International Association for Food Protection®

WEBINAR

PRACTICAL APPLICATIONS OF MICROBIAL MODELLING - WEBINAR SERIES

March 5, 2018 3:00 p.m. EST

Practical Applications of Microbial Modelling Webinar Series

Webinar Series:

Part II of III

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WEBINAR

Practical Applications of Microbial Modelling Webinar Series

Webinar Series:

Part II of III

- …and by the following Professional Development Groups:
  - Microbial Modelling and Risk Analysis
  - Meat and Poultry Safety and Quality

International Association for Food Protection® WEBINAR

Practical Applications of Microbial Modelling

Webinar Series: Part II of III

International Association for Food Protection®
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Dr. Peter Taormina

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Cincinnati, OH

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This webinar is being recorded and will be available for access by IAFP members at www.foodprotection.org within one week.

Agenda

- Introduction
  - Dr. Peter Taormina
- Modelling of inactivation: models and meta-analysis
  - Dr. Marcel Zwietering
- Practical Use of Tertiary Models: I'm having a challenging food safety day….now what?
  - Dr. Betsy Booren
- Questions and Answers

Dr. Marcel Zwietering

Professor
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Olsson, Frank, Weeda, Terman, and Matz PC

Washington, DC

Modelling of inactivation: models and meta-analysis

Dr. Marcel Zwietering

Modelling of inactivation: models and meta-analysis

Marcel Zwietering & Heidy den Besten

Webinar March 5

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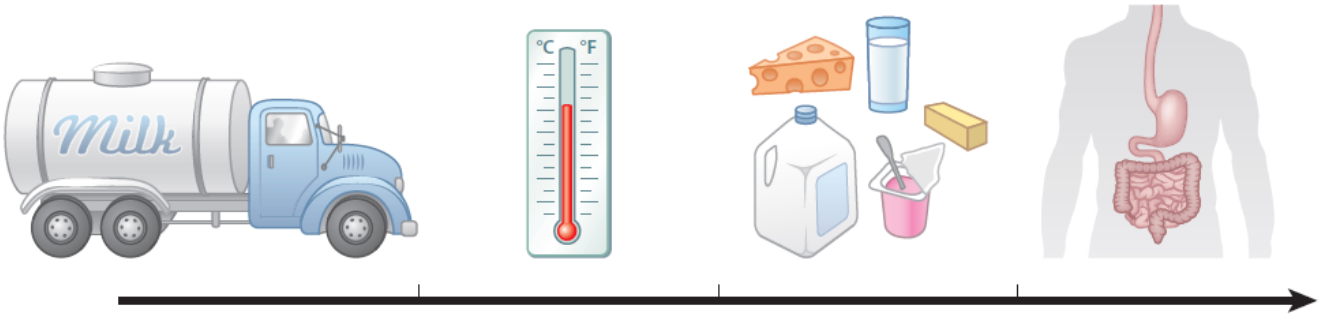
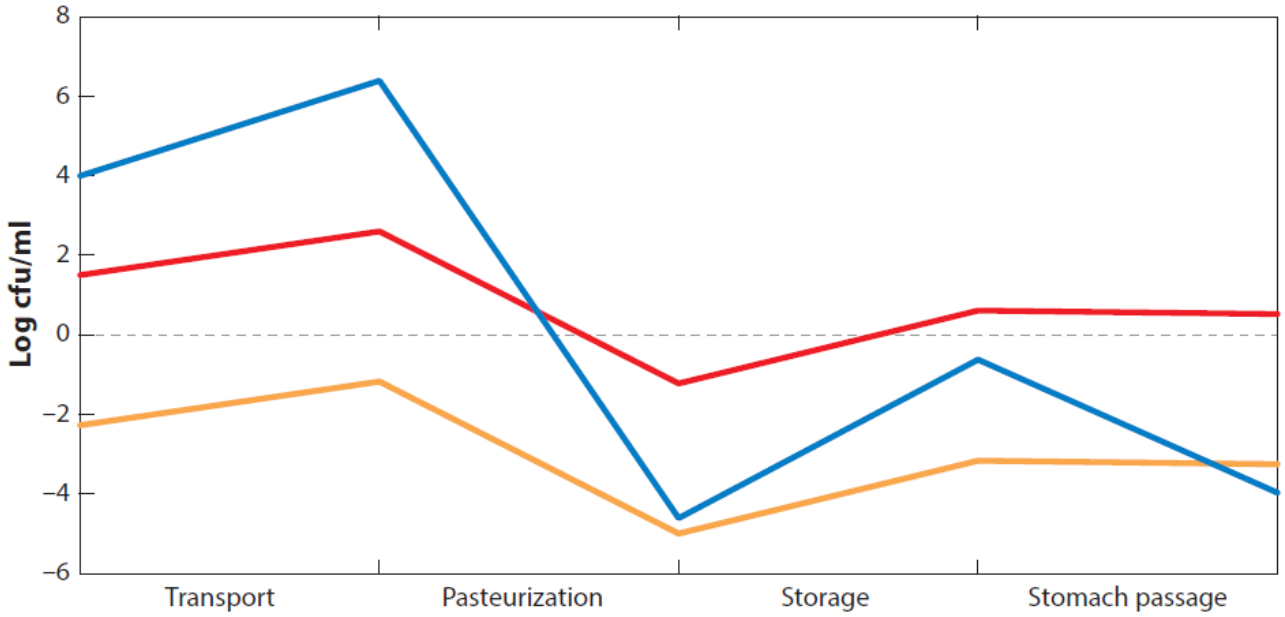
For modelling chains inactivation is relevant

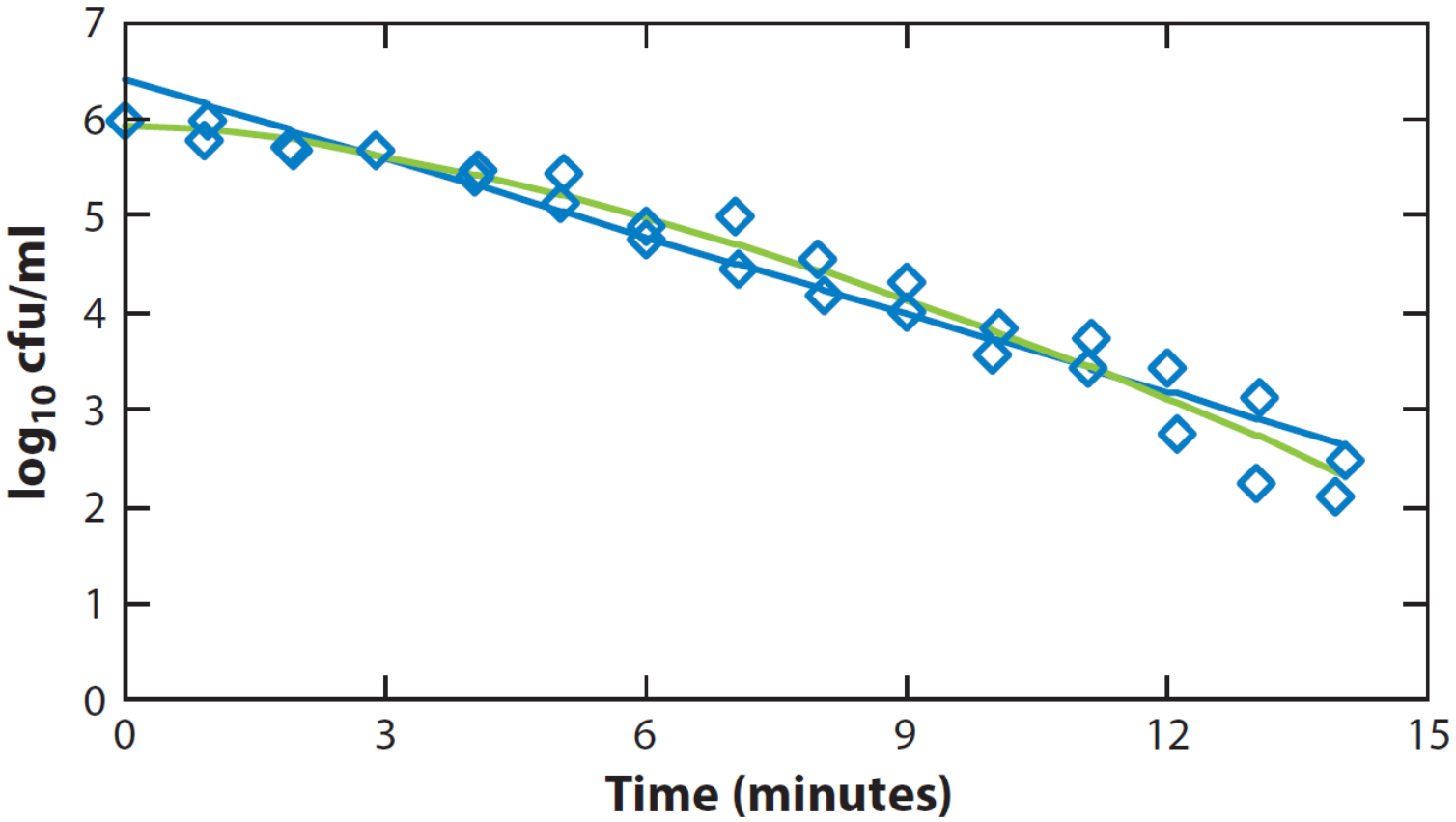




Abee et al., 2016

Primary inactivation models

$$\log_{10} N(t) = \log_{10} N(0) - \frac{t}{D}$$ Bigelow (1921)



Inactivation models: Is inactivation linear ?

$$\log_{10} N(t) = \log_{10} N(0) - \frac{t}{D}$$ Bigelow (1921)

$$\log_{10} N(t) = \log_{10} N(0) - \frac{1}{2.303}\left(\frac{t}{\alpha}\right)^{\beta}$$ Weibull (1951)

$$\log_{10} N(t) = \log_{10} N(0) - \left(\frac{t}{\delta}\right)^{\beta}$$ Mafart (2002)

$$\log_{10} N(t) = \log_{10} N(0) - \Delta\left(\frac{t}{\Delta D}\right)^{\beta}$$ Metselaar (2013)

$$\log_{10} N(t) = \log_{10} N(0) - 6\left(\frac{t}{t_{6D}}\right)^{\beta}$$

Thermal inactivation kinetics: summary (Tom's slide from webinar part I)

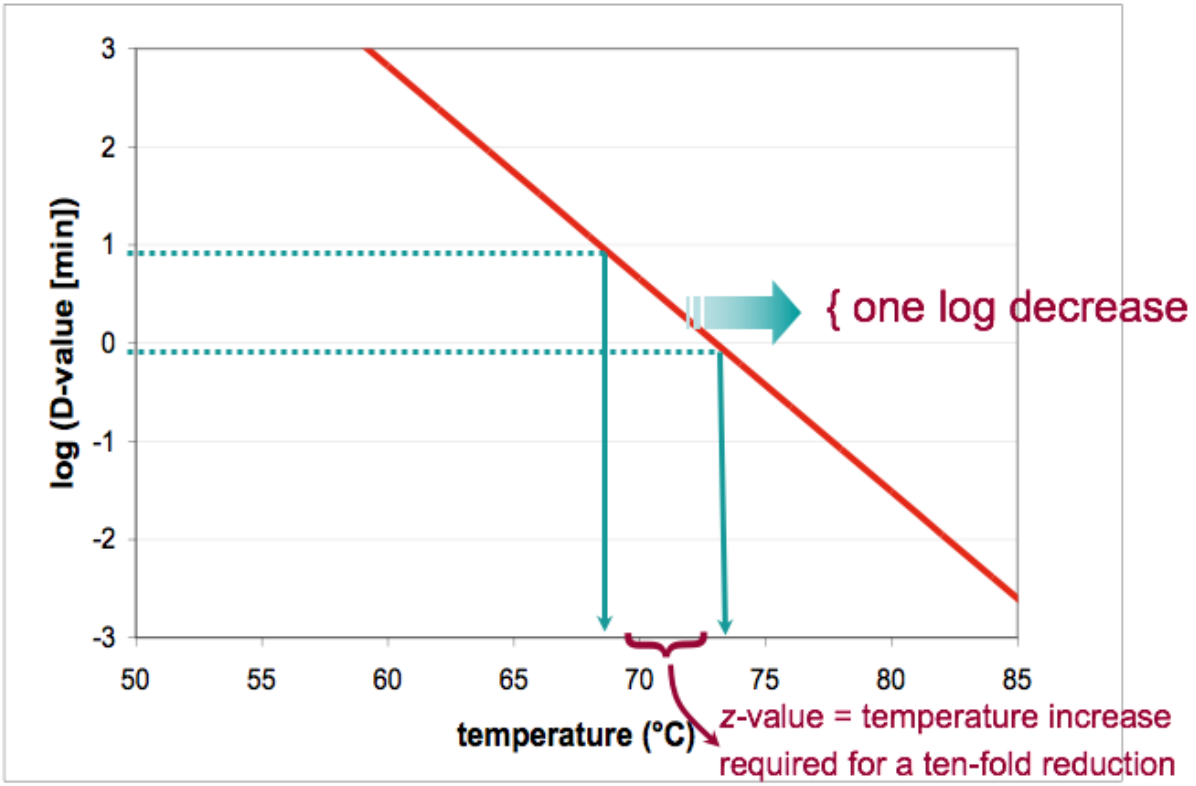
D-value

time required at given temperature to reduce microbial load by a factor of of 10

z-value

temperature increase required to reduce D-value by a factor of 10

Analogous terms (D_p, Z_p, Z_{pH}) proposed for other lethal factors



Secondary inactivation models

$$D = D_{ref}\, 10^{\frac{T_{ref}-T}{z}}$$

Mafart (2000)

$$D = D_{ref}\, 10^{\frac{T_{ref}-T}{z}} \cdot 10^{\frac{pH_{ref}-pH}{z_{pH}}} \cdot 10^{\frac{a_{w,ref}-a_w}{z_{a_w}}}$$

Effect of influencing factors

experimental error
reproduction
non-linearity
T, pH, a_w
product
population diversity
strain diversity
history

What are main effects?

Compare and Prioritize!

Laboratory conditions: practical conditions

Research Article

Extreme Heat Resistance of Food Borne Pathogens *Campylobacter jejuni, Escherichia coli*, and *Salmonella typhimurium* on Chicken Breast Fillet during Cooking

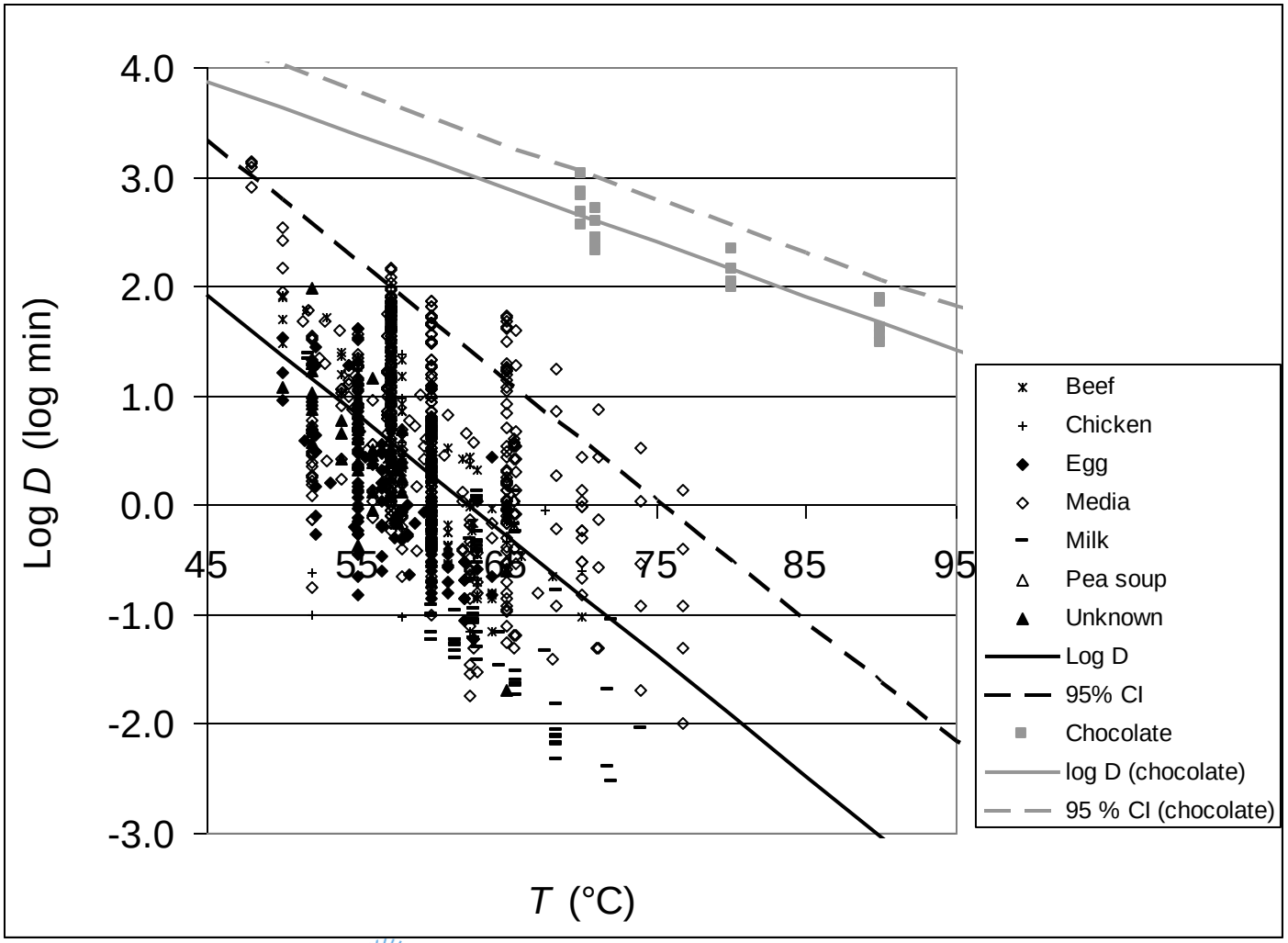
Aarieke E.I. de Jong,[1,2] Esther D. van Asselt,[1,3] Marcel H. Zwietering,[4] Maarten J. Nauta,[1,5] and Rob de Jonge[1]

Meta-analysis:D and z values micro-organisms

Table 1
Mean log D-values at reference temperature T_{ref}, upper 95% PI for log D_{ref}- and z-values for various pathogens

Micro-organism	Product	T_{ref} (°C)	z (°C)	Log D_{ref} (mean) (min)	Log D_{ref} (95% PI) (min)	σ	n
Bacillus cereus	Various	120	12.8	−1.38	−0.28	0.56	465
Listeria monocytogenes	Various	70	7.0	−1.06			
Listeria monocytogenes	Salted (10%)	70	9.2	0.18			
types (BCEF)							
Clostridium botulinum proteolytic type G	Various	120	34.0	−0.60	−0.22	0.18	24
Salmonella spp.	Various	70	9.1	−0.83			
Salmonella spp.	Chocolate	70	20.4	2.65			
Listeria monocytogenes	Salted (10%)	70	9.2	0.18	0.78	0.29	27
Salmonella spp.	Various	70	9.1	−0.83	0.59	0.72	1141
Salmonella spp.	Chocolate	70	20.4	2.65	3.04	0.19	20
Staphylococcus aureus	Various	70	8.8	−0.59	0.33	0.47	204
Streptococcus pyogenes	Various	70	9.2	−1.45	−0.15	0.57	11
Vibrio cholerae	Crabmeat	70	16.7	−0.25	0.34	0.19	5
Vibrio cholerae	Peptone water	70	21.8	−0.72	−0.48	0.05	4
Vibrio parahaemolyticus, *Vibrio vulnificus*	Various	70	8.5	−2.24	−1.30	0.46	34
Yersinia enterocolitica	Various	70	6.2	−1.80	−0.91	0.44	63

Meta-analysis:D and z values micro-organisms



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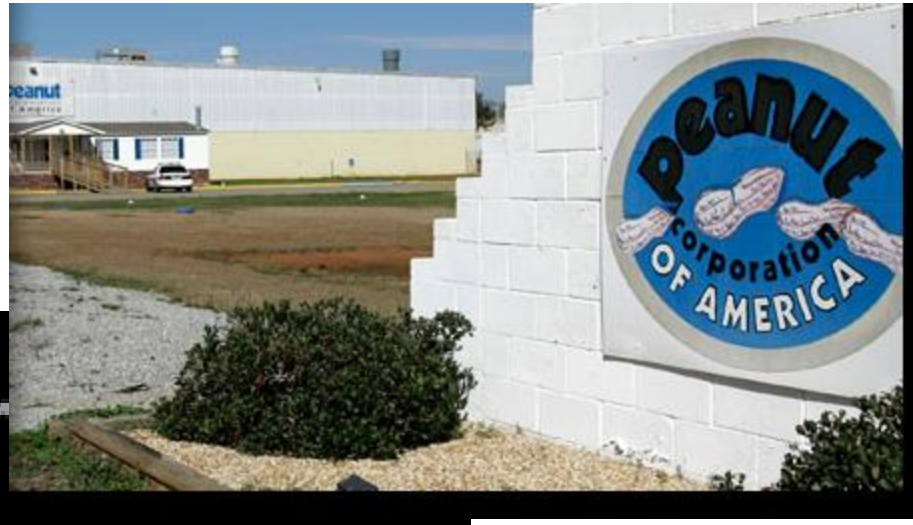
A systematic approach to determine global thermal inactivation parameters for various food pathogens

Esther D. van Asselt [1], Marcel H. Zwietering *

Meta-analysis: Bench marking



2008-09: Peanut butter

Salmonella typhimurium in peanut butter and peanut paste.

Salmonella sickens more than 500 people in 43 states and possibly kills eight. The outbreak is linked to contaminated peanut butter and peanut paste shipped from the Blakely, Ga., plant of the Peanut Corporation of America. A Food and Drug Administration preliminary investigation found that it had products that tested positive for salmonella retested until they were negative and then shipped them to customers on at least

Journal of Food Protection, Vol. 72, No. 8, 2009, Pages 1596–1601

Thermal Inactivation of *Salmonella* in Peanut Butter

LI MA,[1] GUODONG ZHANG,[1] PETER GERNER-SMIDT,[2] VIJAYA MANTRIPRAGADA,[1] IFEOMA EZEOKE,[1] AND MICHAEL P. DOYLE[1]*

TABLE 1. *D-values and z-values of* Salmonella *in peanut butter at 71, 77, 83, and 90°C*

Temp (°C)	Outbreak isolates
71	29.3 ± 2.9 (0.85) A a
77	21.0 ± 4.2 (0.98) B a
83	16.0 ± 0.7 (0.93) C ab
90	13.4 ± 0.9 (0.91) C a
z-values (°C)	55.9 (0.97)

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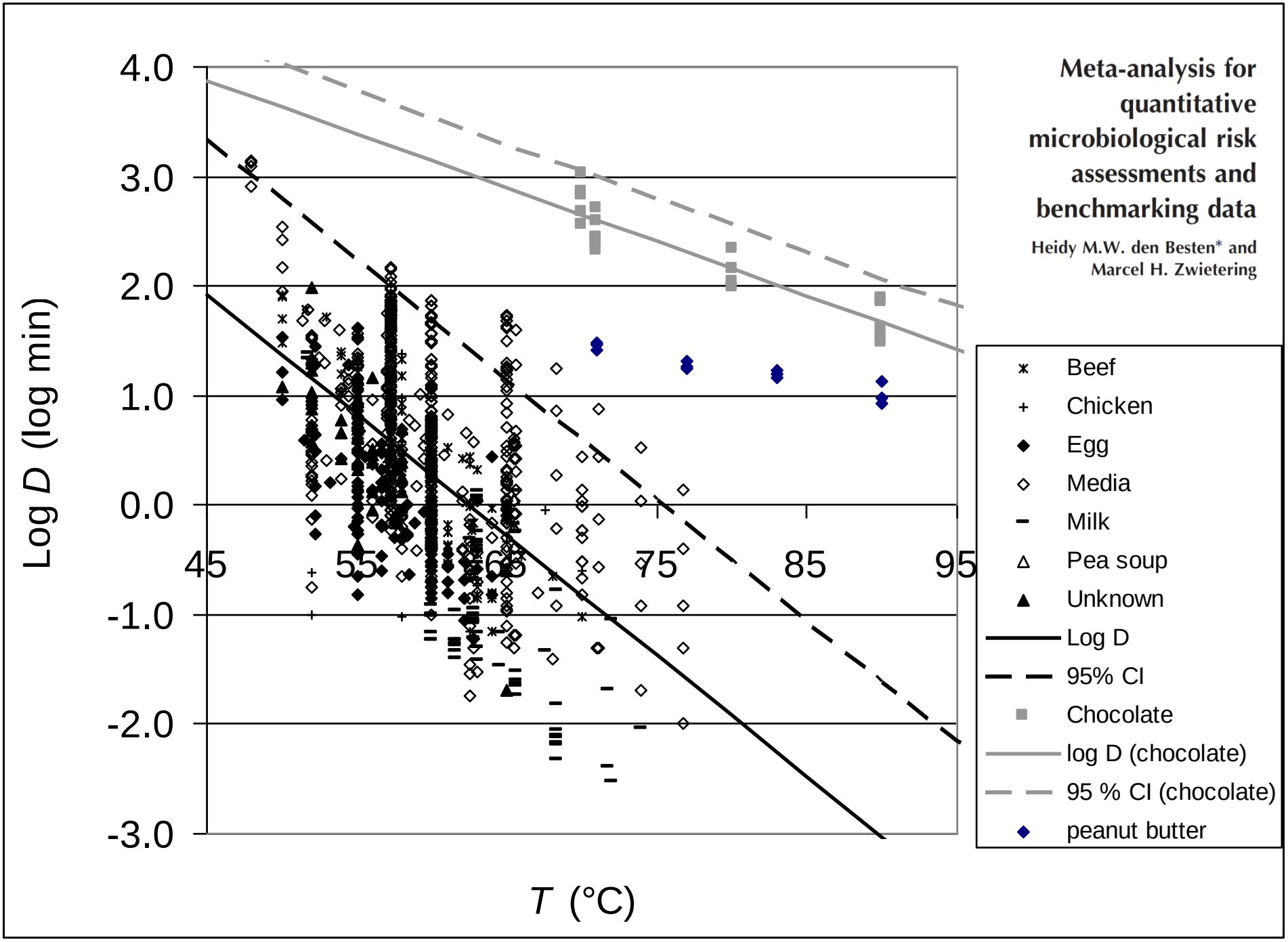
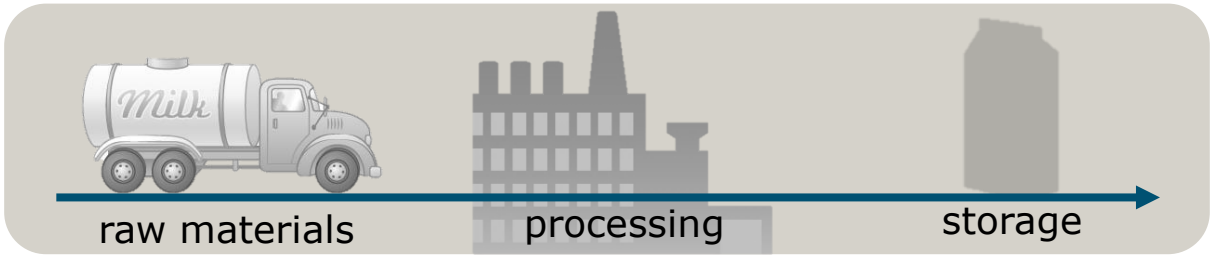

Meta-analysis for quantitative microbiological risk assessments and benchmarking data
Heidy M.W. den Besten* and Marcel H. Zwietering
Log D (log min)
T (°C)
4.0
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
45
55
65
75
85
95
Beef
Chicken
Egg
Media
Milk
Pea soup
Unknown
Log D
95% CI
Chocolate
log D (chocolate)
95 % CI (chocolate)
peanut butter

Comparison of variability sources



- Why quantification of variability?
  - Rank importance
  - Realistic prediction

sources

experimental

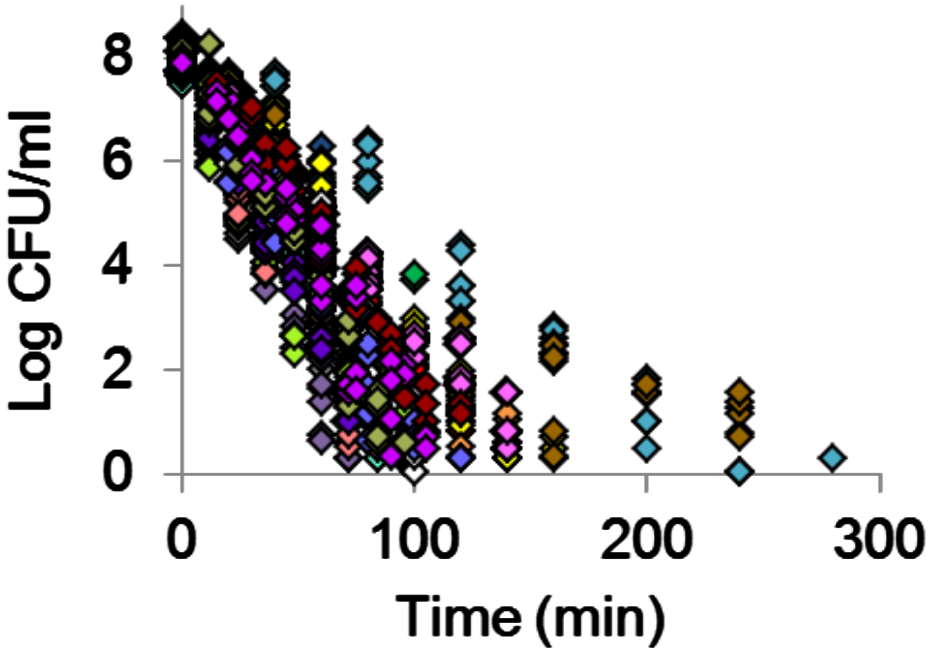
reproduction

strain

history

heterogeneity

Variability between strains



- Experimental
- Biological
- Strain

Contents lists available at ScienceDirect

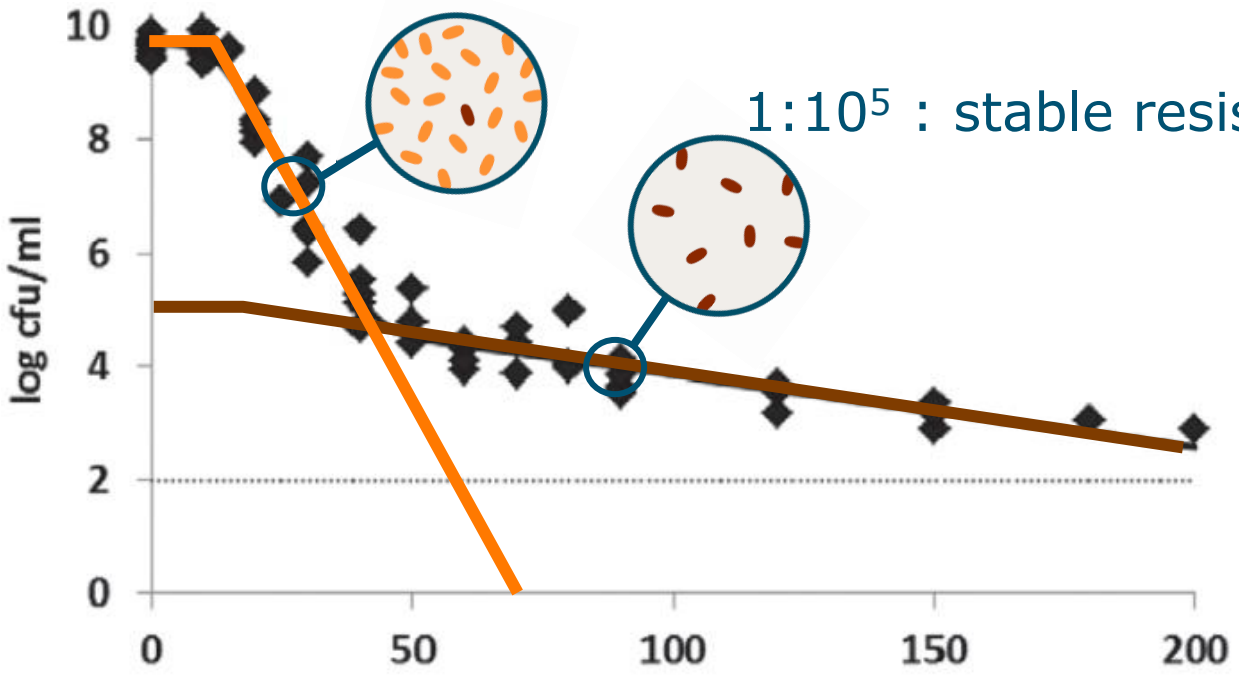
International Journal of Food Microbiology

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

Quantifying variability on thermal resistance of *Listeria monocytogenes*

D.C. Aryani [a,b], H.M.W. den Besten [a,b,*], W.C. Hazeleger [b], M.H. Zwietering [a,b]

Modelling and investigating mechanisms



Good fitting of biphasic model points to population heterogeneity

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Isolation and quantification of highly acid resistant variants of *Listeria monocytogenes*

Karin I. Metselaar [a,b,c], Heidy M.W. den Besten [b], Tjakko Abee [a,b], Roy Moezelaar [a,c,1], Marcel H. Zwietering [a,b,*]

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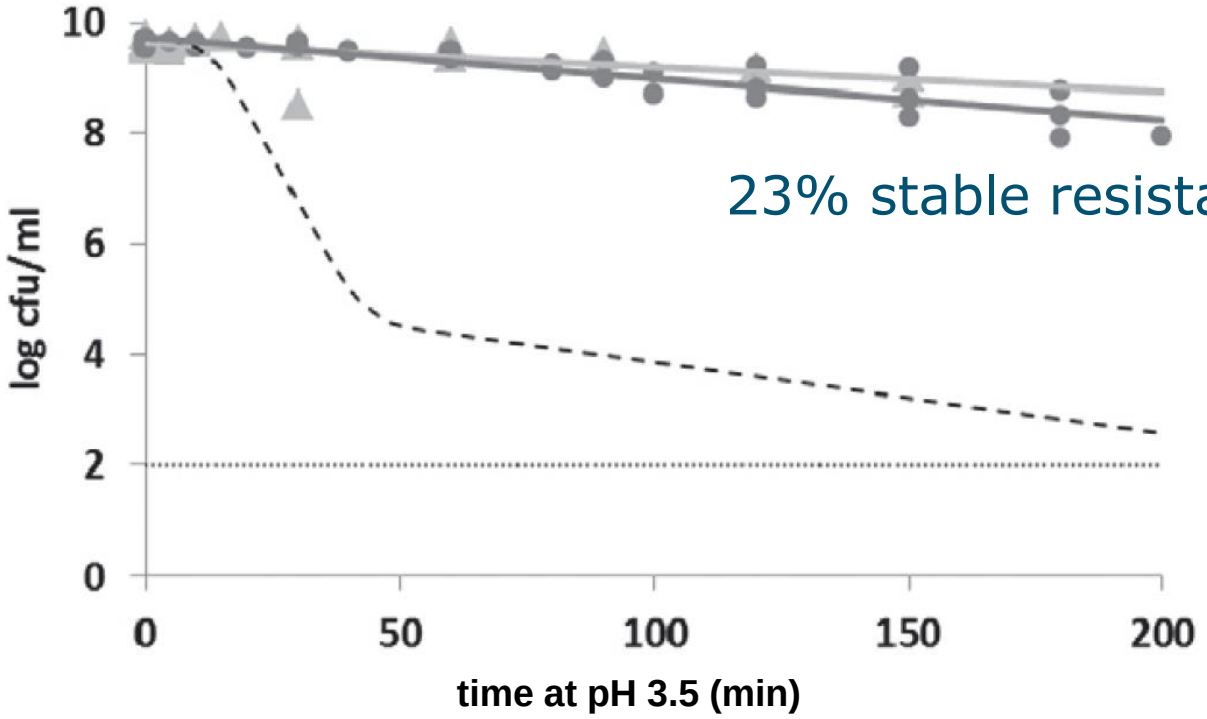
Modelling and investigating mechanisms



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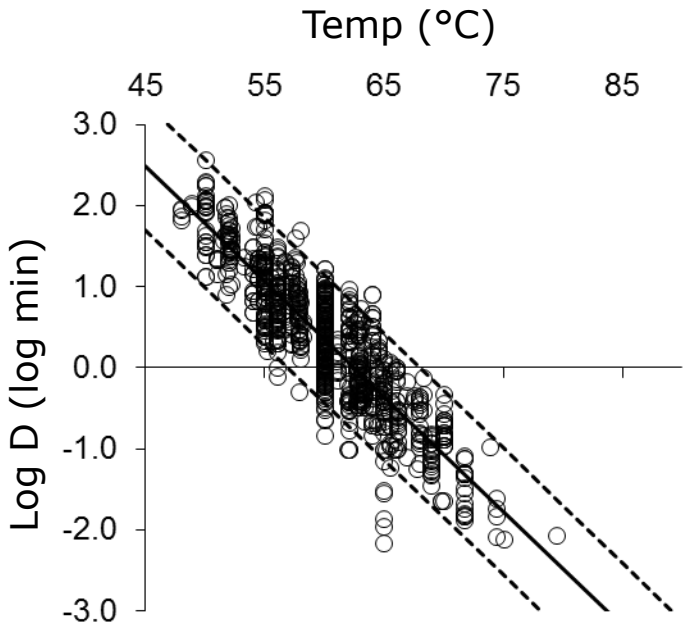
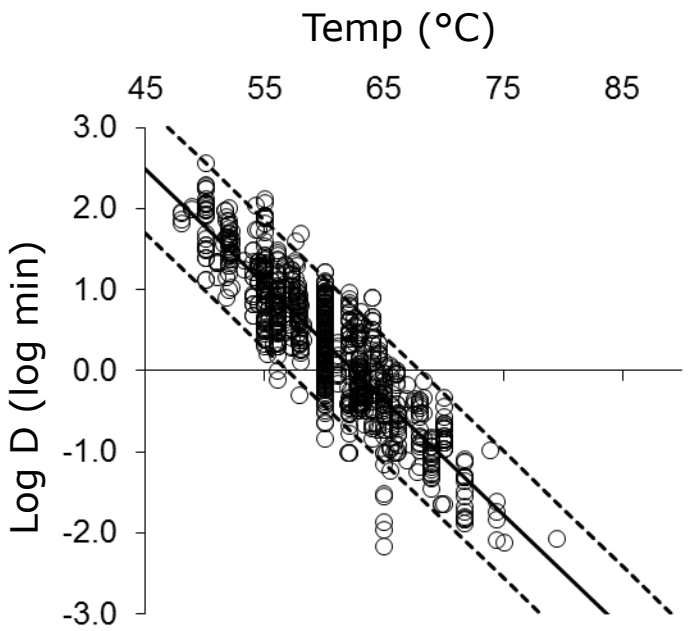
Benchmarking

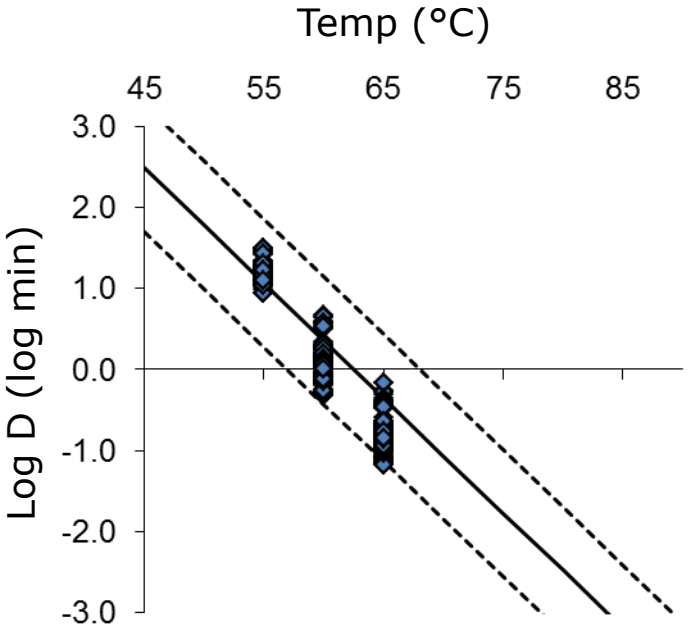


Van Asselt & Zwietering, 2006

Benchmarking

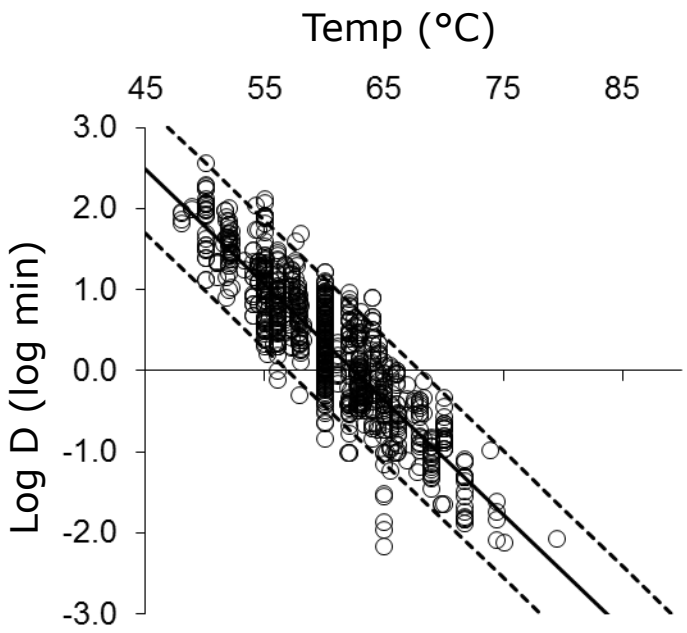


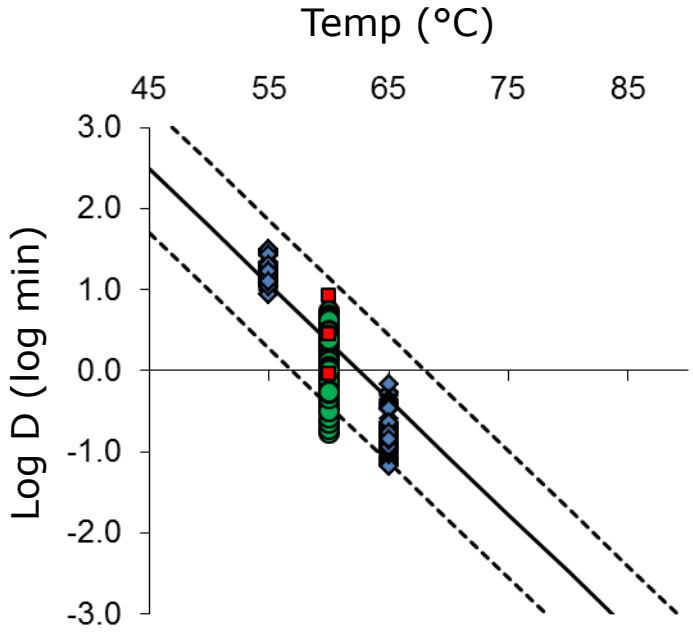
Van Asselt & Zwietering, 2006



20 strains

Benchmarking



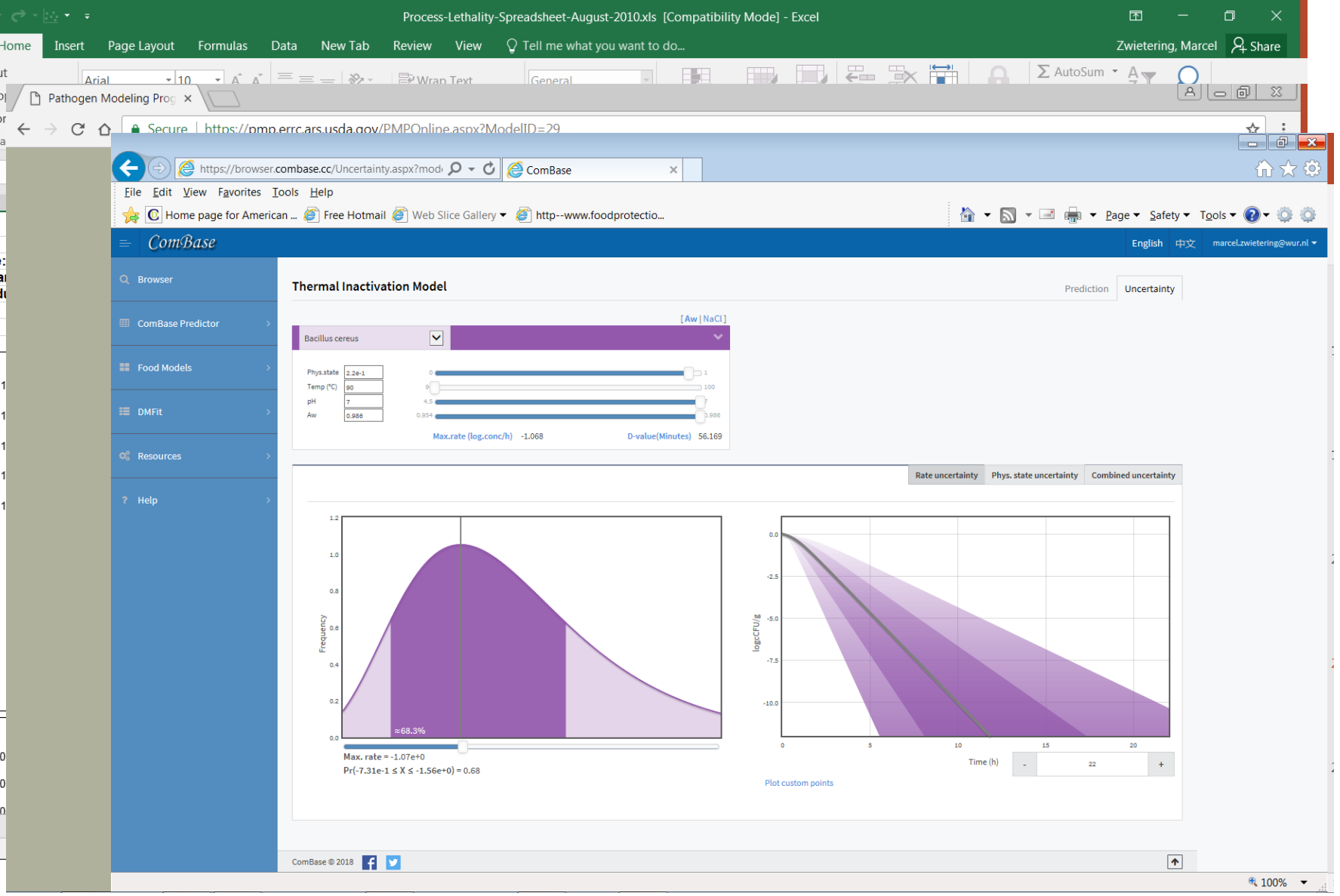


History: pH, aw, low T, growth phase
LO28 and variants

- All variability as found in literature: fail–safe extremes
- Indeed, these extremes can be easily encountered

Predictive modeling tool example

- Foundation for Meat and Poultry Research and Education's Process Lethality Determination Spreadsheet (Formerly AMI Foundation)
  - http://meatpoultryfoundation.org/content/process-lethality-spreadsheet
- PMP
  - https://pmp.errc.ars.usda.gov/default.aspx
- Combase
  - https://www.combase.cc



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Conclusion

model reparameterisation and comparison

meta-analysis

experimental error
reproduction
non-linearity
T, pH, aw
product
population diversity
history
strain diversity

"All models are wrong …… some are useful"

Many models are correct …… but they are not perfect

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Practical Use of Tertiary Models

I'm having a challenging food safety day….now what?

Dr. Betsy Booren

Why Have We Chosen This Approach?

- Discussions after the last webinar led organizers to develop this "practical example".
  - This is a completely fictional situation.
  - Any similarity from actual food safety events is purely coincidental.

- The intent to demonstrate how predictive modeling can be used by the food industry.

Review: Types of Tertiary Models

- Bacterial Transfer
- Survival
- Growth
- Inactivation

Review: Available Tools

- Baseline Software Tool
- Bioinactivation SE
- ComBase Predictor
- Dairy products safety predictor
- DMRI – predictive models for meat
- E. coli Inactivation in Fermented Meats Model
- EcSF – E. coli SafeFerment
- FDA-iRISK®
- Food Spoilage and Safety Predictor (FSSP)
- *FISHMAP*
- GroPIN
- Listeria Control Model 2012
- Listeria Meat Model
- Microbial Responses Viewer (MRV)
- MicroHibro: Predictive Models
- MLA Refrigeration Index Calculator
- PMM-Lab
- Process lethality determination spreadsheet
- Perfringens Predictor
- Praedicere
- Salmonella predictions
- Shelf Stability Predictor
- SWEETSHELF
- Sym'Previus
- Therm 2.0

Some Examples of Predictive Models

- USDA Pathogen Modeling Program
  - https://pmp.errc.ars.usda.gov
- ComBase
  - https://www.combase.cc
- DANISH MEAT RESEARCH INSTITUTE PREDICTIVE MODELS FOR MEAT
  - http://dmripredict.dk

Situation

- Food Company XYZ produces the following product:
  - Fully cooked breaded stuffed pork cutlet
    - Comminuted pork meat
    - Stuffed with Swiss cheese and spinach mixture (pH 5.6)
    - Breaded
    - Cooked on a continuous impingement oven
    - Temperature at geometric center reaches 170°F (73.8°C)
      - Actual product temperature at geometric center is 170°F (73.8°C) for 1.5 minutes.
    - Frozen individually, packaged
    - Has validated reheating instructions
    - Frozen shelf-life of 8 months.

Situation

- Establishment has determined their cooking process provides a 5-log reduction of *Salmonella*
  - The establishment based this determination on supplier history of pork products and ongoing verification activities support that heating process will provide 5-log reduction of *Salmonella* during the cooking process.
- Company has conducted oven validation studies to determine critical parameters for oven settings to achieve the 5-log reduction of *Salmonella*
  - Using FSIS's Appendix A as additional scientific support the Time/Temperature parameters indicate that a greater than 5 log lethality of *Salmonella* is achieved

Situation

- This is a FSIS regulated product, so RTE products are considered adulterated if they are contaminated with *L. monocytogenes*.

Situation

- Food Company XYZ was notified by Supplier ABC that Swiss cheese and spinach mixture produced on Day XX may be exposed with *Listeria monocytogenes*.
  - Food Contact Surface was found to be positive for *L. monocytogenes*

- Food Company XYZ's Food Safety Team begins process of identifying product that may have contained the exposed Swiss cheese and spinach mixture.
  - Identifies 1 day of production potentially affected, but only 1 shift is currently in commerce.

Food Company XYZ Investigation

Disclaimer

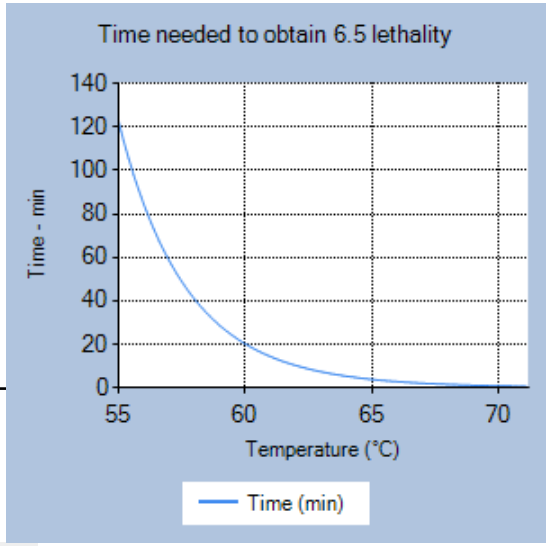
- In this scenario, I am only focusing on the scientific thought process…there are other regulatory requirements that may need to be met, but are not being discussed during this webinar. I am only focusing on the use of predictive modeling with that limited view of information.

Next Steps

- Food safety team has notified customer with the potential contaminated product and has them hold the product.
  - The in-commerce product is at a customer's 3rd Party Cold Storage Facility
  - Food Safety Team has product on hold brought back to their establishment.
- Food safety team determines modeling, among other activities, is needed to provide scientific evidence that product is safe and wholesome, and meets regulatory RTE requirements.

USDA Pathogen Modeling Program

- Used Heat Inactivation Model
  - *Salmonella* – ground beef
    - No sodium lactate or sodium diacetate



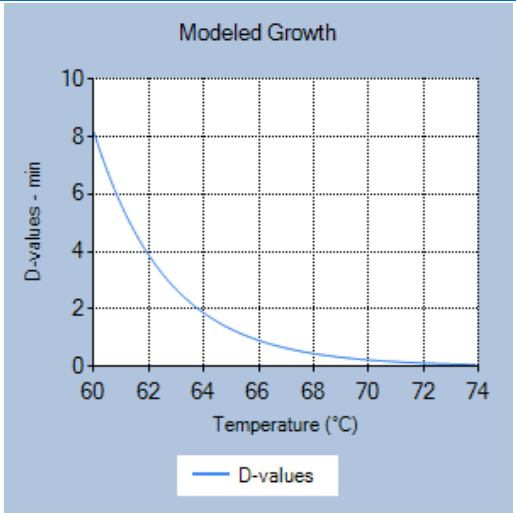
MODELED INACTIVATION

Temperature (°C)	Time for 6.5 lethality (min)
70.9	0.63
71.0	0.61
71.1	0.60

- Actual product temperature at geometric center is 74°C for 1.5 minutes.

USDA Pathogen Modeling Program

- Used Heat Inactivation Model
  - *L. monocytogenes* – ground beef
    - No sodium lactate or sodium diacetate



MODELED INACTIVATION	
Temperature (°C)	D-value (min)
73.7	0.06
73.8	0.06
73.9	0.06

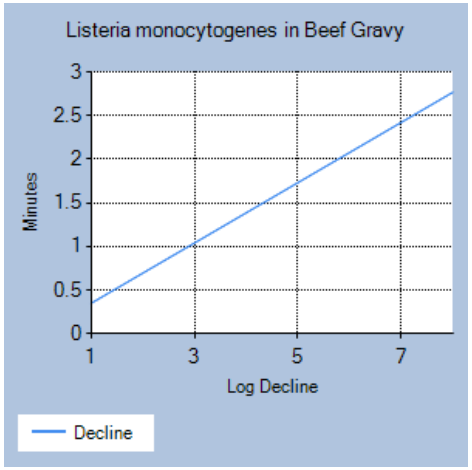
  - Assume 5-log reduction is adequate for safety, approximately 0.3 minutes are needed to achieve a 5 log reduction.
    - Actual product temperature at geometric center is 74°C for 1.5 minutes.

USDA Pathogen Modeling Program

- Used Heat Inactivation Model
  - *L. monocytogenes* – simulated beef gravy
    - pH: 5.6; Temp Range: 65°C; No salt or phosphate;
    - Log Reduction: 5.0

MODELED DECLINE

Log Decline	Minutes
1.00	0.35
2.00	0.69
3.00	1.04
4.00	1.39
5.00	1.73



USDA Pathogen Modeling Program

- Used Heat Inactivation Model

Assume 5-log reduction is adequate for safety

  - Approximately 1.73 minutes at 65°C with pH at 5.6 are needed to achieve a 5 log reduction.
    - Actual product temperature is 74°C for 1.5 minutes.
    - Would need to extrapolate the model

Time out

Model Selection

- In my experience, this is a common process taken as the food product type doesn't fit exact model parameters
- Intent was to demonstrate challenges of selecting the right model.

- Another approach, would be to select a model that doesn't specify a food matrix and use other critical parameters of the food (e.g. pH, water activity, salt concentration, fat level) for modeling parameters

Time In

Food Company XYZ - Now What?

- Confusing, conflicting data.
- Food safety team engages with a process authority to review modeling data, internal oven validation studies, and formulation data.
  - Conclusion, the food matrixes in the predictive modeling tools were not precise to the food being evaluated.
  - Other modeling using intrinsic properties of food were conducted.
  - Oven validation studies demonstrated internal temperatures were accurate under conditions the study were conducted.
    - Food Safety Team has reviewed records and oven conditions during production were the same as oven validation study.

Food Company XYZ - Now What?

- Food Safety Team believes evidence supports cooking process did eliminate any potential contamination from the Swiss cheese and spinach mixture.
- In abundance of caution, Food Safety Team has decided to do product testing.
  - International Commission on Microbiological Specifications for Foods (ICMSF) Sampling Plans for *L. monocytogenes*: Case 11
    - Testing Results were all negative.

Food Company XYZ - Now What?

- Product is deemed safe and could enter commerce

- Reminder: In this scenario, I am only focusing on the scientific thought process…there are other regulatory requirements that may need to be meet, but are not being discussed during this webinar. I am only focusing on the use of predictive modeling with that limited view of information.

Food Company XYZ - Closing Out the Internal Investigation

Review of Food Safety Event

- Food Company XYZ internal policy is to bring together Food Safety Team to do a review of food safety event and do a "lessons learned"
- During review, questions are raised regarding favorable conditions regarding staphylococcal enterotoxin growth in batter for the breading
- Currently, batter temperature is monitored and following critical limits are set:
  - Hydrated batter mix should not be held for more than 8 hours, cumulatively, at temperatures between 50°F (10°C) and 70°F (21.1°C); and
  - Hydrated batter mix should not be held for more than 3 hours, cumulatively, at temperatures above 70°F (21.1°C).

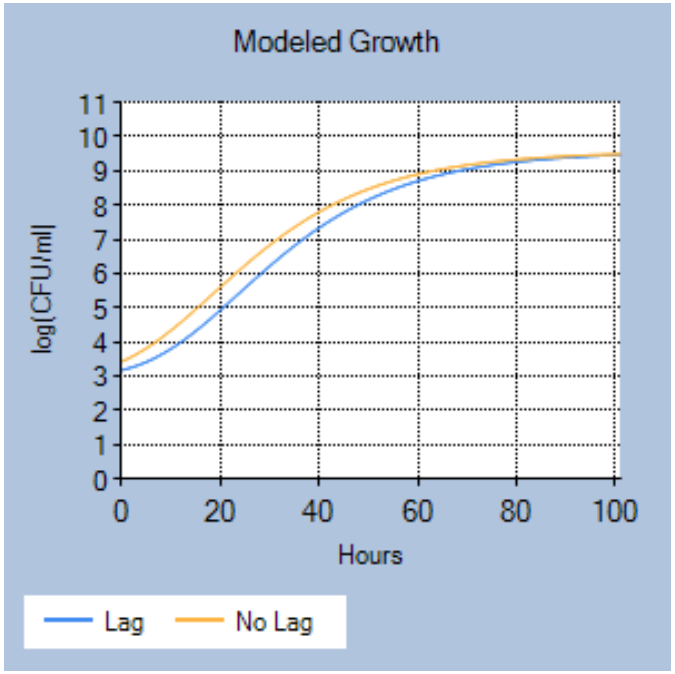
Review of Food Safety Event

- Hydrated batter is held in jacketed tank during production day and them pumped for application to meat product.
- Concern was raised that could the temperature of certain equipment surfaces may cause a situation where hydrated batter mix was held in conditions favorable for staphylococcal enterotoxin development.
  - Could lead to contamination of product prior to cooking.

USDA Pathogen Modeling Program

- Growth Model: *Staphylococcus aureus* (Broth Culture, Aerobic)
  - Aerobic conditions; Temperature: 21.0°C (69.8°F); pH: 6.0; Sodium Chloride: 2.5 %; and Initial Load: 3 log (CFU/mL)

Modeled Growth Parameters
Lag Phase Duration: 5.18 (hours)
Generation Time: 2.30 (hours)
Growth Rate: 0.131 (log(cfu/ml)/h)
Max Population Density: 9.57(log(cfu/ml))



USDA Pathogen Modeling Program

- Growth Model: *Staphylococcus aureus* (Broth Culture, Aerobic)
  - Aerobic conditions; Temperature: 21.0°C (69.8°F); pH: 6.0; Sodium Chloride: 2.5 %; and Initial Load: 3 log (CFU/mL)

MODELED GROWTH

Hours	log(CFU/ml)	
	Lag	No Lag
13.60	4.17	4.79
13.80	4.19	4.81
14.00	4.22	4.84
14.20	4.24	4.86
14.40	4.26	4.89
14.60	4.28	4.91
14.80	4.31	4.94
15.00	4.33	4.96
15.20	4.35	4.99
15.40	4.37	5.01

USDA Pathogen Modeling Program

- Growth Model: *Staphylococcus aureus* (Broth Culture, Aerobic)
  - Aerobic conditions; Temperature: 29.4°C (85°F); pH: 6.0; Sodium Chloride: 2.5 %; and Initial Load: 3 log (CFU/mL)

MODELED GROWTH

Hours	log(CFU/ml)	
	Lag	No Lag
3.60	3.86	4.39
3.80	3.91	4.46
4.00	3.97	4.53
4.20	4.03	4.60
4.40	4.09	4.67
4.60	4.15	4.74
4.80	4.22	4.81
5.00	4.28	4.88
5.20	4.35	4.96
5.40	4.41	5.03

USDA Pathogen Modeling Program

- Growth Model: *Staphylococcus aureus* (Broth Culture, Aerobic)
  - Aerobic conditions; Temperature: 42°C (107.6°F); pH: 6.0; Sodium Chloride: 2.5 %; and Initial Load: 3 log (CFU/mL)

MODELED GROWTH

Hours	log(CFU/ml)	
	Lag	No Lag
0.00	3.06	3.43
0.20	3.08	3.50
0.40	3.10	3.57
0.60	3.13	3.65
0.80	3.16	3.74
1.00	3.19	3.83
1.20	3.23	3.93
1.40	3.28	4.03
1.60	3.33	4.14
1.80	3.39	4.25
2.00	3.45	4.36
2.20	3.52	4.48
2.40	3.59	4.61
2.60	3.67	4.73
2.80	3.76	4.86
3.00	3.85	4.99

Now what?

- Predictive Modeling indicates in certain scenarios a possibility that staphylococcal enterotoxin may develop.
- Food Safety Team is reanalyzing Food Safety Program to address this issue.

Summary

- Predictive Modeling is a valuable tool for the food industry to use.
  - It can be used in a variety of situations to access food safety risk.
  - It is important to understand the limitations of predictive modeling to make the best food safety assessment.

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QUESTIONS & ANSWERS

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Q Laboratories

Practical Applications of Microbial Modelling Webinar Series

Future Sessions

- ✔ Part I – Overview & Practical Applications
  - November 29, 2017 (Q&A Document Now Available!)
    https://www.foodprotection.org/upl/downloads/library/qa-11-29-webinar.pdf
- ✔ Part II – Inactivation
  - March 5, 2018
- ☐ Part III – Risk Modeling
  - Spring 2018