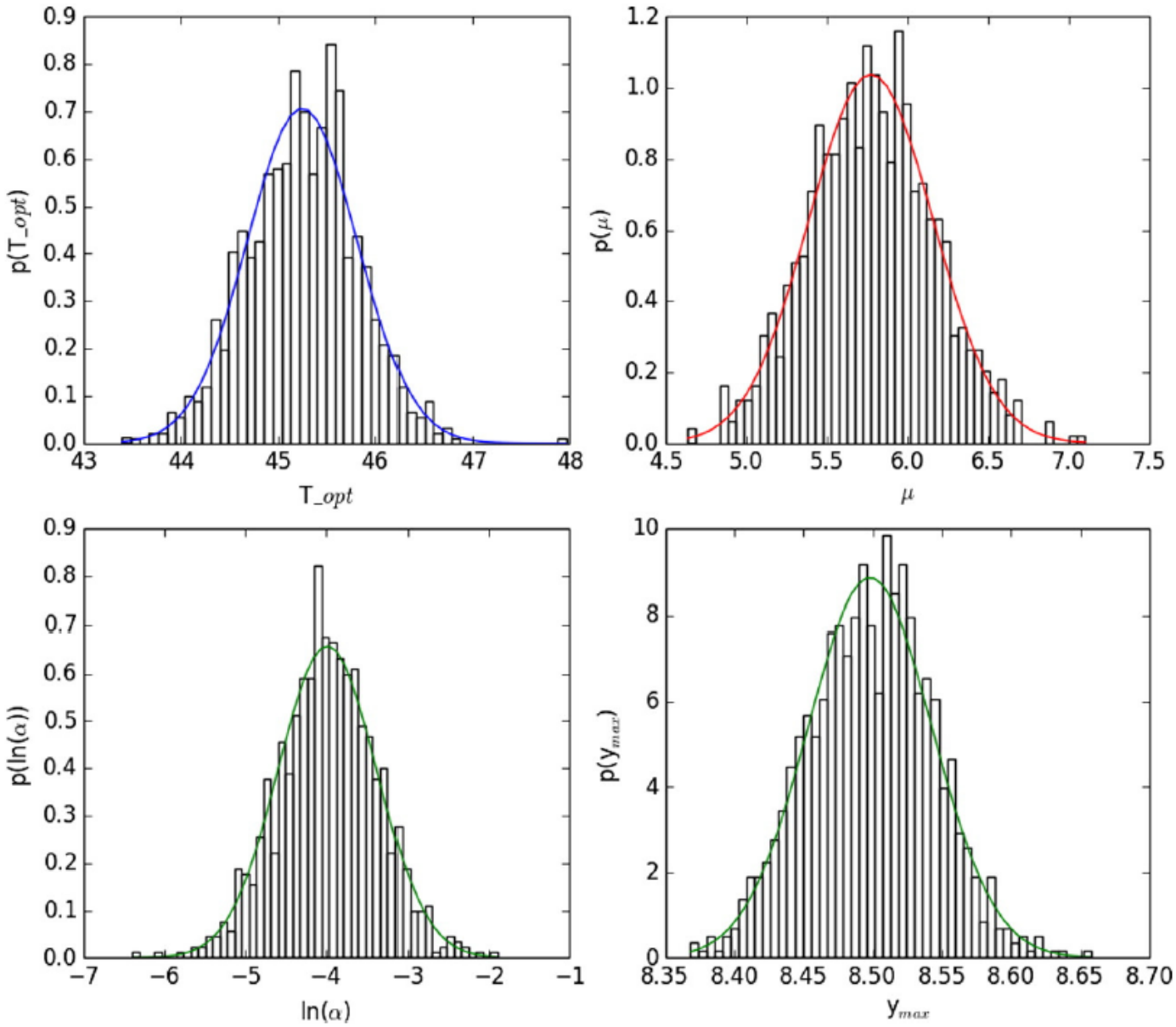
ABSTRACT

The objective of this research was to develop a new one-step methodology that uses a dynamic approach to directly construct a tertiary model for prediction of the growth of *Clostridium perfringens* in cooked beef. This methodology was based on simultaneous numerical analysis and optimization of both primary and secondary models using multiple dynamic growth curves obtained under different conditions. Once the models were constructed, the bootstrap method was used to calculate the 95% confidence intervals of kinetic parameters, and a Monte Carlo

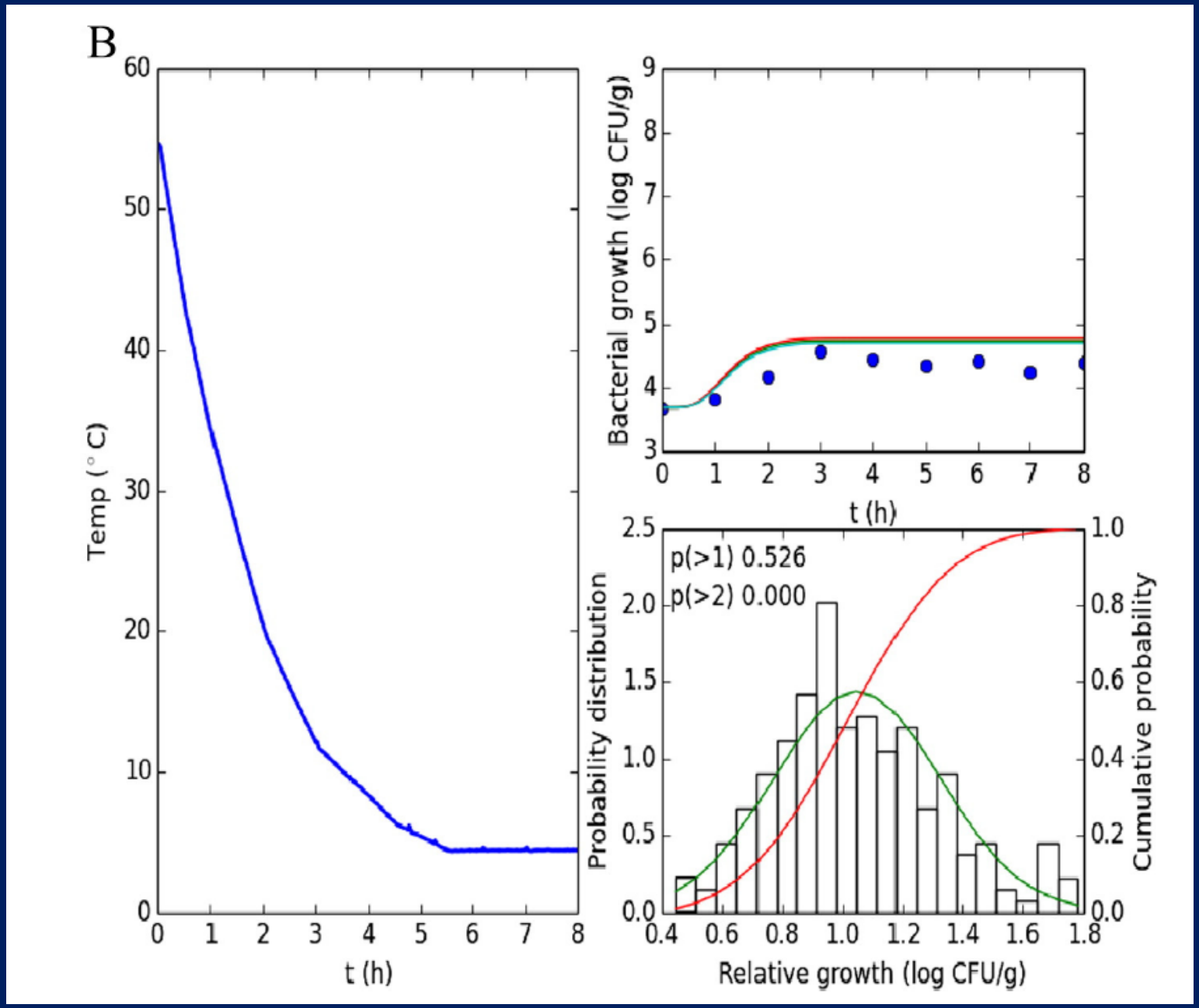
One-step dynamic inverse analysis



One-step dynamic inverse analysis (2)



Prediction/validation



Prediction/validation (2)

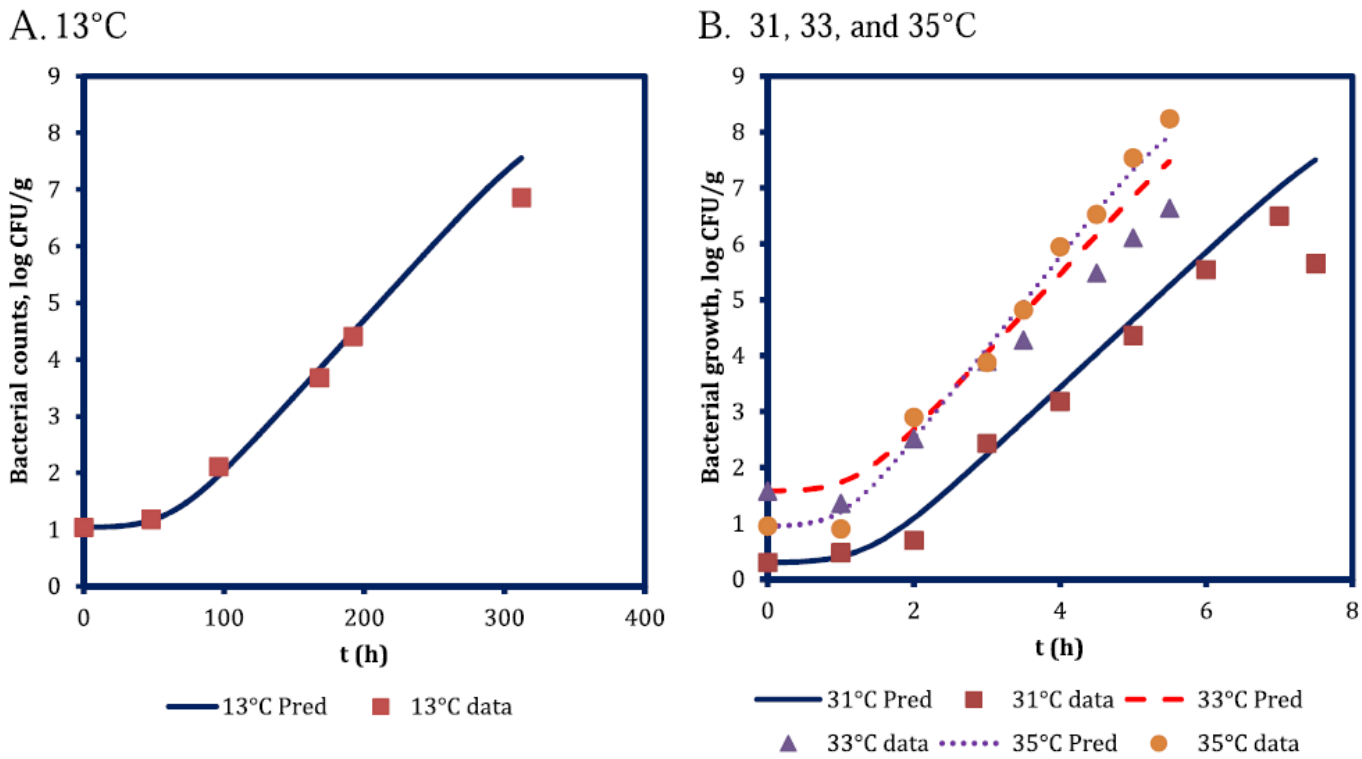


Fig. 8. Monte Carlo simulation and validation with isothermal growth curves downloaded from Combase (www.combase.cc). A: 13 °C; B: 31, 33, and 35 °C. Pred: predicted growth curves; data: raw data.

One-step dynamic analysis of growth of *C. perfringens* in cooked beef (2)

Direct Dynamic Kinetic Analysis and Computer Simulation of Growth of *Clostridium perfringens* in Cooked Turkey during Cooling

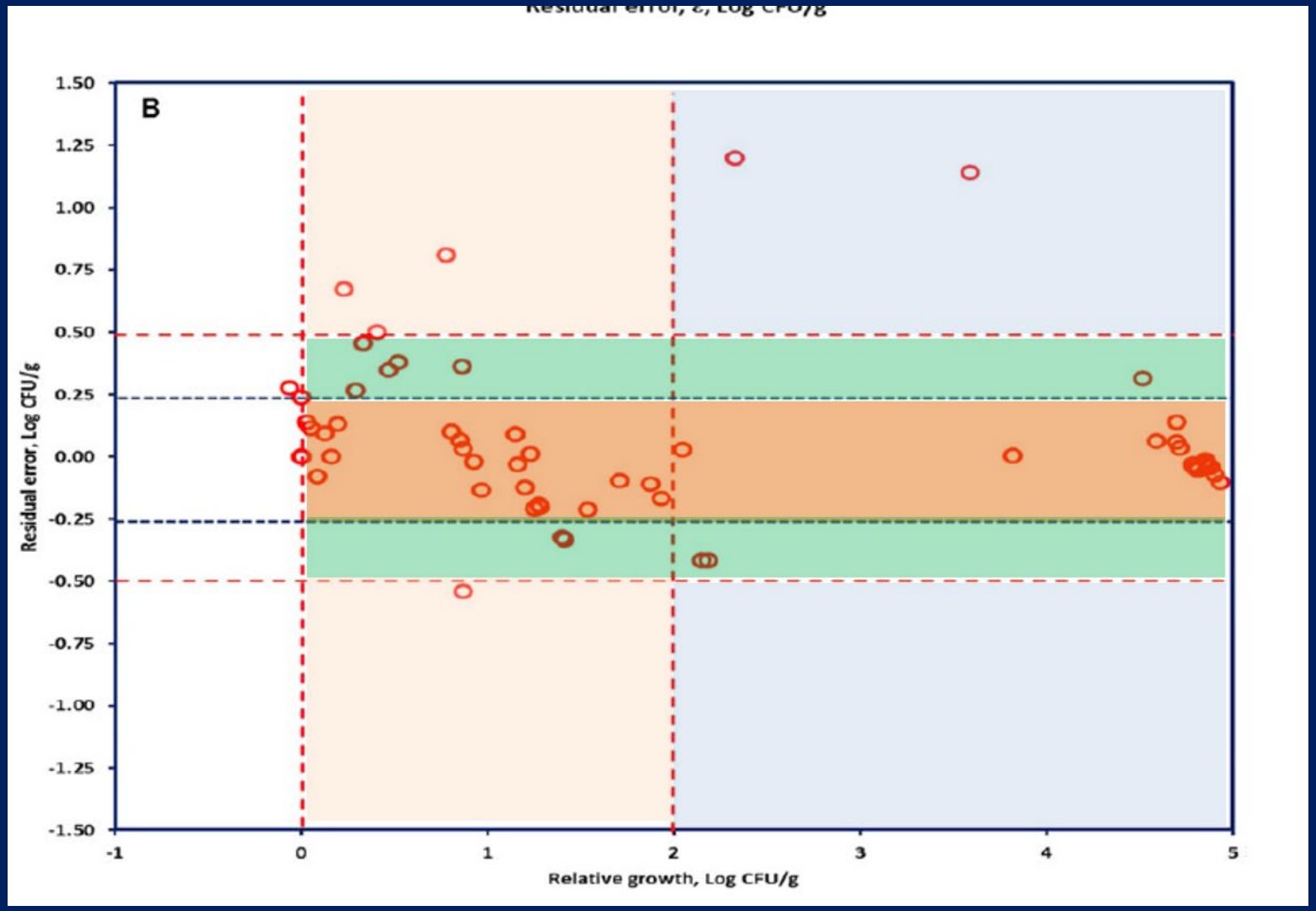
Lihan Huang and Bryan T. Vinyard

M692 Journal of Food Science • Vol. 81, Nr. 3, 2016

Abstract: This research applied a new 1-step methodology to directly construct a tertiary model that describes the growth of *Clostridium perfringens* in cooked turkey meat under dynamically cooling conditions. The kinetic parameters of the growth models were determined by numerical analysis and optimization using multiple dynamic growth curves.
The models and kinetic parameters were validated using independent growth curves obtained under various cooling conditions. The results showed that the residual errors (ε) of the predictions followed a Laplace distribution that is symmetric with respect to $\varepsilon = 0$. For residual errors, 90.6% are within ±0.5 Log CFU/g and 73.4% are ±0.25 Log CFU/g for all growth curves used for validation. For relative growth <1.0 Log CFU/g, 88.9% of the residual errors are within ±0.5 Log CFU/g, and 63.0% are within ±0.25 Log CFU/g. For relative growth of <2.0 Log CFU/g, 92.7% of the residual errors are within ±0.5 Log CFU/g, and 70.3% are within ±0.25 Log CFU/g. The scale and distribution of residual errors clearly suggests that the models and estimated kinetic parameters are reasonably accurate in predicting the growth of *C. perfringens*.
Monte Carlo simulation was used to estimate the probabilities of >1.0 and 2.0 Log CFU/g relative growth of *C. perfringens* in the final products at the end of cooling. This probabilistic process analysis approach provides a new alternative for estimating and managing the risk of a product and can help the food industry and regulatory agencies assess the safety of cooked meat in the event of cooling deviation.

Keywords: bootstrap, *C. perfringens*, dynamic modeling, Monte Carlo analysis, numerical analysis, optimization

Residual errors of prediction



Performance of CP dynamic model

Table 1–Criteria used to characterize the accuracy of predictions.

Criteria	Accurate prediction	Fail-safe prediction	Fail-dangerous prediction
Criterion 1	$-1.0 \leq \varepsilon \leq 0.5$	$\varepsilon < -1.0$	$\varepsilon > 0.5$
Criterion 2	$-1.0 \leq \varepsilon \leq 1.0$	$\varepsilon < -1.0$	$\varepsilon > 1.0$
Criterion 3	$-0.5 \leq \varepsilon \leq 0.5$	$\varepsilon < -0.5$	$\varepsilon > 0.5$

Table 3–Classification of prediction accuracy for isothermal and dynamic heating conditions.

Temperature profiles	Criterion	Accurate	Fail-safe	Fail-dangerous	Accurate and fail-safe
Isothermal beef	1	79.3%	2.8%	17.9%	82.1%
	2	92.6%	2.8%	4.6%	95.4%
	3	66.7%	15.4%	17.9%	82.1%
Isothermal poultry	1	85.8%	8.0%	6.2%	93.8%
	2	91.5%	8.0%	0.5%	99.5%
	3	66.5%	27.2%	6.2%	99.5%
Dynamic heating beef	1	91.2%	8.8%	0	100%
	2	91.2%	8.8%	0	100%
	3	56.1%	43.9%	0	100%

Table 4–Classification of prediction accuracy for dynamic cooling conditions.

Temperature profiles	Criterion	Accurate	Fail-safe	Fail-dangerous	Accurate and fail-safe
Single-rate beef	1	56.6%	41.0%	2.4%	97.6%
	2	59.0%	41.0%	0.0%	100%
	3	22.9%	74.7%	2.4%	97.6%
Single-rate poultry	1	43.7%	56.3%	0.0%	100%
	2	43.7%	56.3%	0.0%	100%
	3	6.2%	93.8%	0.0%	100%
Single-rate pork	1	36.7%	63.3%	0.0%	100%
	2	36.7%	63.3%	0.0%	100%
	3	14.3%	85.7%	0.0%	100%
Single-rate combined	1	47.5%	51.5%	1.0%	99.0%
	2	48.5%	51.5%	0.0%	100.0%
	3	15.3%	83.7%	1.0%	99.0%
Dual-rate beef	1	66.7%	33.3%	0.0%	100%
	2	66.7%	33.3%	0.0%	100%
	3	50.0%	50.0%	0.0%	100%

New Dynamic CP models

Point-by-point comparison

- Documented temperature profiles
- Continuous curves, either isothermal or dynamic curves, compared point-by-point
- Very accurate (internal and external validation)

Single-point comparison

- No documented temperature profiles
- Initial point and final point
- Only relative growth is reported
- Dynamic curves
- Not as accurate
- These data are not so scientifically convincing

One-Step Dynamic Analysis – LM in Pork (1)

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Dynamic kinetic analysis of growth of *Listeria monocytogenes* in a simulated comminuted, non-cured cooked pork product☆

Lihan Huang

US. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038 USA

ARTICLE INFO

Article history:
Received 22 March 2016
Received in revised form
25 June 2016
Accepted 28 June 2016
Available online 29 June 2016

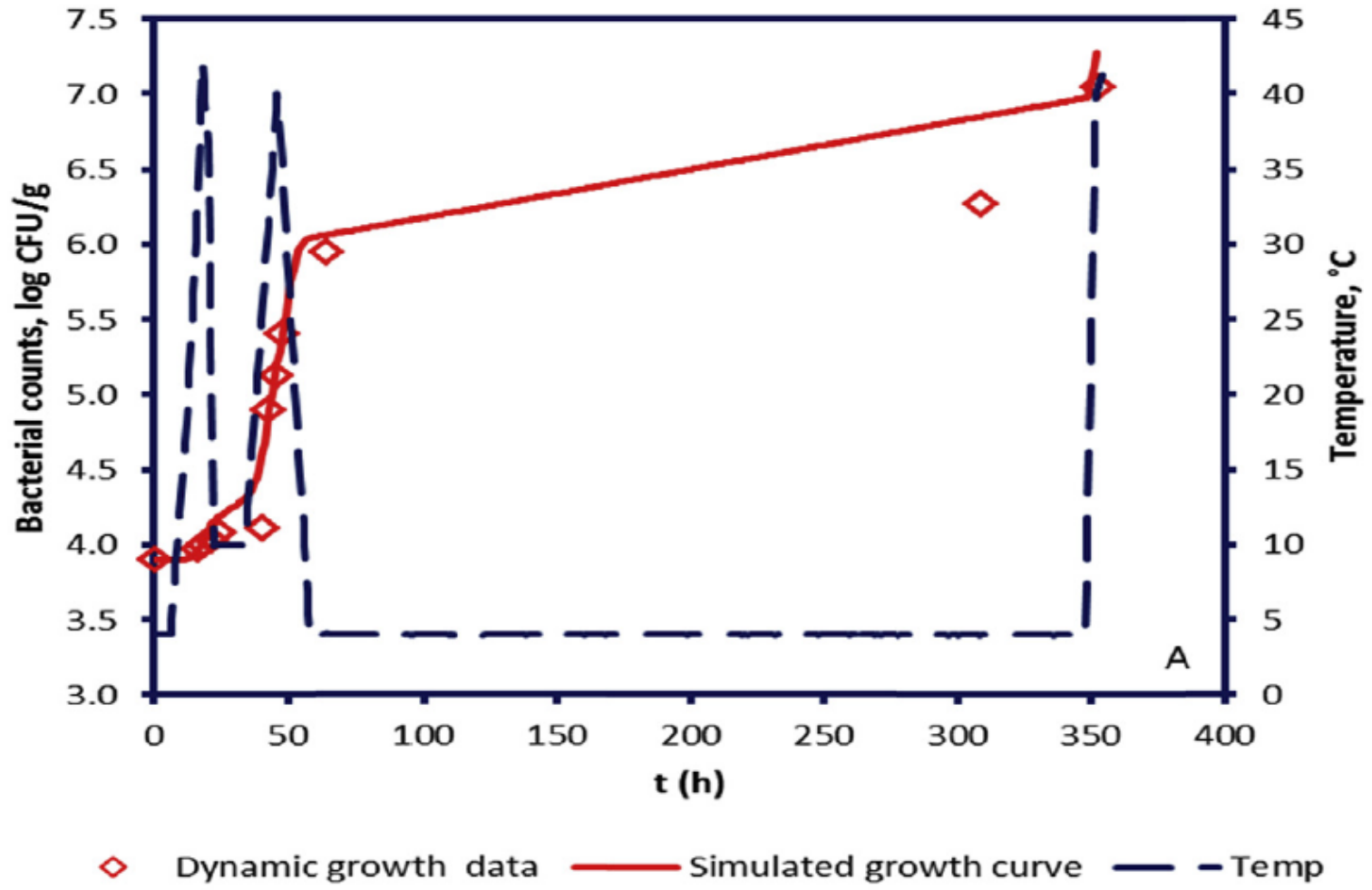
Keywords:
Predictive modeling
Dynamic analysis
Listeria monocytogenes

ABSTRACT

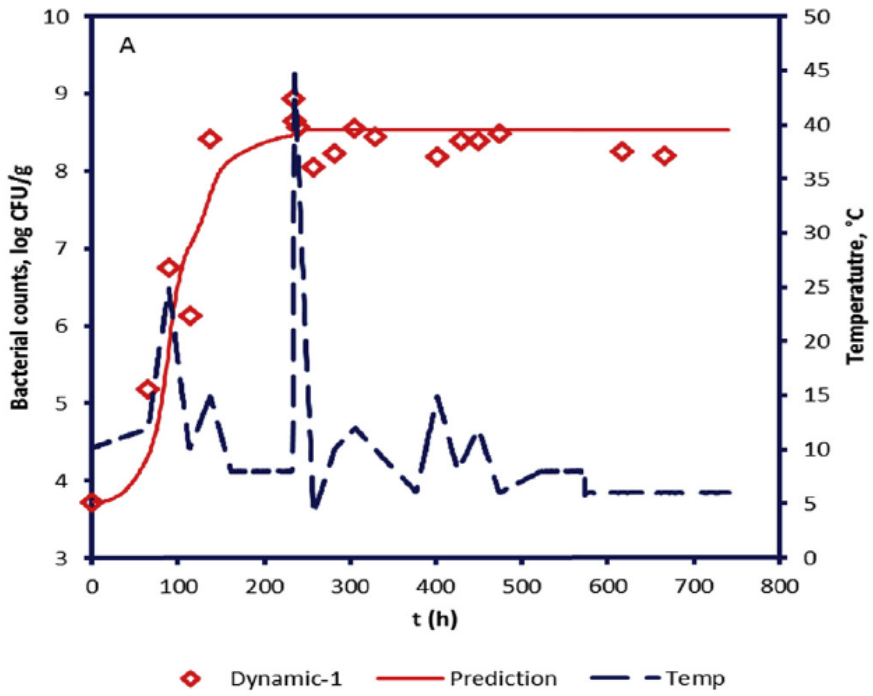
The objective of this study was to directly construct a tertiary growth model for *Listeria monocytogenes* in a simulated comminuted, non-cured cooked pork product and simultaneously determine the kinetic parameters using a combination of dynamic and isothermal growth curves. Growth studies were conducted using a cocktail of 5 strains of *L. monocytogenes* in cooked pork under both dynamic and isothermal temperature profiles designed to examine the effect of temperature on bacterial growth.

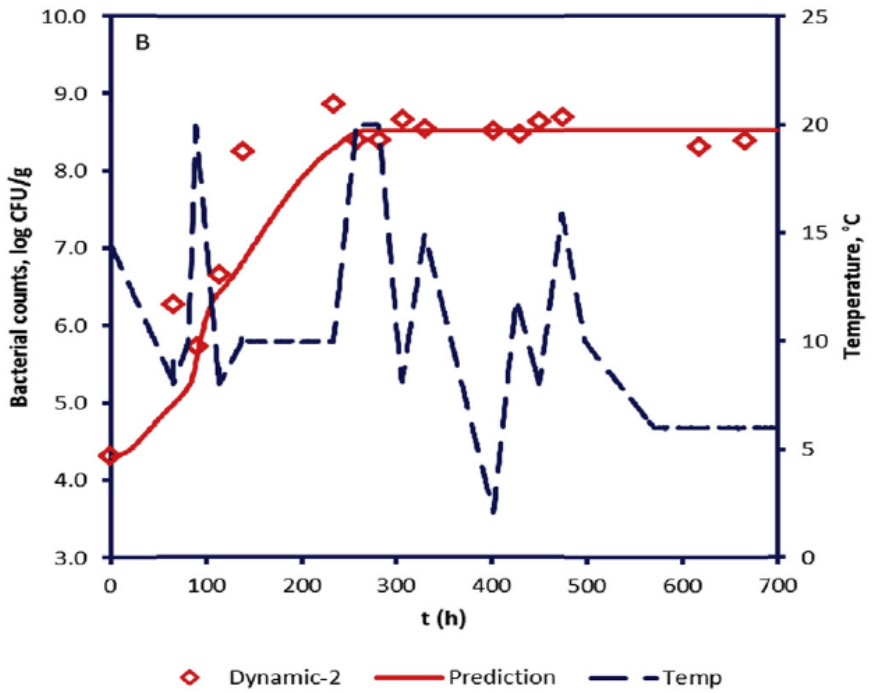
A direct kinetic analysis method was used to construct the growth models and determine the kinetic parameters. The bacterial growth was simulated by a set of differential equations, and the temperature effect was evaluated by the cardinal parameters model. A numerical analysis and optimization method was used to simultaneously solve the different equations and search for the best fits of kinetic parameters for the growth curves and models. The estimated minimum, optimum, and maximum growth

One-Step Dynamic Analysis – LM in Pork (2)



One-Step Dynamic Analysis – LM in Pork (3)





One-Step Dynamic Analysis – LM in Pork (4)

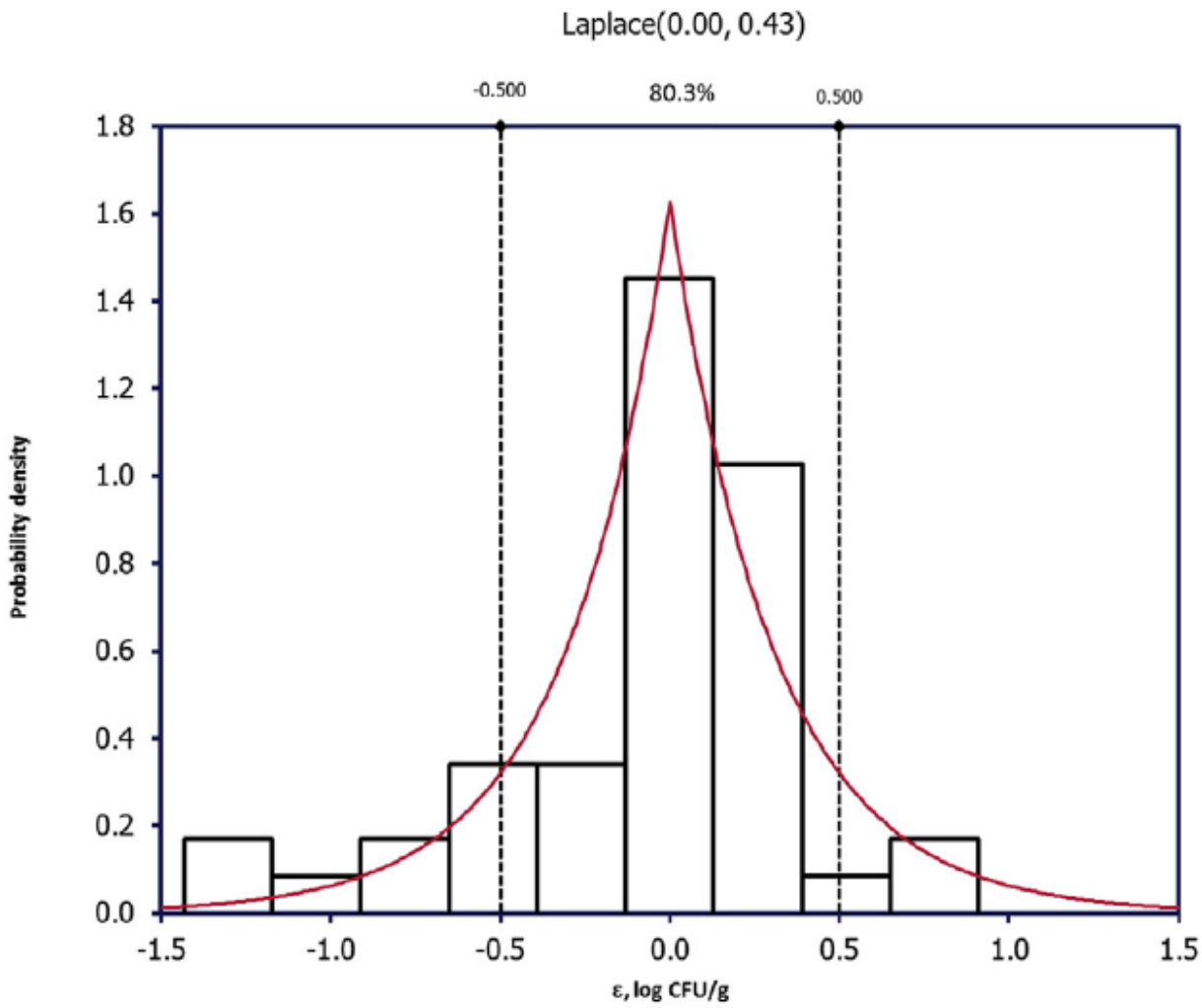


Fig. 5. Distribution of residual errors of validation in Fig. 4 using laboratory-generated dynamic and isothermal growth curves. The unit of ε is log CFU/g.

One-Step Dynamic Analysis – SE in Liquid Egg Whites (1)

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Dynamic analysis of growth of *Salmonella* Enteritidis in liquid egg whites☆

Lihan Huang*, Cheng-An Hwang

U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA, 19038, USA

ARTICLE INFO

Article history:
Received 13 February 2017
Received in revised form
26 April 2017
Accepted 29 April 2017
Available online 30 April 2017

ABSTRACT

Salmonella Enteritidis (SE) is a common foodborne pathogen associated with eggs and egg products. This research was conducted to study the kinetics of growth and survival of SE in liquid egg whites (LEW). A dynamic temperature profile that exposed SE to suboptimal temperatures and below the minimum growth temperature (T_{min}) was used with two isothermal conditions to develop kinetic models. One-step dynamic analysis was used to directly construct a tertiary model for describing the growth and survival of SE and determine the kinetic parameters.

One-Step Dynamic Analysis – SE in Liquid Egg Whites (2)

Dynamic temperature profiles

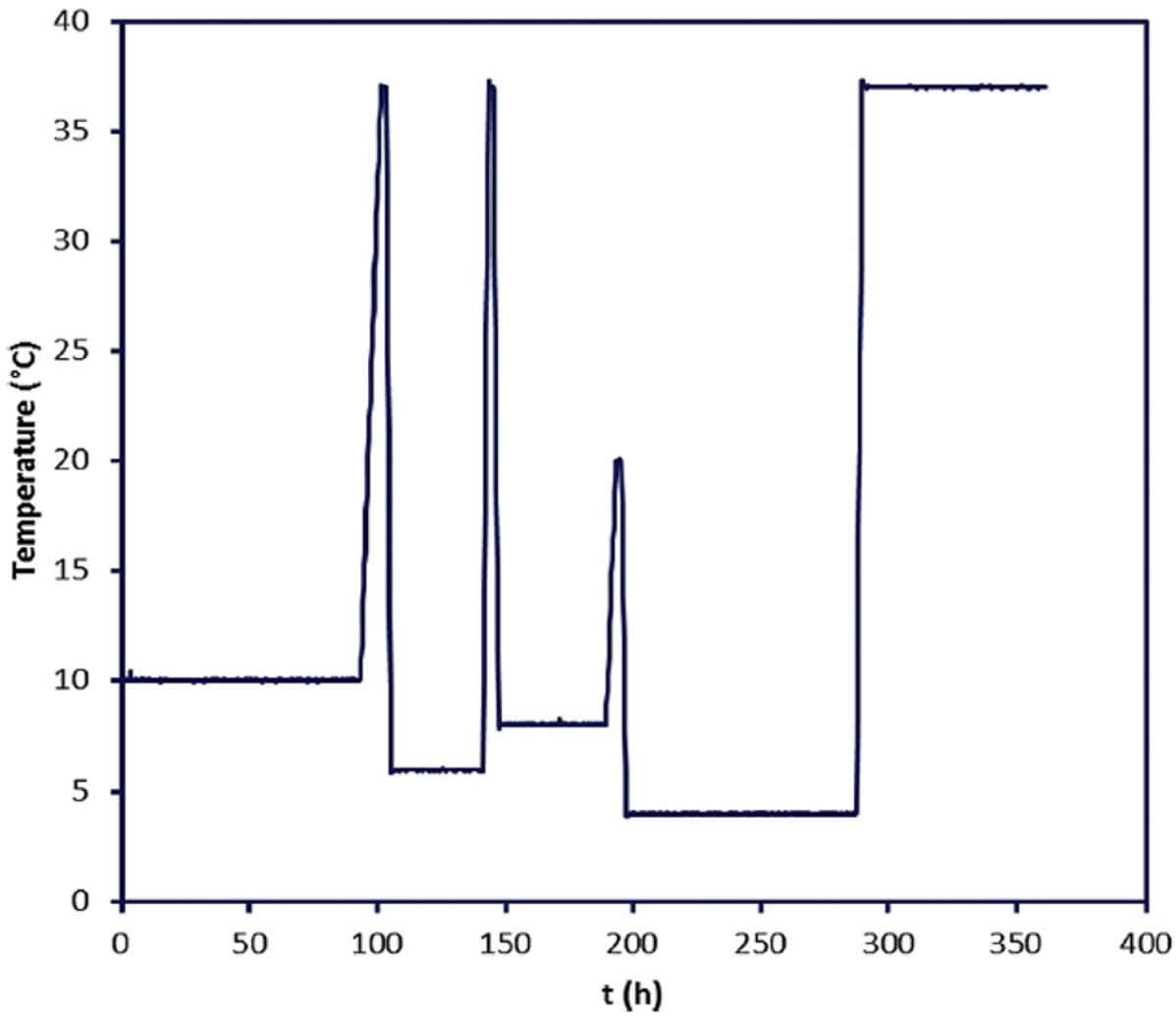


Fig. 1. The dynamic temperature profile used to determine the kinetic parameters in growth models.

One-Step Dynamic Analysis – SE in Liquid Egg Whites (3)

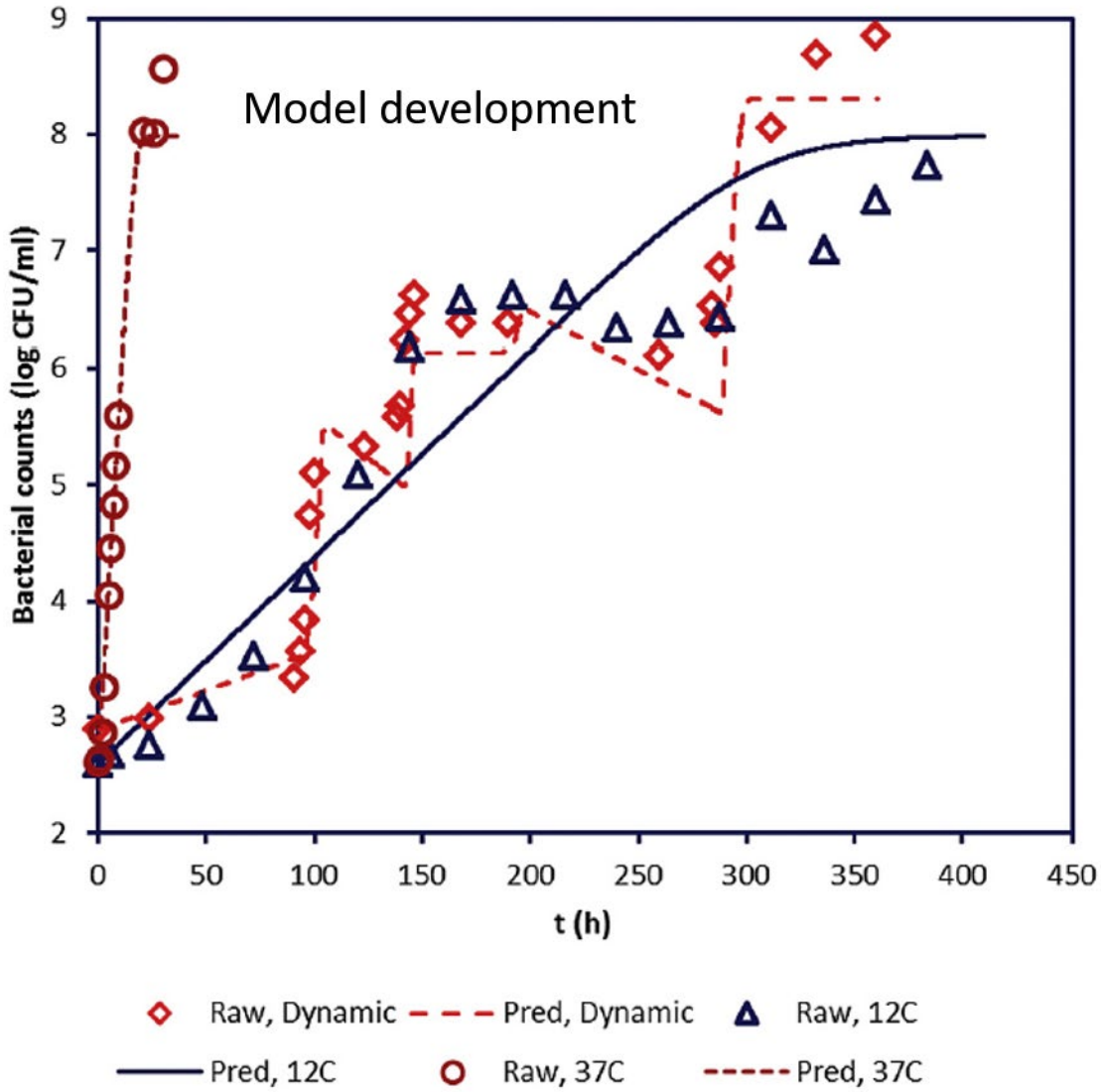
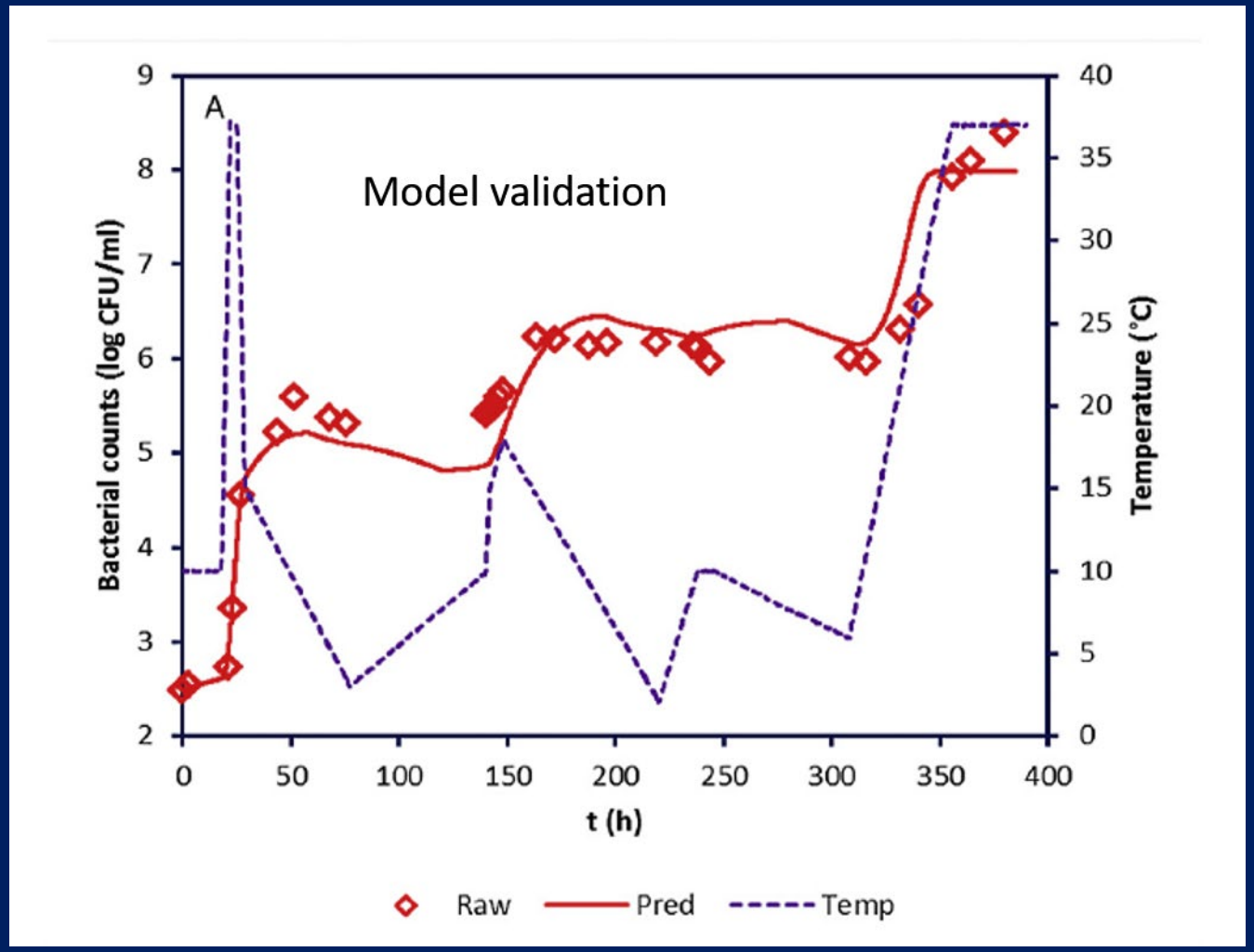


Fig. 2. Growth and survival of SE under both dynamic and isothermal temperature profiles and mathematical modeling.

One-Step Dynamic Analysis – SE in Liquid Egg Whites (4)



One-Step Dynamic Analysis : *Salmonella* Paratyphi A in roasted chicken

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Growth and survival of *Salmonella* Paratyphi A in roasted marinated chicken during refrigerated storage: Effect of temperature abuse and computer simulation for cold chain management☆

Miaoyun Li [a, **], Lihan Huang [b, *], Qianqian Yuan [a]

[a] College of Food Science and Technology, Henan Agricultural University, No. 95 Wenhua Road, Zhengzhou, Henan Province, 450002, PR China
[b] U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

ARTICLE INFO

Article history:
Received 5 October 2016
Received in revised form
14 November 2016
Accepted 15 November 2016
Available online 23 November 2016

Keywords:
Dynamic modeling
Salmonella Paratyphi A
Predictive microbiology

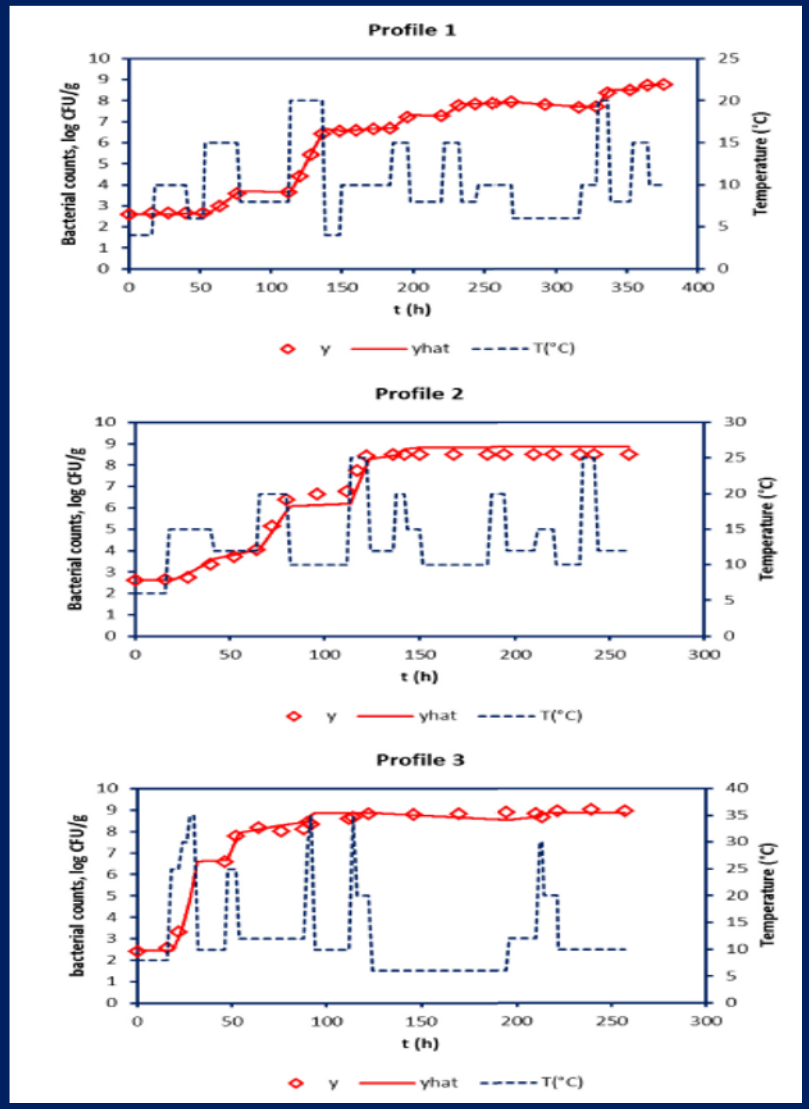
ABSTRACT

This research was conducted to evaluate the feasibility of using a one-step dynamic numerical analysis and optimization method to directly construct a tertiary model to describe the growth and survival of *Salmonella* Paratyphi A (SPA) in a marinated roasted chicken product. Multiple dynamic growth and survival curves obtained under different fluctuating temperature conditions between 4 and 35 °C were used to determine the growth kinetics of SPA. In combination with appropriate secondary models, the study examined both growth and survival of SPA simultaneously by an integrated one-step approach using a set of differential equations.

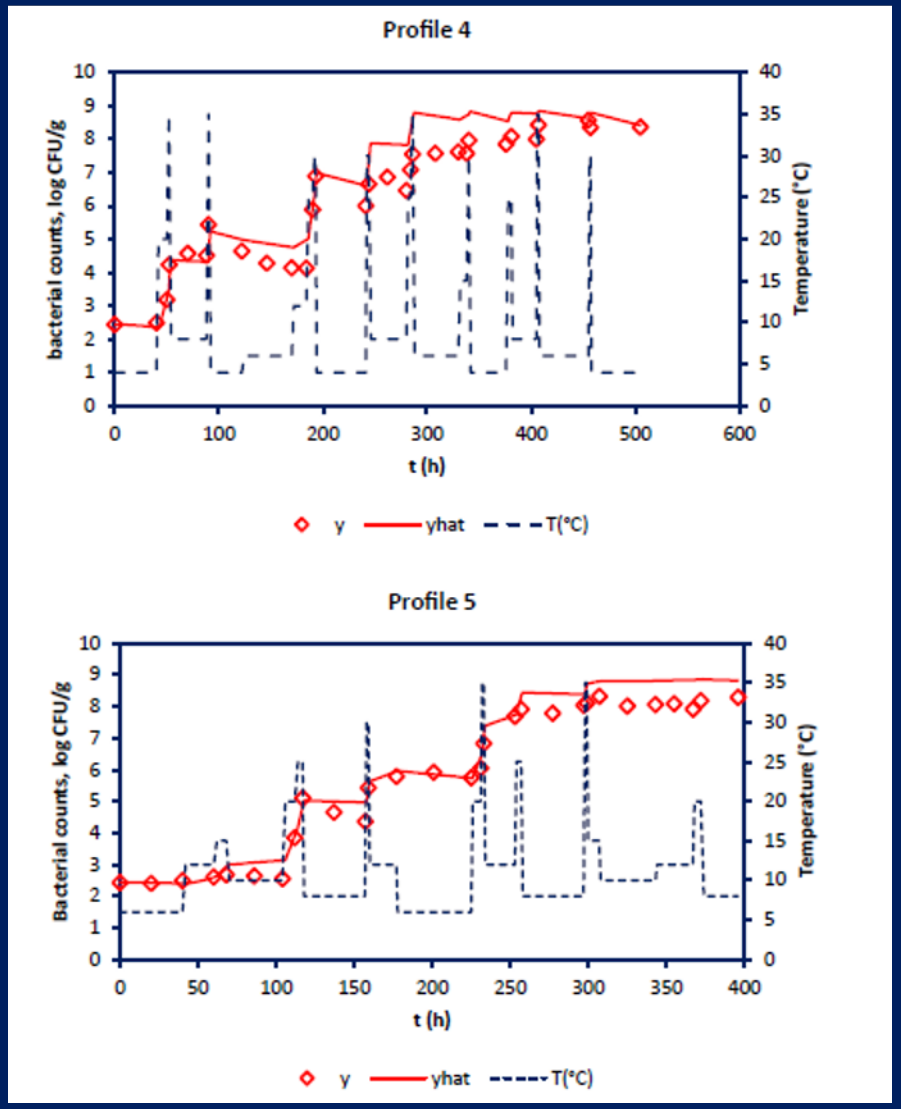
The estimated minimum growth temperature (T_{min}) of SPA was 8.91 °C, matching well with the growth characteristics of this microorganism. The growth at temperatures above T_{min} and the survival below T_{min} was accurately simulated by the predictive models. For model development, the root mean

One-Step Dynamic Analysis : *Salmonella* Paratyphi A in roasted chicken (2)

Model development



Model validation



Growth and survival of *Bacillus cereus* from spores in cooked rice – One-step dynamic analysis and predictive modeling (1)

ELSEVIER

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Growth and survival of *Bacillus cereus* from spores in cooked rice – One-step dynamic analysis and predictive modeling☆

Cheng-An Hwang*, Lihan Huang

U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA, 19038, USA

ARTICLE INFO

Keywords:
Dynamic modeling
One-step analysis
Temperature
Bacillus cereus
Rice

ABSTRACT

Bacillus cereus is a spore-forming foodborne pathogen that can produce toxins causing emetic or diarrheal intoxication. Food poisoning caused by *B. cereus* is a significant public health concern as it is frequently associated with the consumption of starch-based food products, which are a staple food worldwide. The objective of this study was to investigate the growth and survival of *B. cereus* in cooked rice under changing temperatures between 1 and 48 °C. A one-step dynamic analysis was used to directly construct a tertiary model to describe the growth and survival of *B. cereus* and estimate the kinetic parameters.

Growth and survival of *Bacillus cereus* from spores in cooked rice – One-step dynamic analysis and predictive modeling (2)

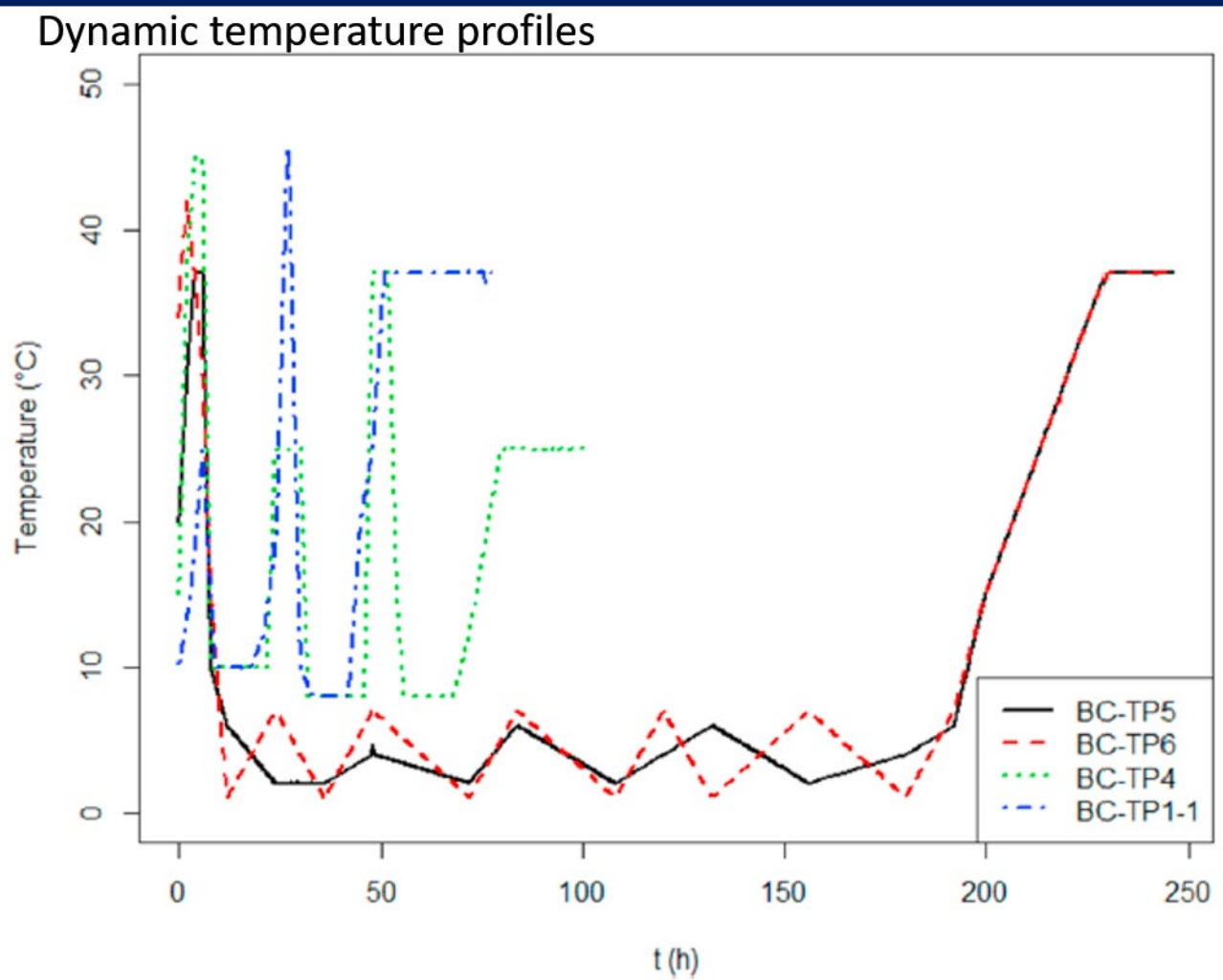


Fig. 1. Dynamic temperature profiles (TP 1-1, 4, 5, and 6) for the determination of kinetic parameters.

Growth and survival of *Bacillus cereus* from spores in cooked rice – One-step dynamic analysis and predictive modeling (3)

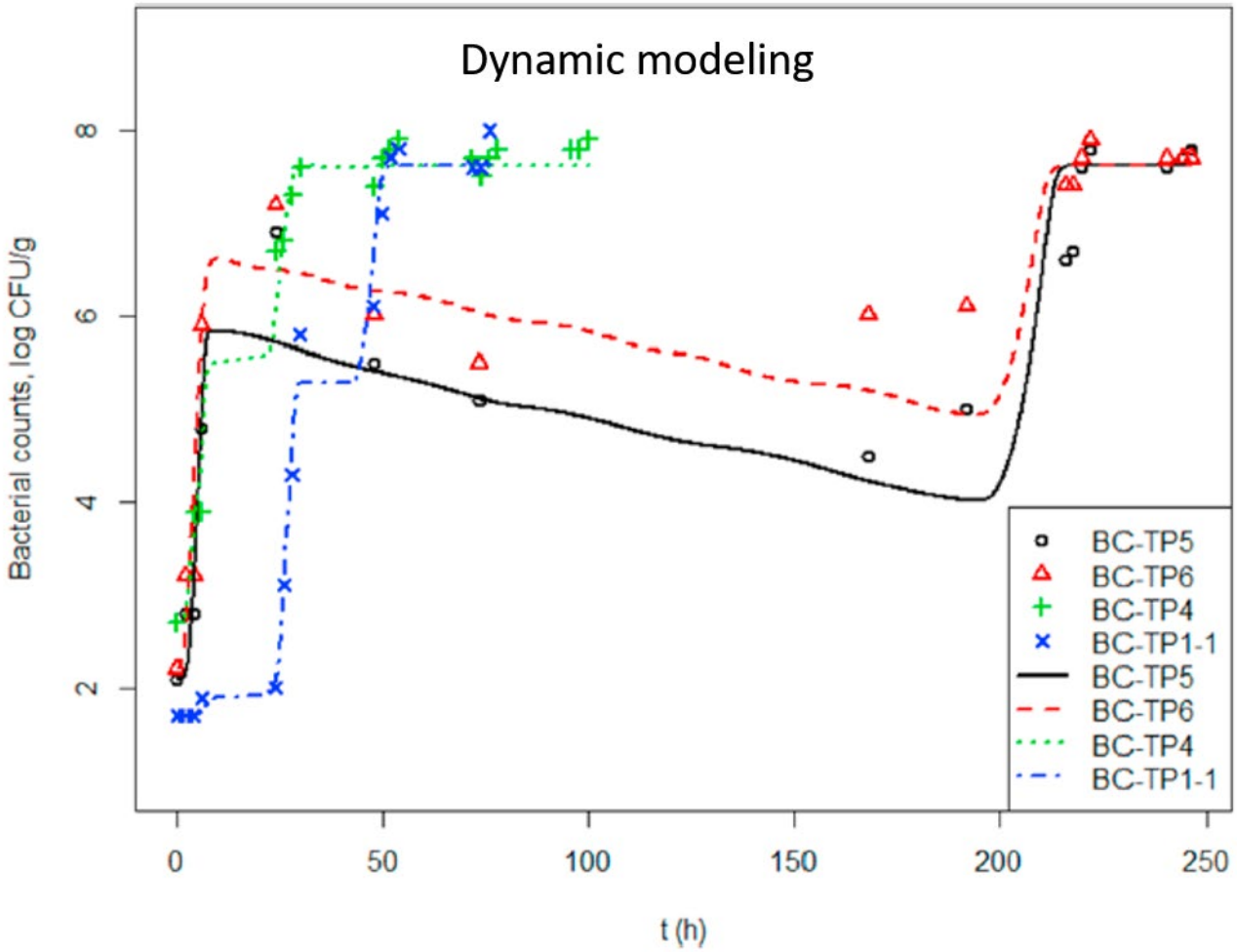


Fig. 2. The growth and survival of *B. cereus* in cooked rice exposed to dynamic temperature profiles (TP1-1, 4, 5, and 6).

Growth and survival of *Bacillus cereus* from spores in cooked rice – One-step dynamic analysis and predictive modeling (4)

Validation

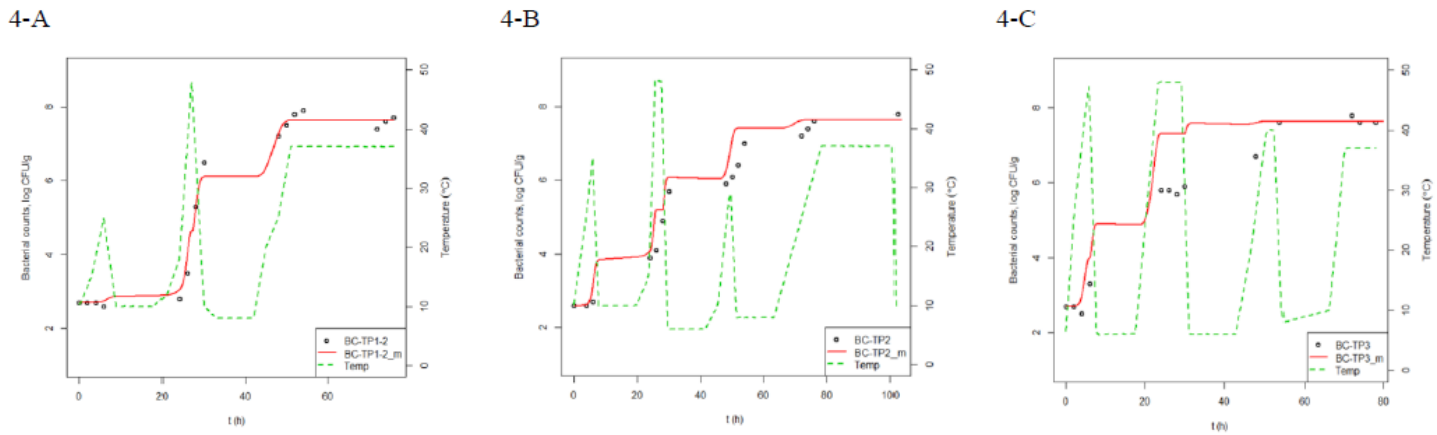


Fig. 4. Validation of the tertiary model. Open cycles: observed data; solid lines: model predictions; and dotted lines: TP 1–2 (4-A), TP 2 (4-B), and TP 3 (4-C).

Growth and survival of *Bacillus cereus* from spores in cooked rice – One-step dynamic analysis and predictive modeling (5)

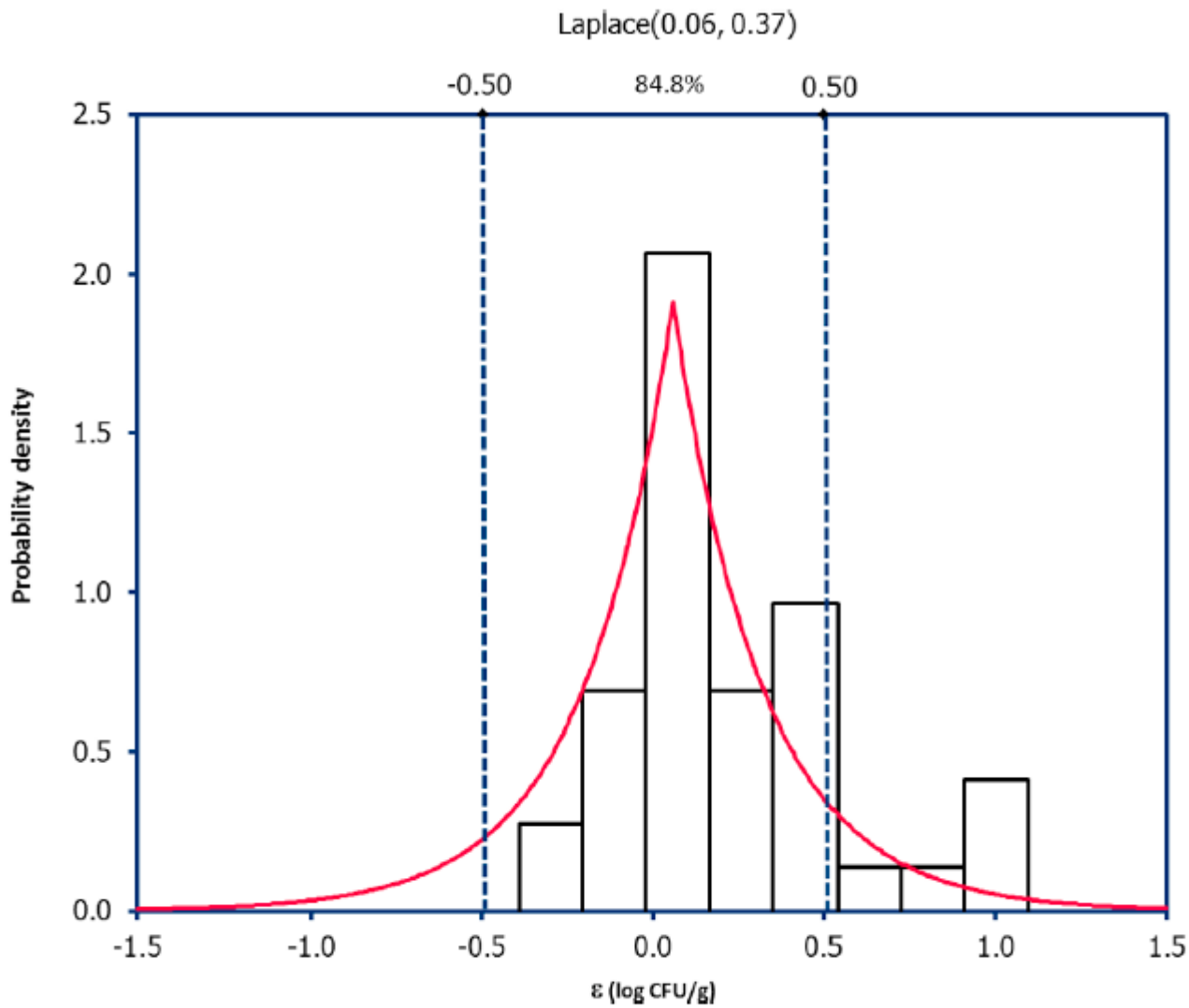


Fig. 5. Distribution of residual errors of prediction.

Dynamic Growth and Survival with Competition (1)

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Dynamic analysis of competitive growth of *Escherichia coli* O157:H7 in raw ground beef☆

Cheng-An Hwang*, Lihan Huang

U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

ARTICLE INFO

Keywords:
Escherichia coli O157:H7
Growth competition
Dynamic modeling
One-step kinetic analysis

ABSTRACT

The objective of this study was to investigate the growth of *Escherichia coli* O157:H7 in raw ground beef under competition from background flora. The growth of *E. coli* O157:H7 was observed in sterile irradiated and non-irradiated raw ground beef under dynamically changing temperature conditions. A one-step dynamic analysis method was used to directly construct tertiary models for describing bacterial growth with and without competition and to estimate the kinetic parameters from dynamic growth curves to prove the hypothesis that the growth of *E. coli* O157:H7 was significantly affected by competition from background flora in raw ground beef.

The one-step dynamic method successfully modelled the growth of *E. coli* O157:H7 and background flora in ground beef and the competition between the two. The estimated minimum growth temperature for *E. coli*

Dynamic Growth and Survival with Competition (2)

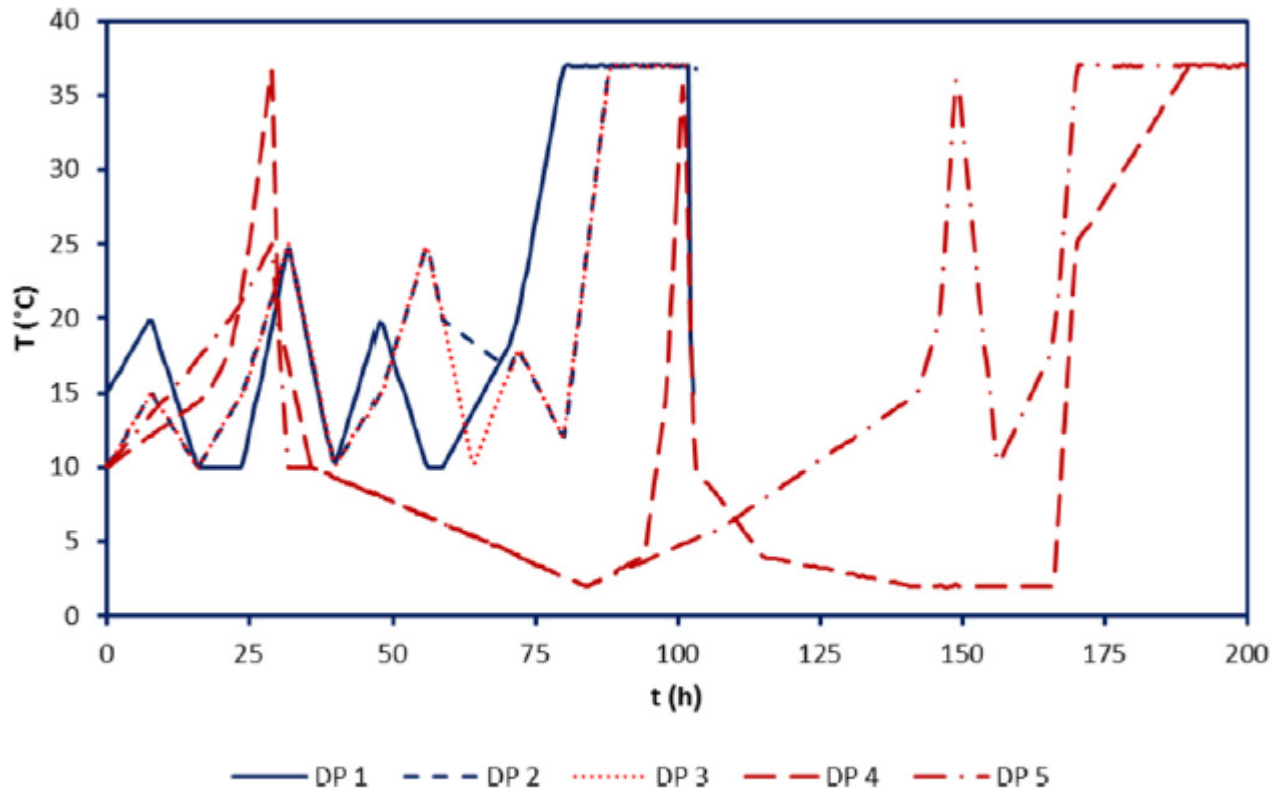

Fig. 1. Dynamic temperature profiles (DP1 – DP5).

Dynamic Growth and Survival with Competition (3)

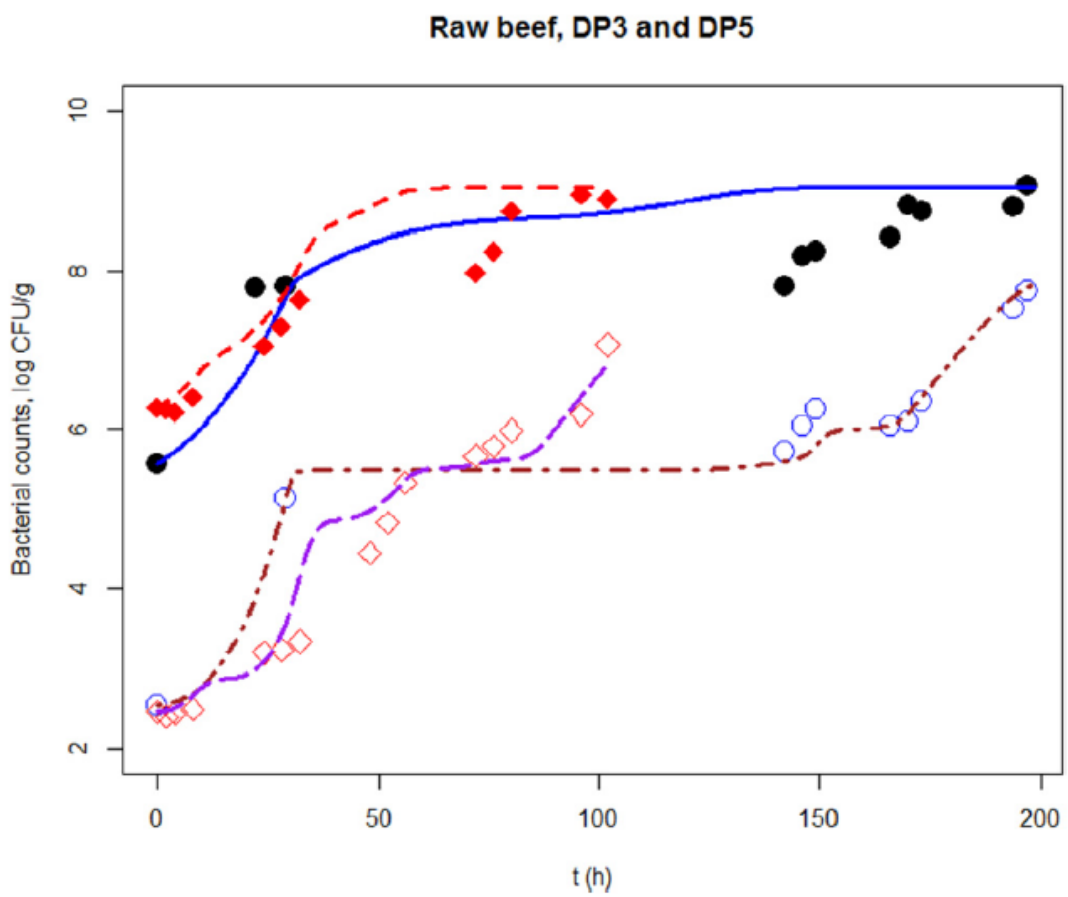

Fig. 3. Growth data and regression curves of background flora and *E. coli* O157:H7 in raw ground beef under DP3 and DP5. Legends: raw data for background flora - solid diamonds (DP3) and solid circles (DP5); regression curves for background flora – dashed line (DP3) and solid line (DP5); raw data for *E. coli* O157:H7 - empty diamonds (DP3) and empty circles (DP5); regression curves for *E. coli* O157:H7 – broken line (DP3) and dash-dotted line (DP5).

Dynamic Growth and Survival with Competition (4)

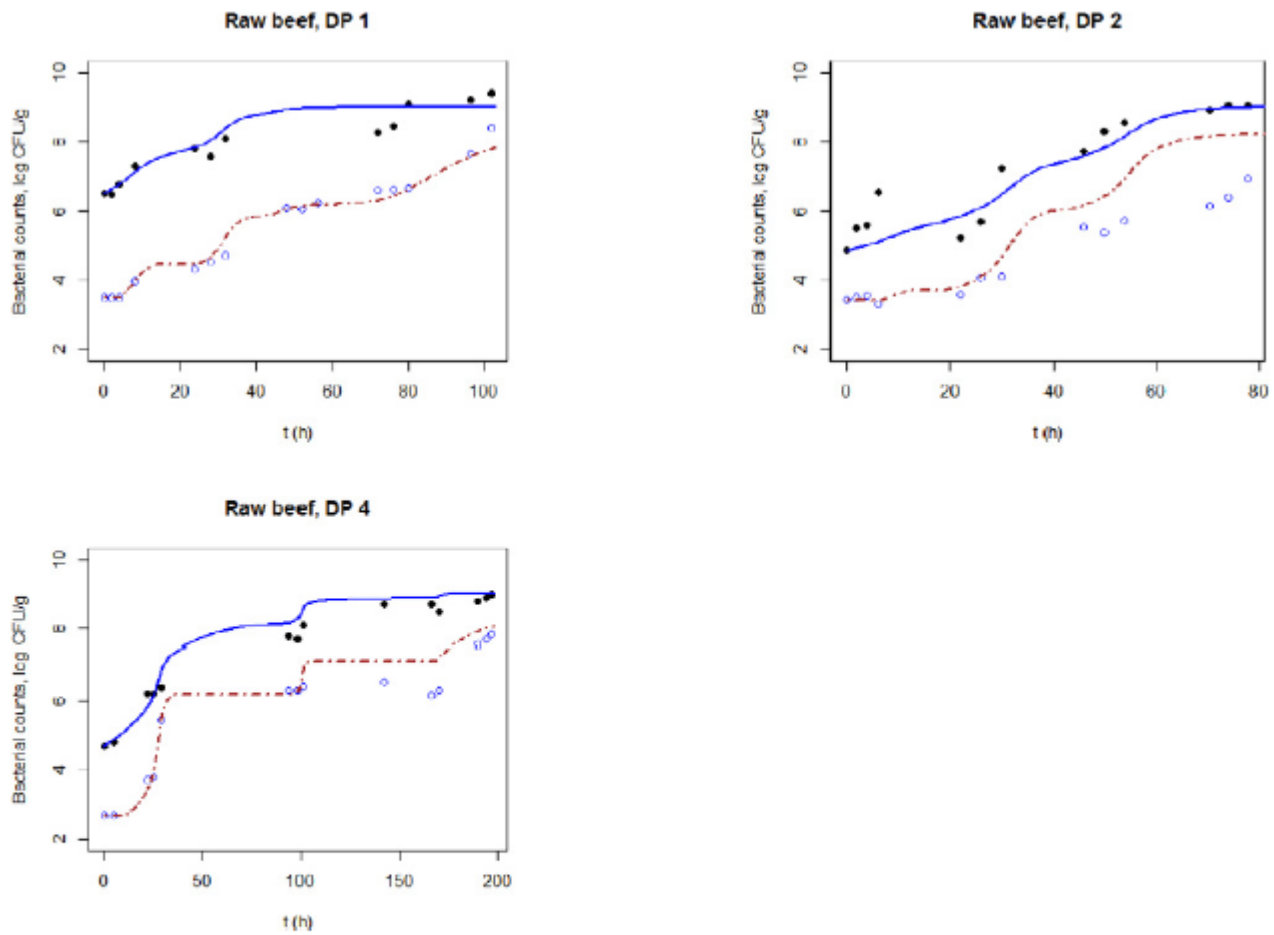

Fig. 6. Predictions of growth of *E. coli* O157:H7 and background flora in raw ground beef under three different dynamic temperature profiles (DP1, DP2, and DP4). Legends: raw data - solid circles (background flora) and empty circles (*E. coli* O157:H7); regression curves – solid line (background flora) and dash-dotted lines (*E. coli* O157:H7).

Dynamic Growth and Survival with Competition/Validation

Journal of Food Science
A Publication of the Institute of Food Technologists

Full Access

Mathematical Modeling of Growth of Non-O157 Shiga Toxin-Producing *Escherichia coli* in Raw Ground Beef

Lihan Huang, Shu-I Tu, John Phillips, Pina Fratamico

First published: 19 April 2012 | https://doi.org/10.1111/j.1750-3841.2012.02647.x | Cited by: 4

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Abstract

Abstract: The objective of this study was to investigate the growth of Shiga toxin-producing *Escherichia coli* (STEC, including serogroups O45, O103, O111, O121, and O145) in raw ground beef and to develop mathematical models to describe the bacterial growth under different temperature conditions. Three primary growth models were evaluated, including the Baranyi model, the Huang 2008 model, and a new growth model that is based on the communication of messenger signals during bacterial growth. A 5 strain cocktail of freshly prepared STEC was inoculated to raw ground beef samples and incubated at temperatures ranging from 10 to 35 °C at 5 °C increments. Minimum relative growth (<1 $\log_{10}$ cfu/g) was observed at 10 °C, whereas at other temperatures, all 3 phases of growth were observed.

Dynamic Growth and Survival with Competition/Validation (2)

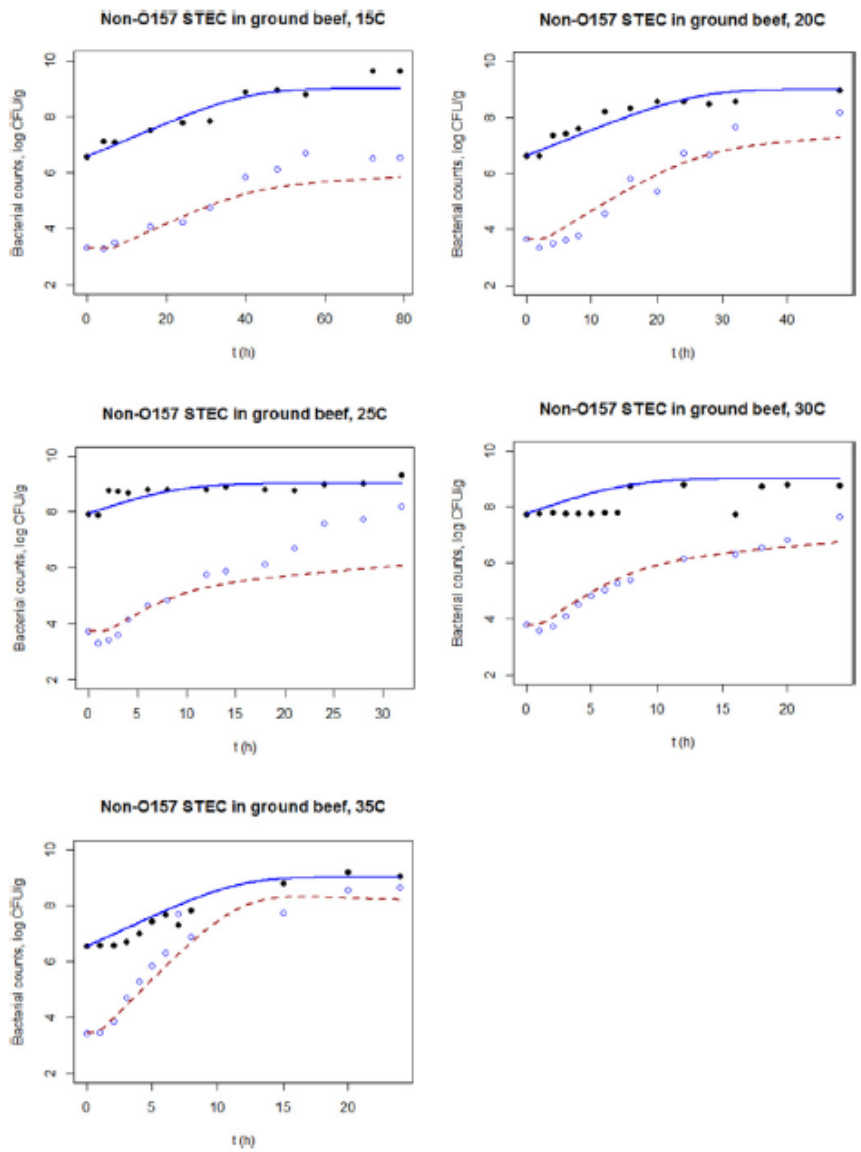


Fig. 7. Prediction of growth of non-O157 STEC and background flora in raw ground beef under isothermal conditions. Legends: raw data - solid circles (background flora) and empty circles (non-O157 STEC); regression curves – solid line (background) and dashed line (non-O157 STEC).

One-Step Dynamic Analysis (1)

- More accurate
- More efficient
- More realistic
- Both growth and survival can be studied
- Unique methodology – we are the only laboratory actively working on this methodology (data collection and analysis)
- Future of predictive modeling

Accuracy of One-Step Dynamic Analysis

- In all validation tests
- 60-85% of residual errors of prediction is within ±0.5 log CFU/g
- Most of the residual errors follows Laplace, logistic, or normal distribution

One-Step Dynamic Analysis (2)

- We have firmly established the methodology
- We have reasonably defined residual errors
- 60-85% of residual errors of prediction is within ±0.5 log CFU/g
- But is that all?
- Can we do better than ±0.5 log CFU/g?
- We have to rely on other modeling methods

Deterministic vs stochastic modeling

- Deterministic – all data are known beforehand
  - The results are the same as long as the conditions are the same
  - You have all the data for prediction and the outcome can be predicted with 100% certainty
- Stochastic – certain things are uncertain
  - You know the likelihood that something is going to happen, but not sure when it will occur
  - If repeated many, many times, the mean of the outcomes may be equal to a deterministic process

Bayesian Analysis

- This is a new area that we are experimenting and have found surprisingly good results
- Bayesian analysis is a statistical paradigm that answers research questions about unknown parameters using probability statements
- Stochastic approach

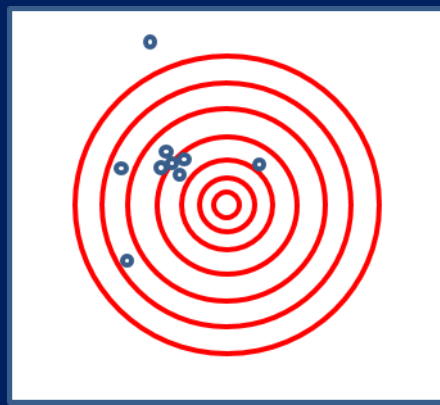
Bayesian analysis is based on Bayes' Theorem

$$P(A|B) = \frac{P(B|A)P(A)}{P(B)}$$

- A and B are two events
- P(A|B): conditional probability: the likelihood of even A occurring given that B is true
- P(B|A): conditional probability: the likelihood of even B occurring given that A is true
- P(A) and P(B): marginal probability: the probabilities of observing A and B independently of each other

Bayesian inference is a method of statistical inference in which Bayes' theorem is used to update the probability for a hypothesis as more evidence or information becomes available

How can we use the existing data (observations) to make more reliable/accurate predictions?

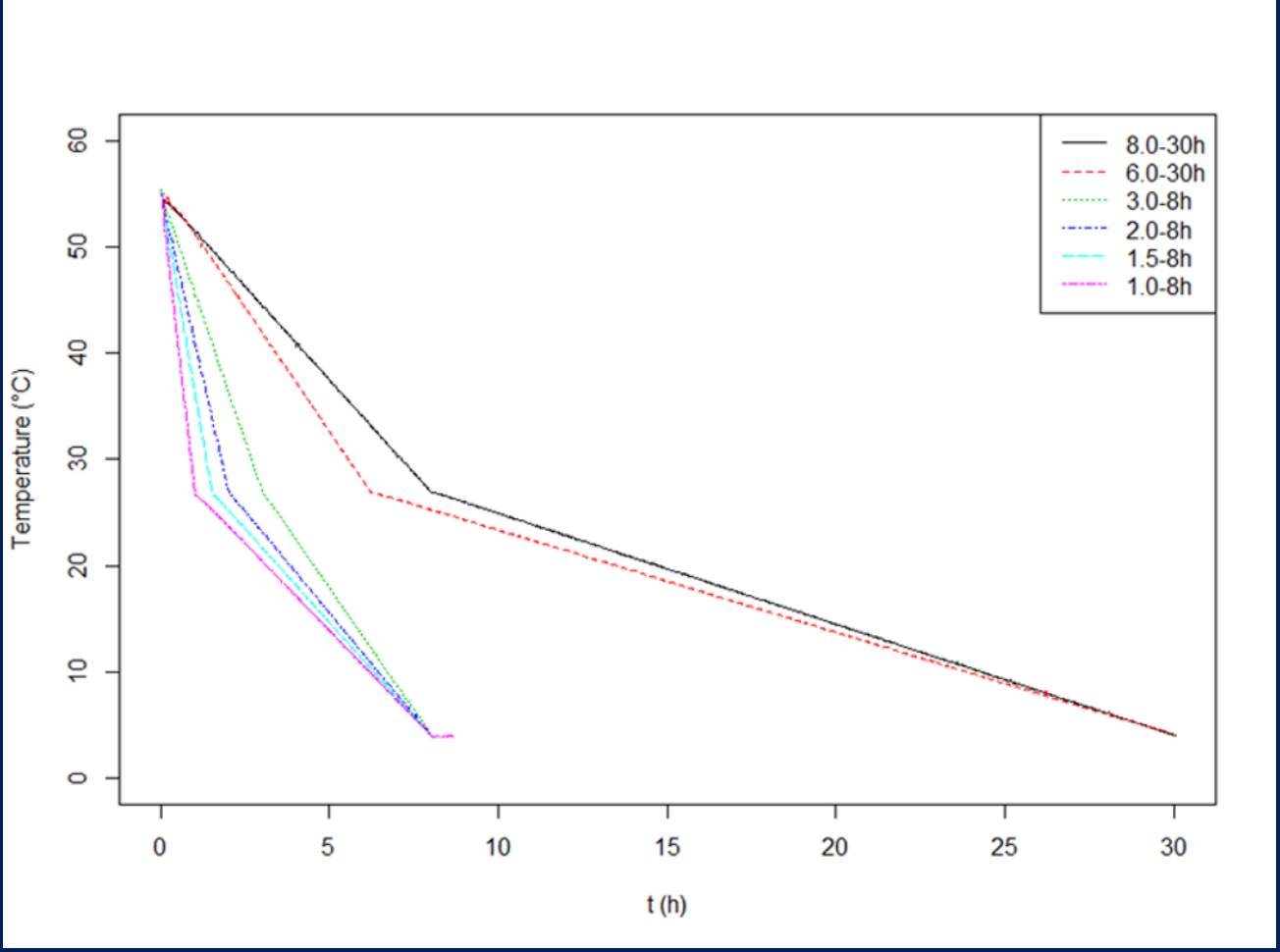


Markov Chain Monte Carlo Simulation

- Markov chain Monte Carlo (MCMC) methods comprise a class of algorithms for sampling from a probability distribution
- It is used to construct posterior distribution
- In Bayesian analysis, the kinetic parameters are not fixed numbers anymore
- They are treated as random numbers

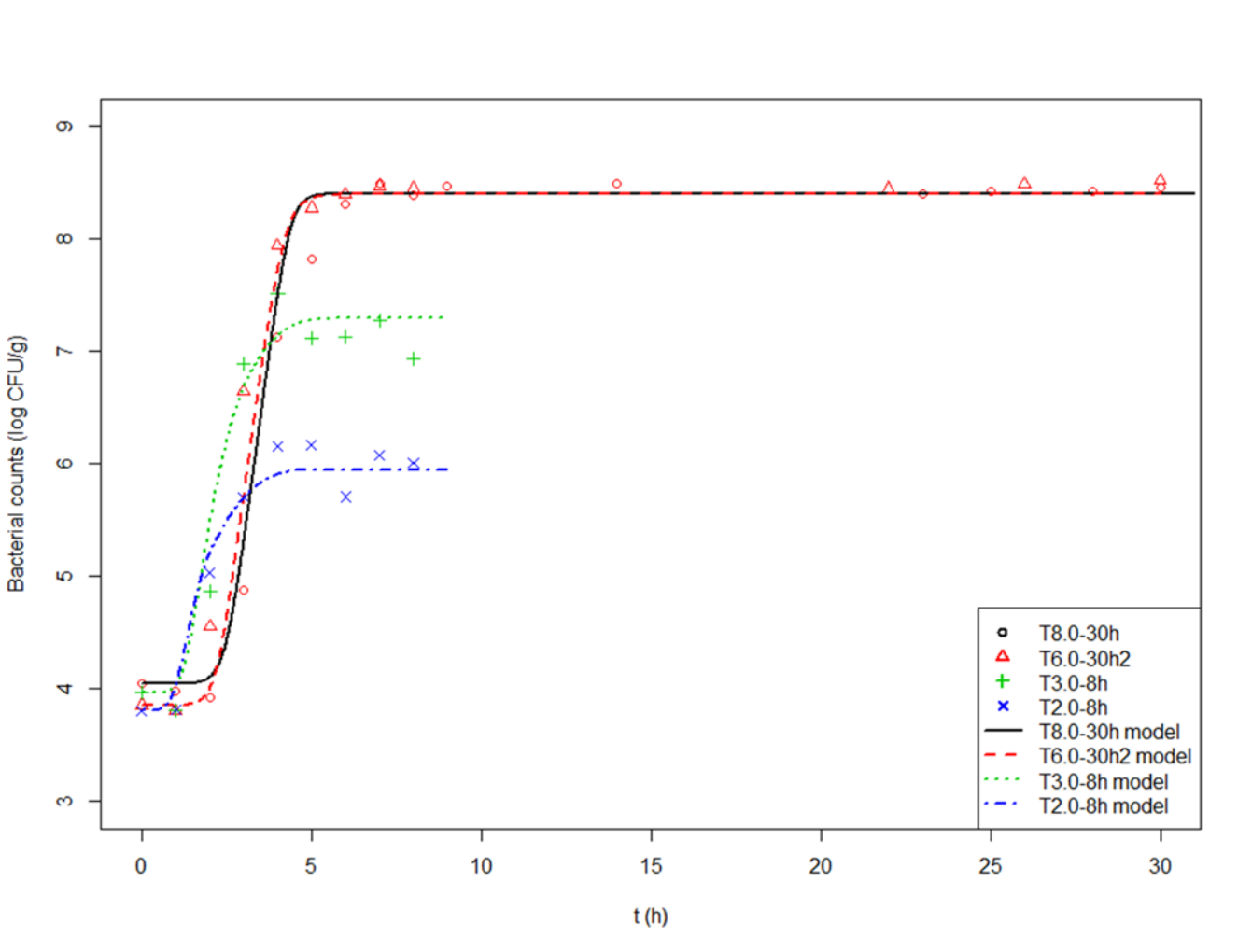
BA and MCMC simulation of growth of *C. perfringens* in cooked chicken

Dynamic cooling

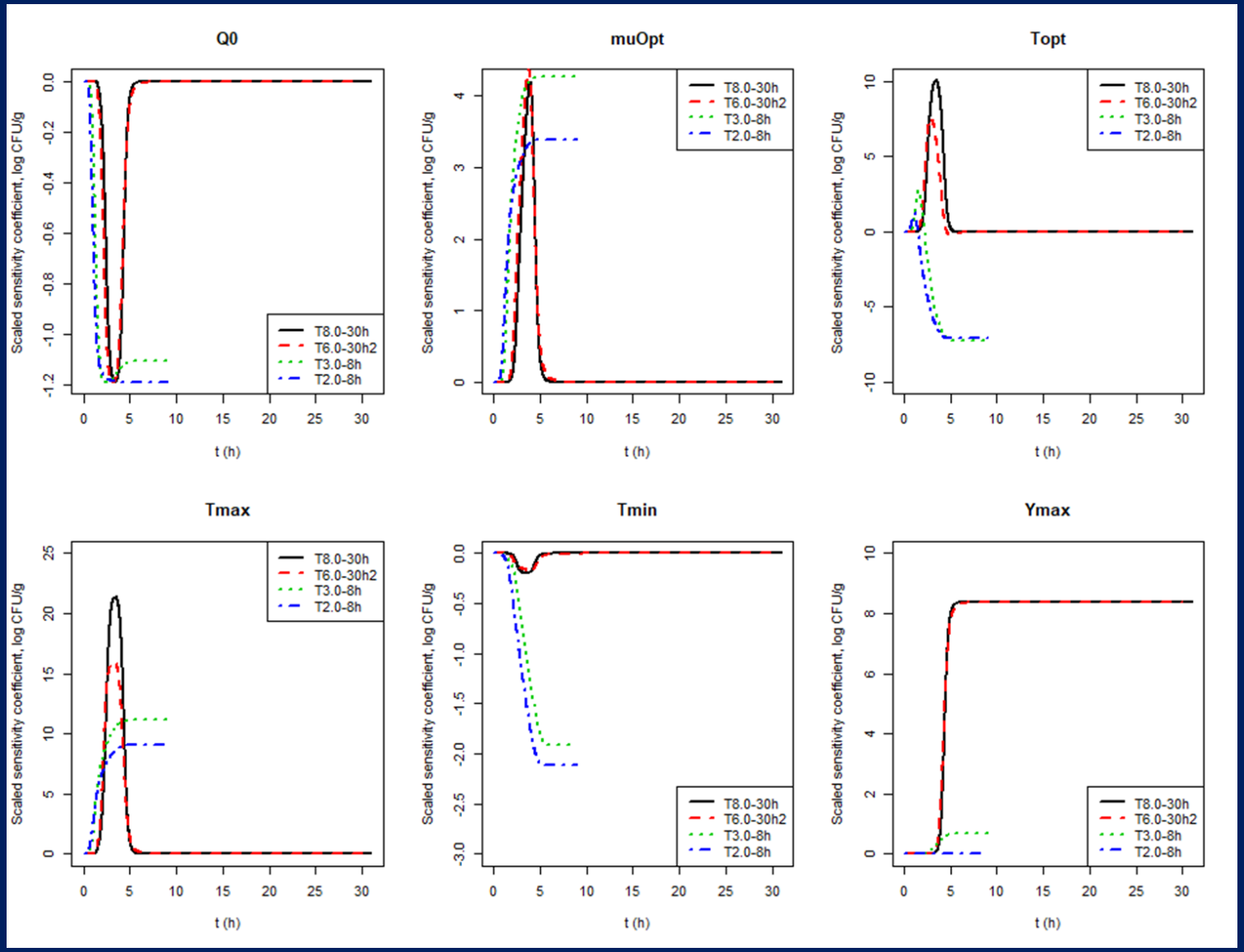


BA and MCMC simulation of growth of *C. perfringens* in cooked chicken (2)

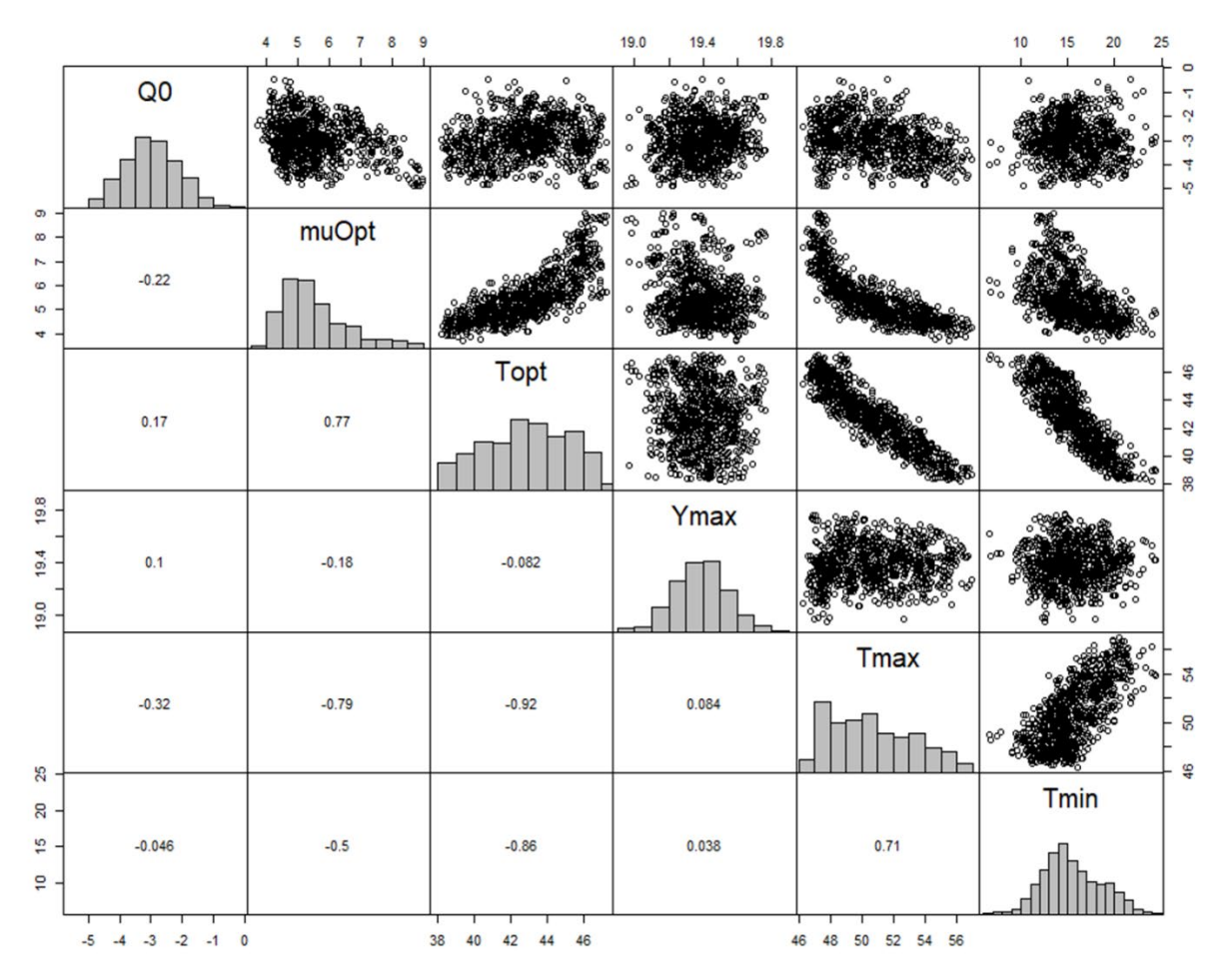
Growth curves



Sensitivity analysis

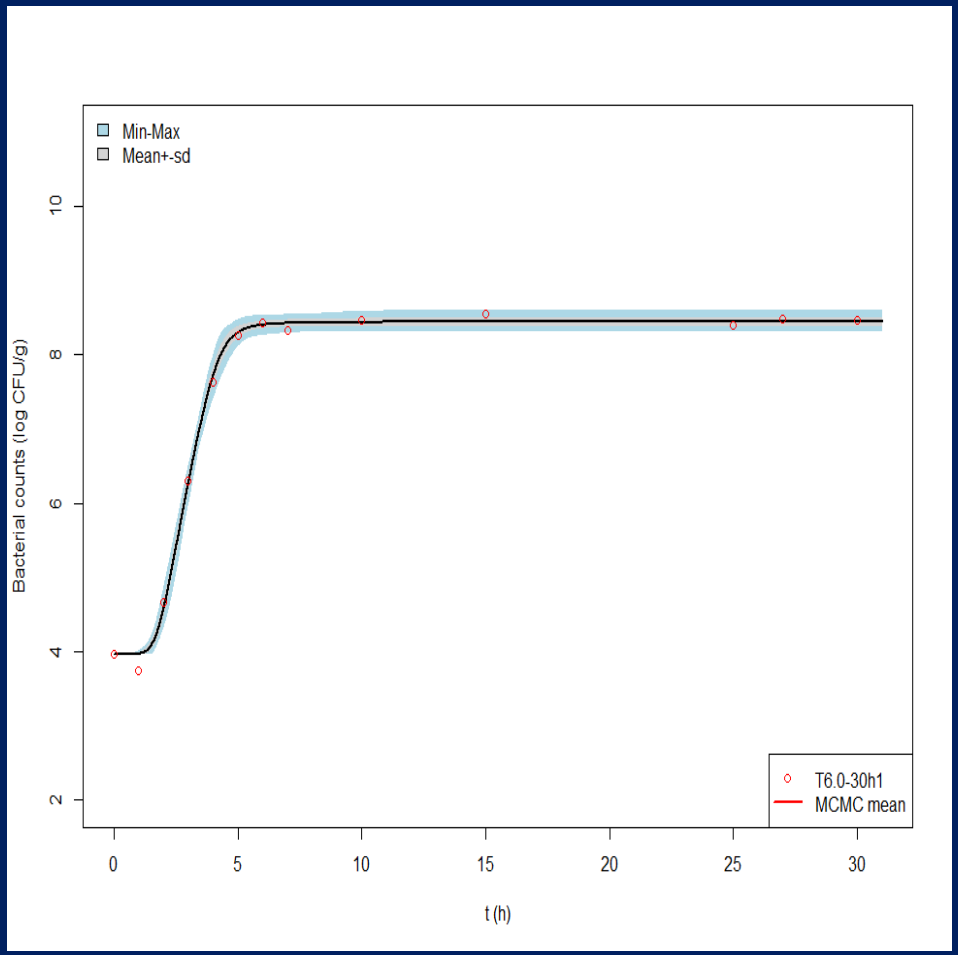


Correlation analysis

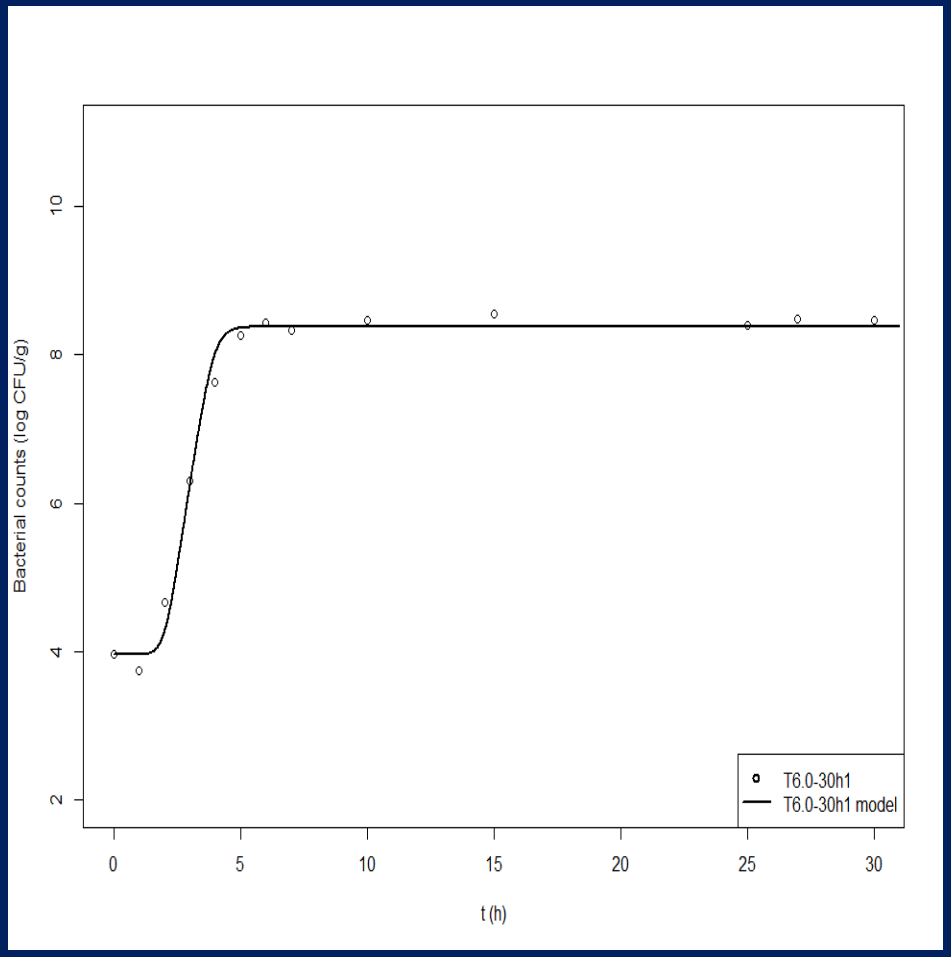


Comparison of results (1)

Bayesian

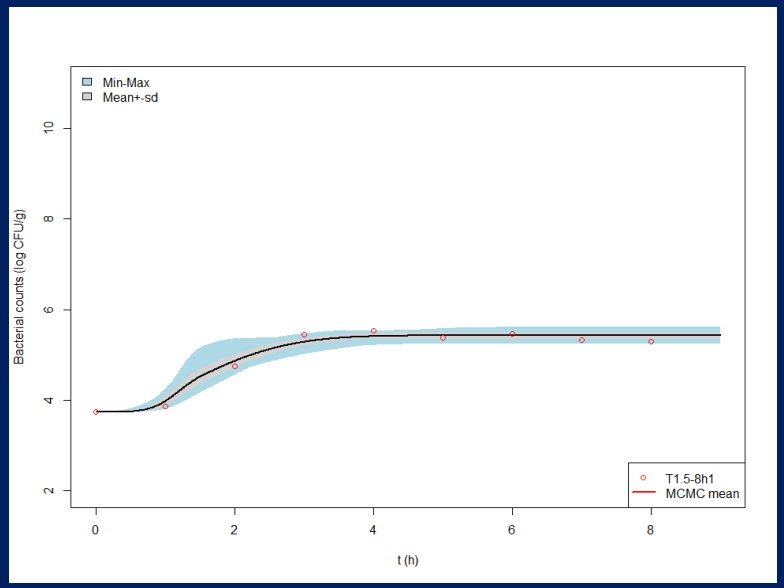


Deterministic

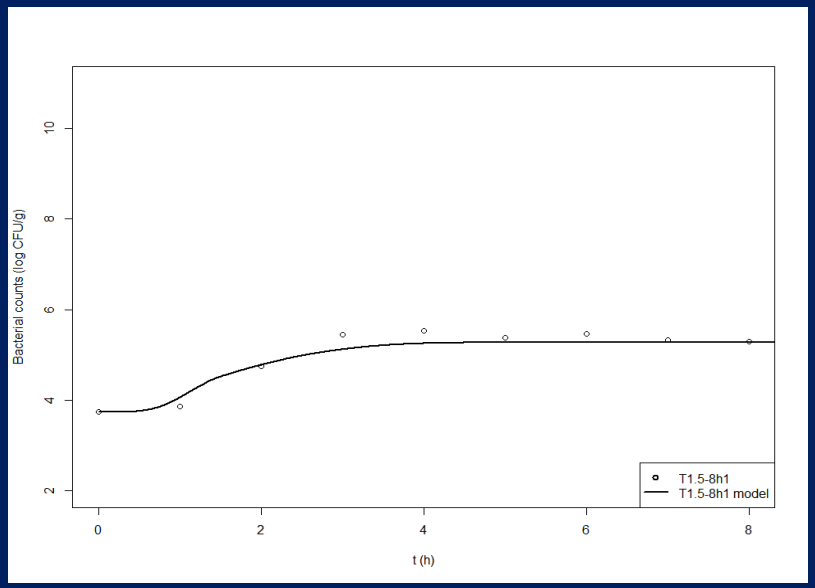


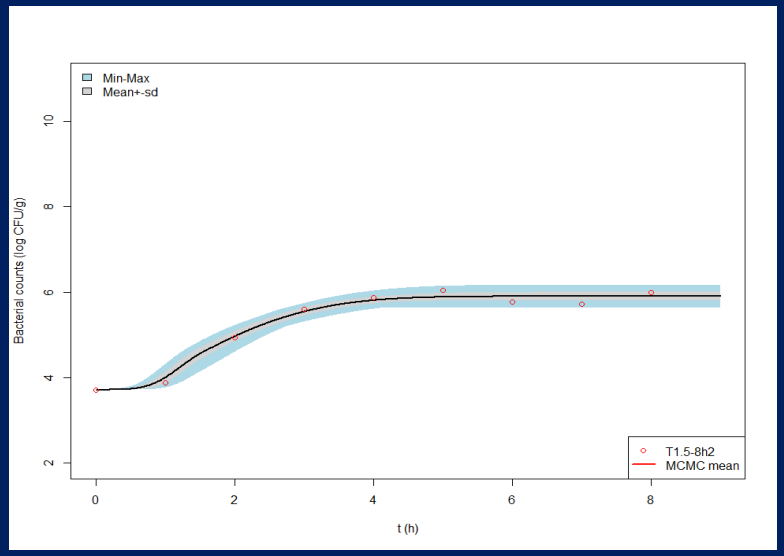
Comparison of results (2)

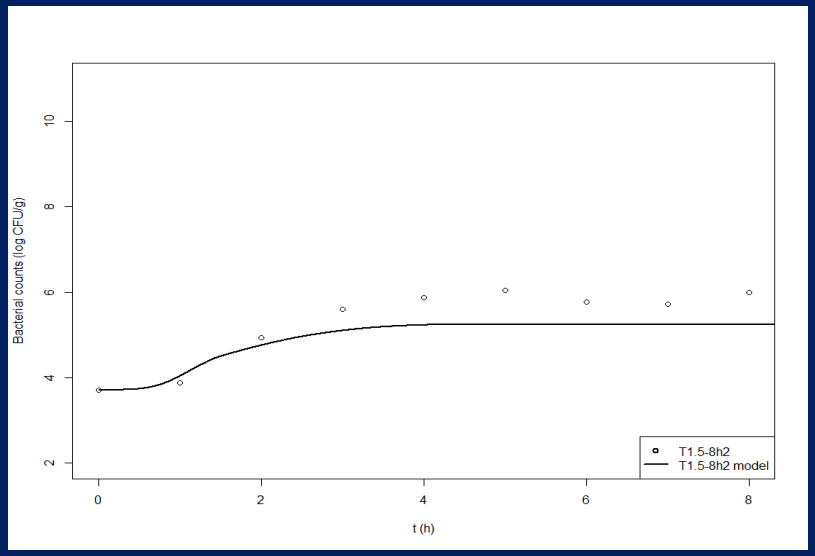
Bayesian



Deterministic

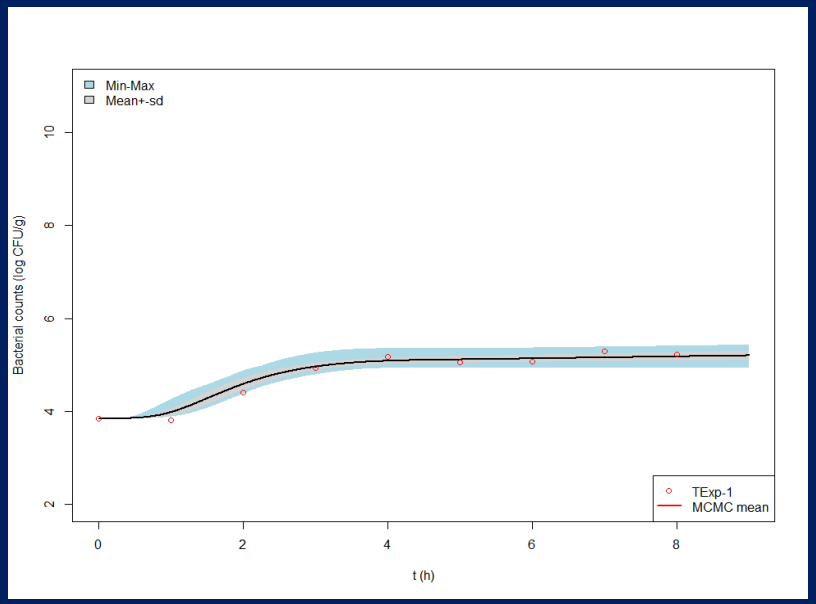




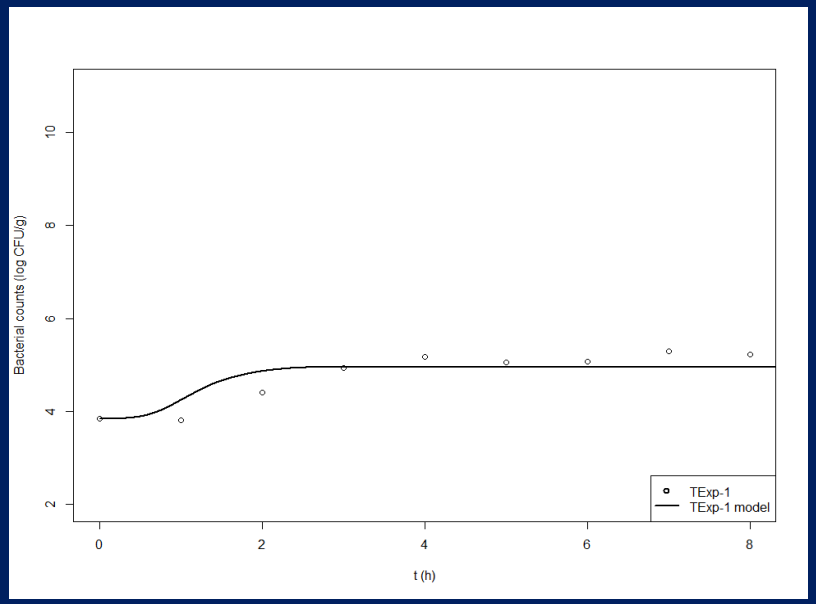


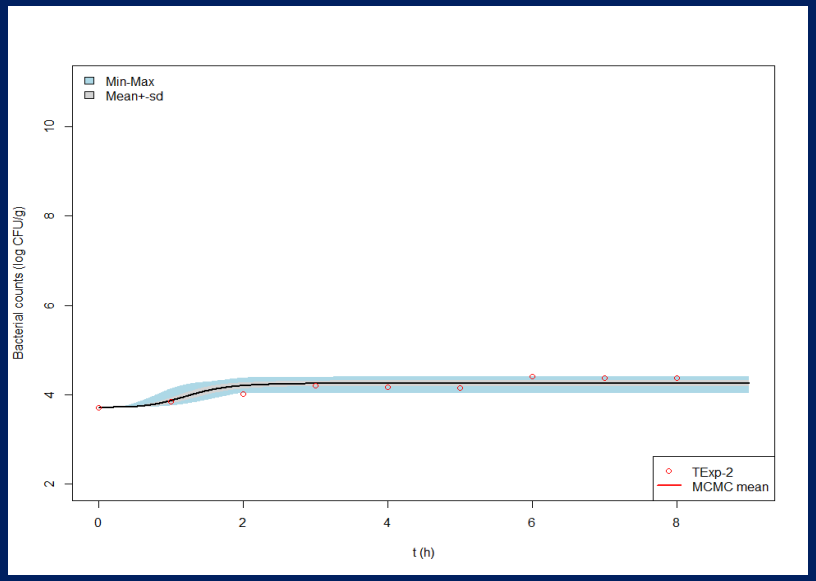
Comparison of results (3)

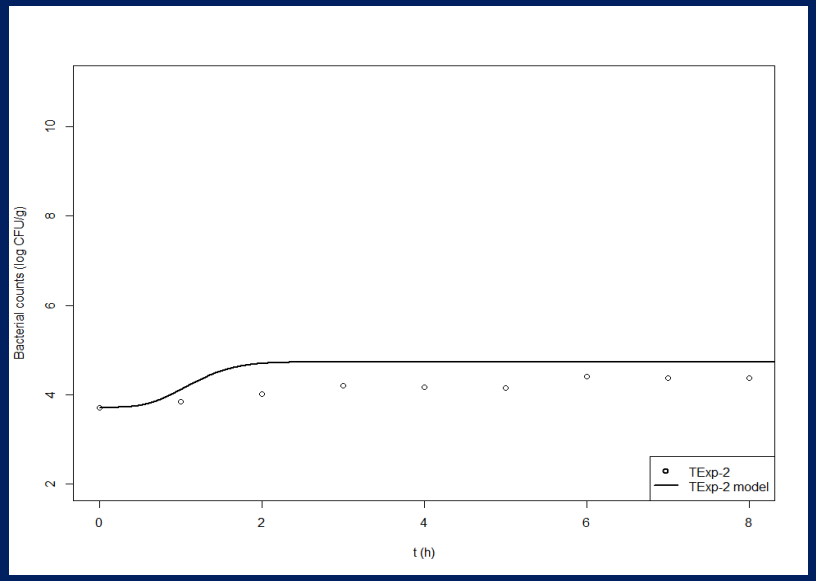
Bayesian



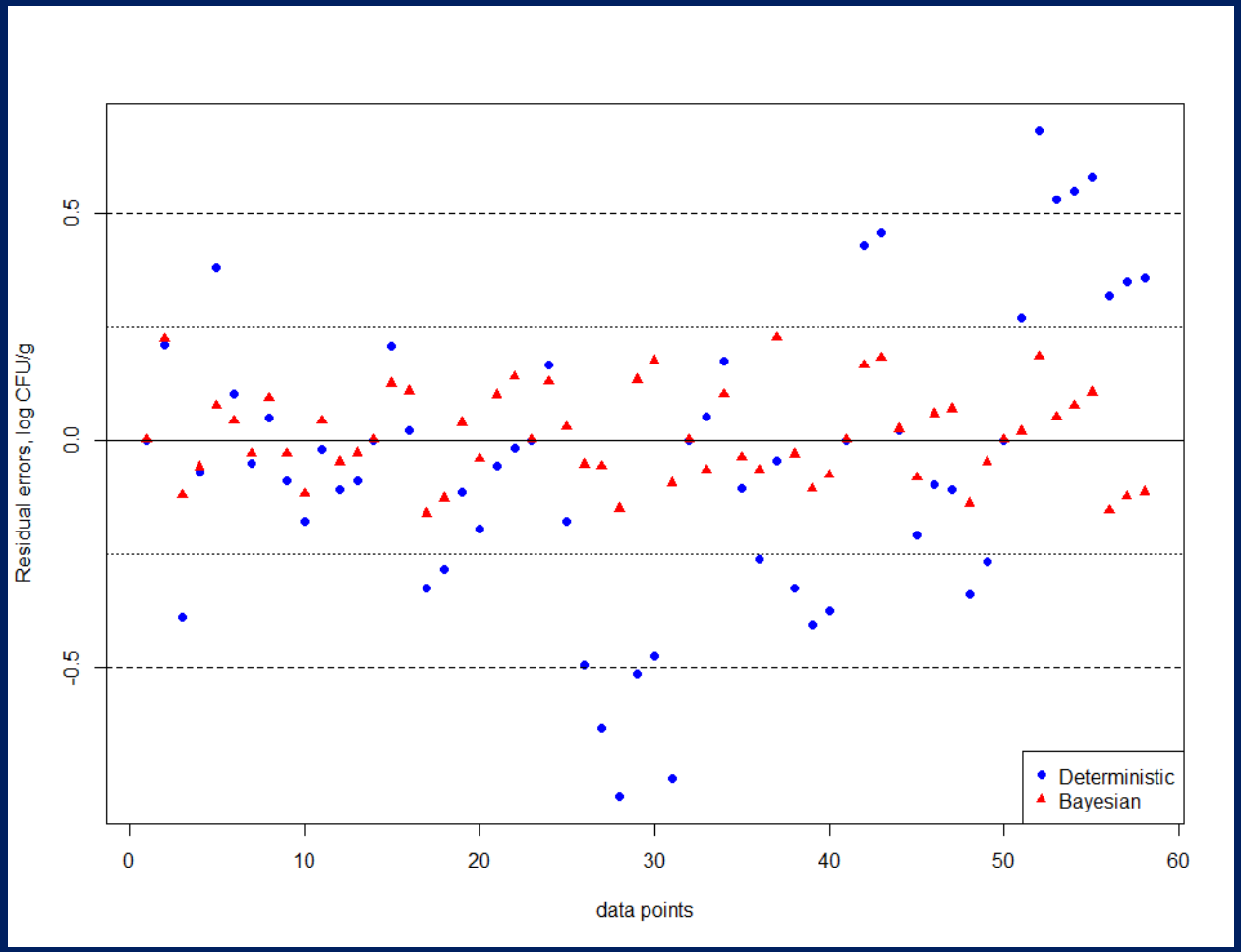
Deterministic



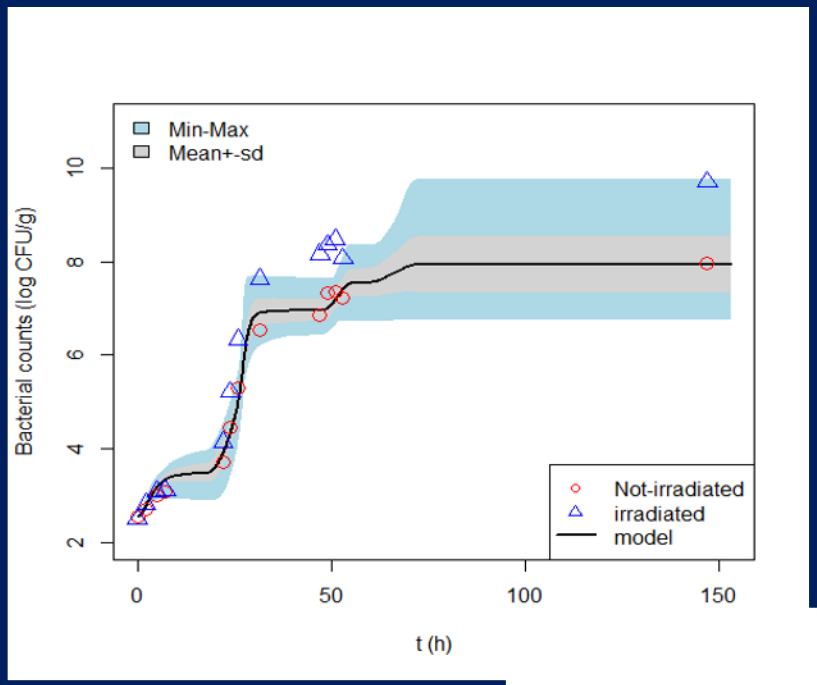


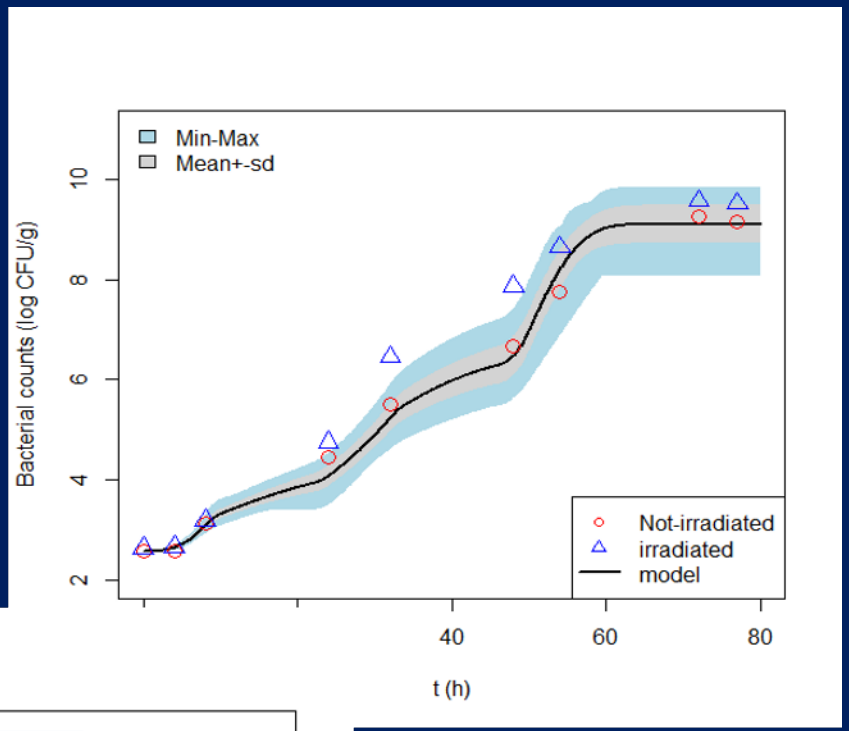


Comparison of residual errors of prediction

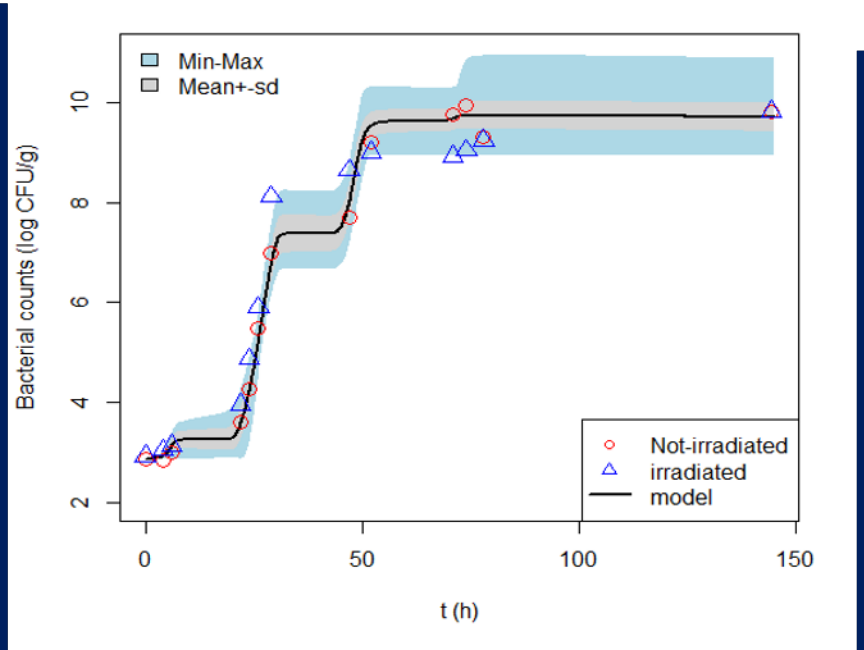


Growth of *Salmonella* in ground beef



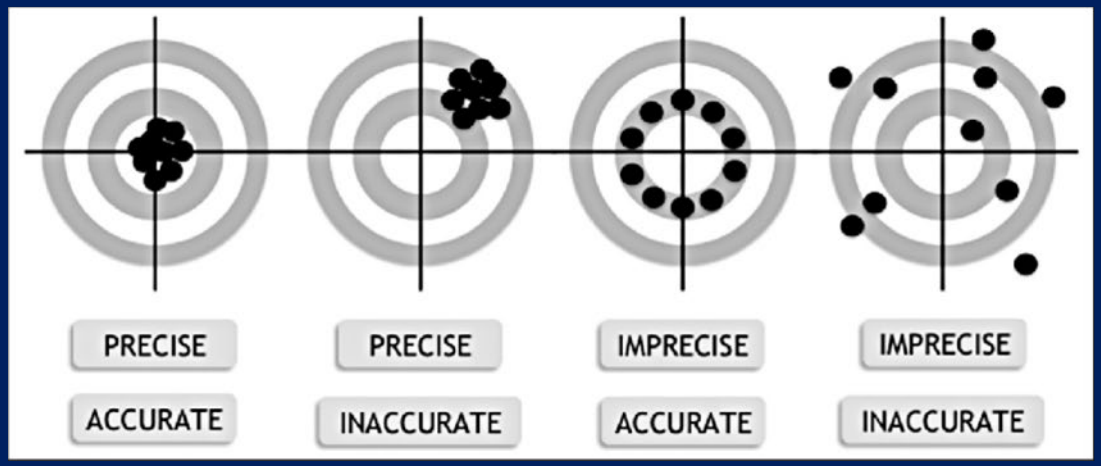


Different dynamic temperature profiles



Significantly improved accuracy and precision

Prediction errors are 100% within ± 0.25 log CFU/g!



Logistic modeling for controlling bacterial growth

- All we have talked about so far is how to model bacterial growth
- There is another very important area – how to use mathematical modeling to assist in controlling bacterial growth?

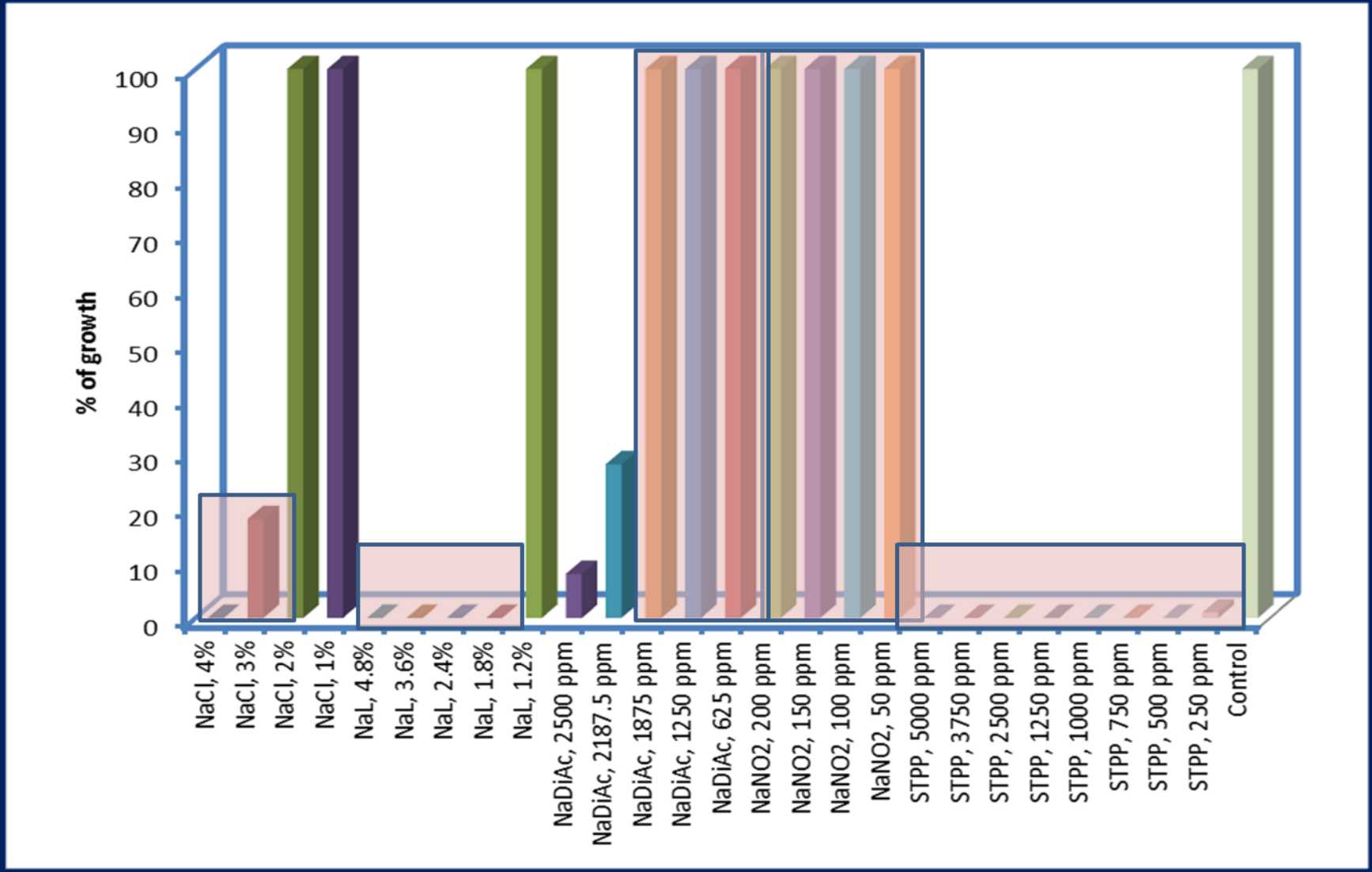
Growth and No Growth Boundary of *C. perfringens* in Cooked Beef

- Apply logistic modeling to define the growth/no growth boundary
- Formulate meat and poultry products that may not support and allow the germination and outgrowth of *C. perfringens*
- Prevent food poisoning outbreaks caused by this microorganism.

Common ingredients

- Ingredients: Sodium chloride (NaCl), sodium diacetate (NaDiAc), sodium nitrite ($NaNO_2$), sodium DL-lactate solution (NaL, 60% w/w), and sodium tripolyphosphate (STPP)
- NaCl (1-4%), NaL (1.2-4.8%), STPP (250-5000 ppm), NaDiAc (0-2500 ppm), and $NaNO_2$ (50-200 ppm).

100 well broth test under optimum temperature

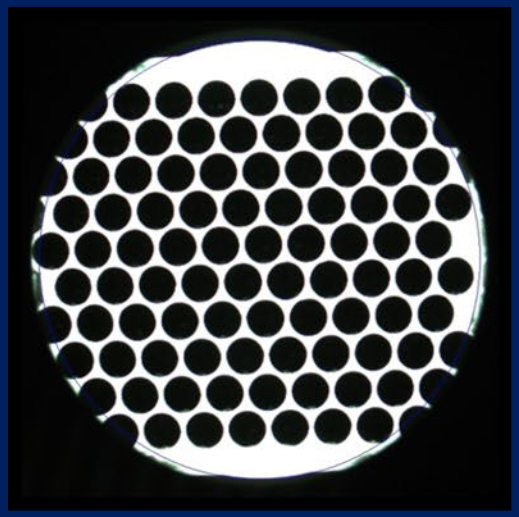


Broth test

- Almost 100% of studies like this are conducted in broth
- However, we found out that it was almost impossible to repeat the results in meat.
- We concluded that the broth test may not produce meaningful results

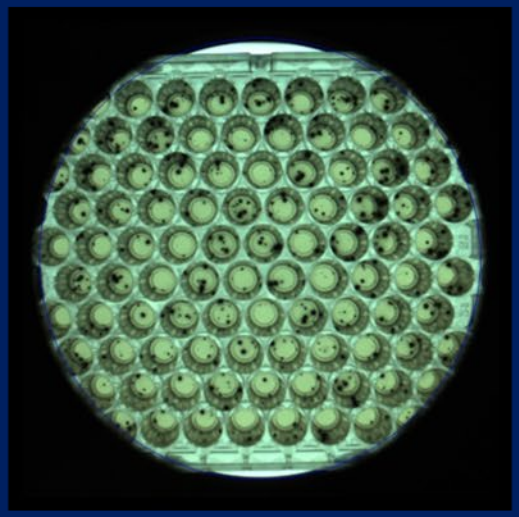
Solid agar test

Positive control



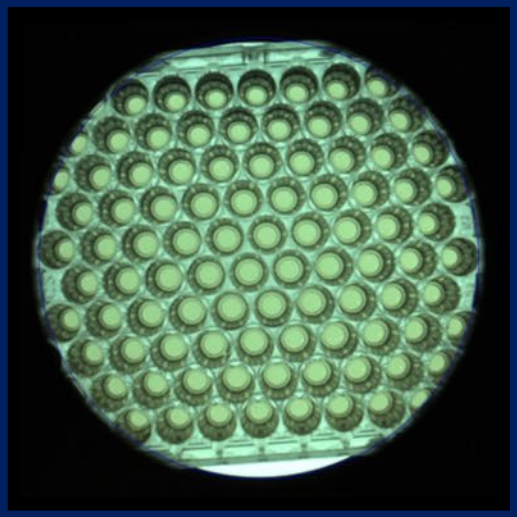
Full growth

Test 38



Partial growth

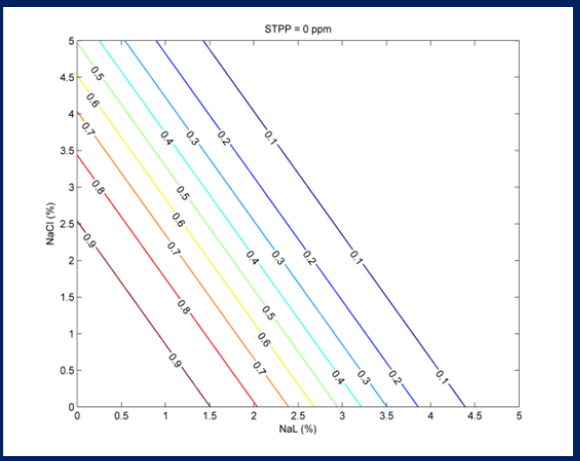
Negative control

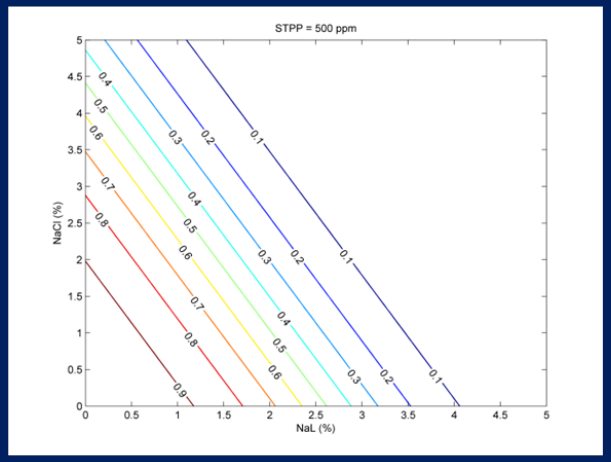


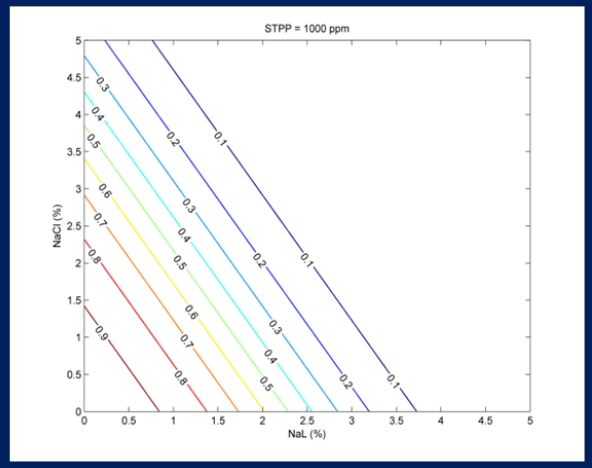
Negative growth

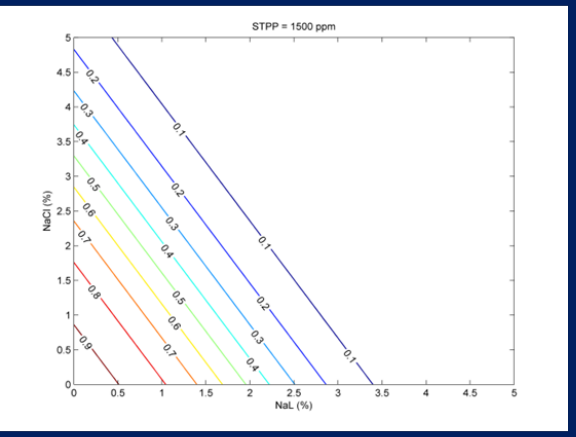
Definition of growth: anything > background reading + 3 SD

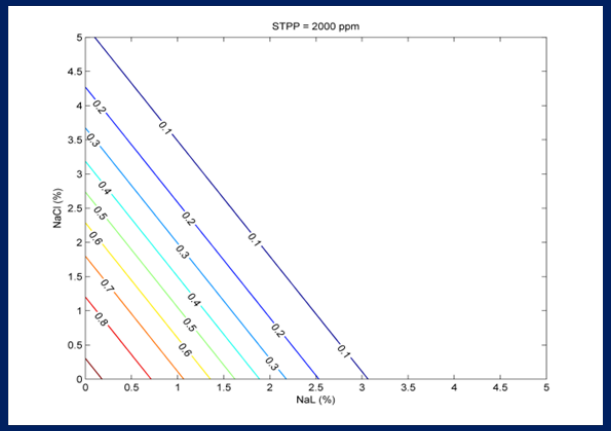
Growth boundaries based on solid agar test

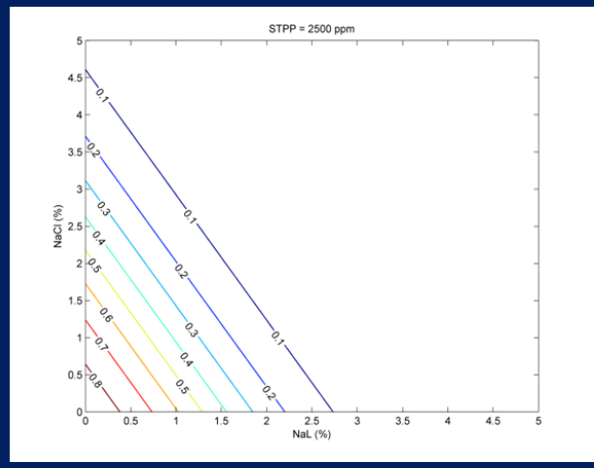




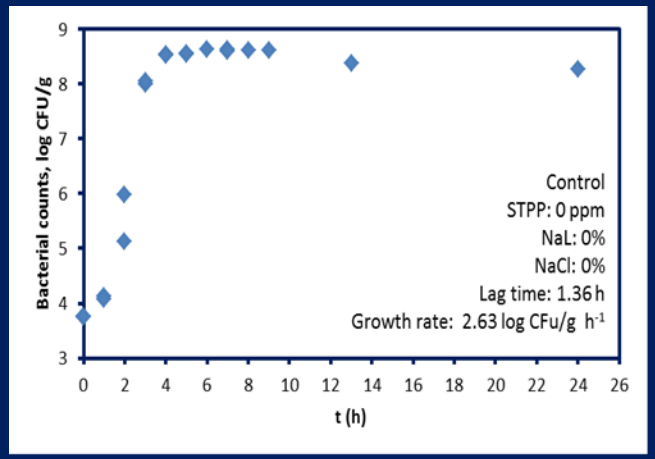


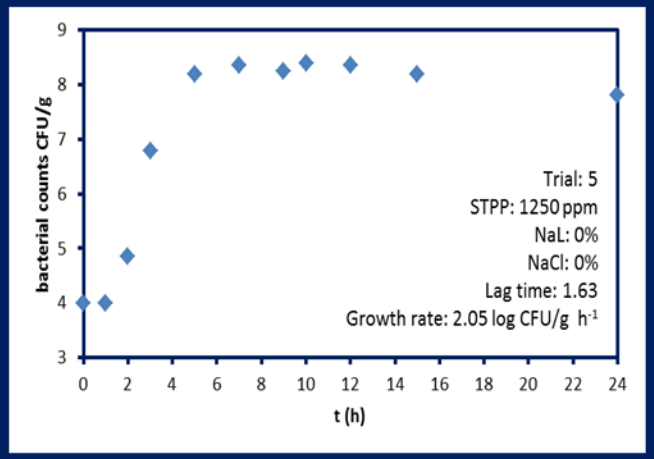


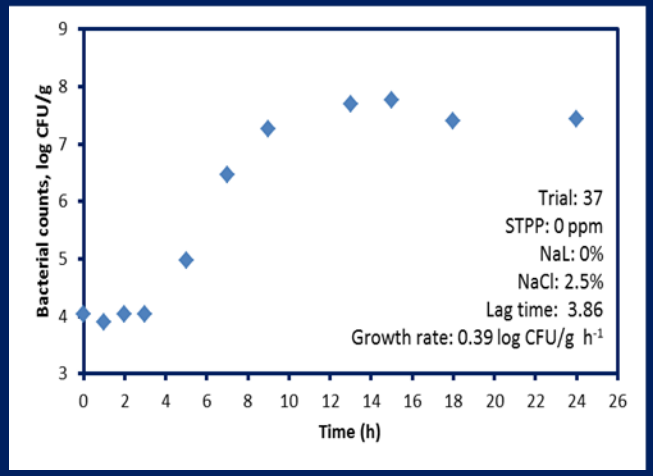


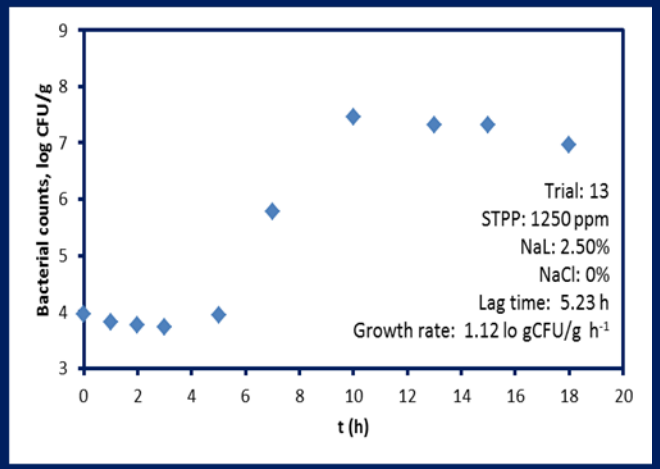


Validation in cooked ground beef (1)

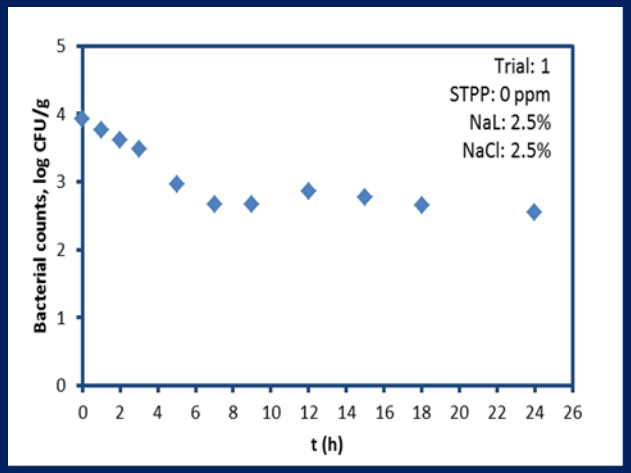


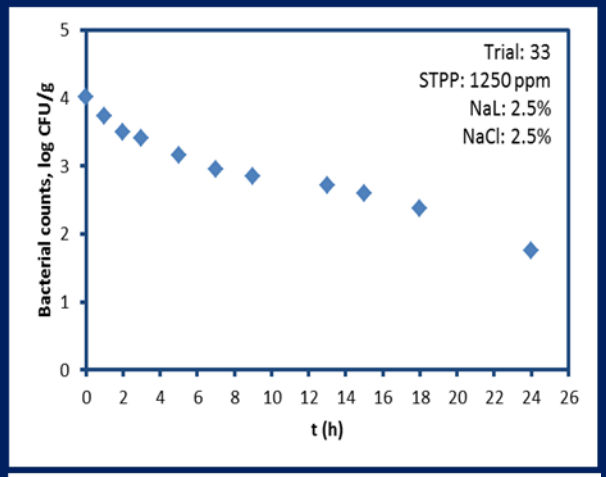


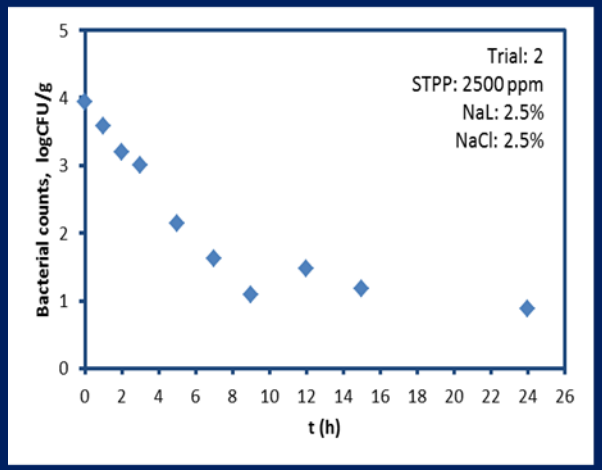


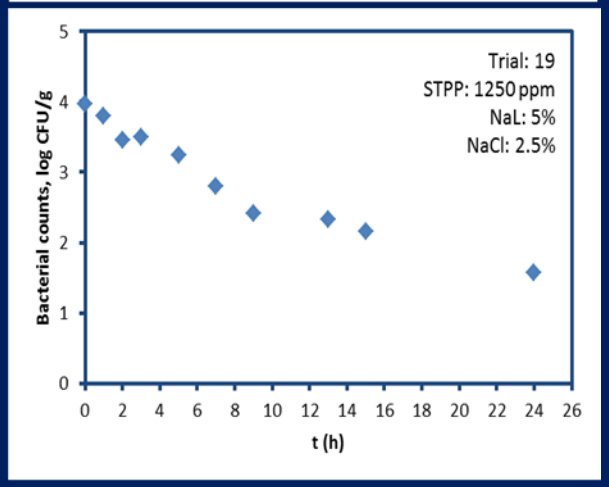


Validation in cooked ground beef (2)

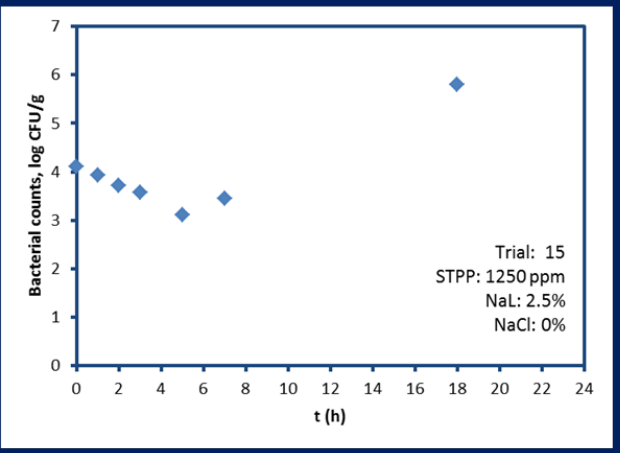


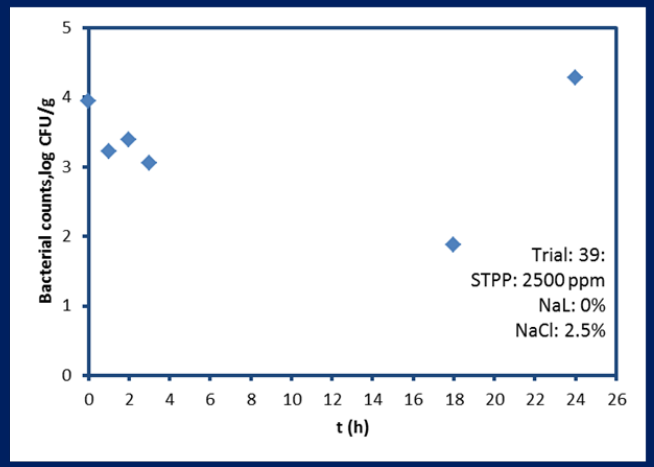


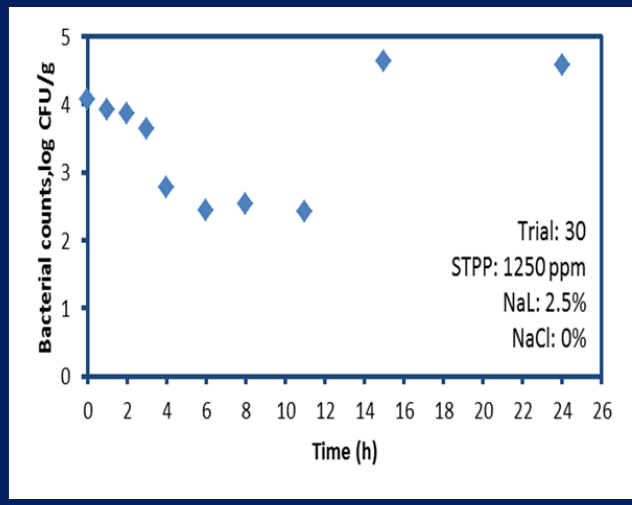




Validation in cooked ground beef (3)







Logistic modeling for controlling growth of *C. perfringens*

- With a threshold of 0.2 in the model, the accuracy of Growth/No Growth prediction in beef is 95.7%, with 4.3% over-prediction of growth events (fail-safe).
- Proper combinations of STPP, NaL, and NaCl could be used to control the growth of *C. perfringens* in cooked beef under the optimum temperature.
- The model can be used to formulate products to prevent the growth of *C. perfringens* during cooling.

Main Research Objectives (2)

1. Development and validation of predictive models for growth of high priority pathogens in processed foods
2. Dynamic simulation and probabilistic modeling of growth of foodborne pathogens in foods
3. Development of an advanced decision support system and software for predictive microbiology and food safety regulations
4. Expand where necessary the ARS curve-fitting (modeling) program also known as the “Integrated Pathogen Modeling Program (IPMP)”

Objective 3

- We have developed advanced techniques in modeling
- We have developed and validated highly accurate predictive models
- Our next step is to make the models available

Three Products

USDA Integrated Pathogen Modeling Program (IPMP)

- A suite of data analysis tools that contains 20+ most frequently used models
- Intelligent, interactive data analysis and model development
- Standardized data analysis and interpretation

IPMP – Global Fit

- One-step data analysis and model development tool for isothermal studies
- Direct construction of predictive models for minimized global errors (more accurate)
- Intelligent, interactive, and automated data analysis

IPMP – Dynamic Prediction

- One-step data dynamic analysis and model development tool
- Direct construction of predictive models from dynamic curves
- More accurate and more efficient model development
- Probabilistic analysis

Our future directions

- Bayesian analysis
  - We will continue to develop and optimize BA methods for predictive modeling
  - Logistic modeling for controlling bacterial growth
- Computational tools

We will try cloud computing

- We have the technical knowhow for modeling and computing
- Cloud computing will not be based on USDA networks
- We are thinking computational resources
  - More powerful
  - Less risk to government network
  - We will need additional $
  - A programmer?

Collaborators

- Many USDA ARS collaborators
- Many international collaborators
  - PRIMA Project (EU Food Safety in the Mediterranean region)

Thank you!

Lihan.Huang@ars.usda.gov
Andy.Hwang@ars.usda.gov