

Comparison of Mathematical Approaches to Predicting Pathogen Growth During Short-Term Temperature Abuse of Raw Meat and Poultry Products

Darand L. Borneman and Steven C. Ingham
University of Wisconsin-Madison



Introduction:

- Since 2000, American wholesale meat and poultry processors have used the mandatory Hazard Analysis Critical Control Point (HACCP) system for ensuring food safety.
- For raw meat processing, growth of pathogens such as *Salmonella* serovars, *Escherichia coli* O157:H7, and *Staphylococcus aureus* is a hazard that is reasonably likely to occur.
- In many small and very small plants with un-refrigerated raw-meat processing areas, the temperature at the processing step designated as the CCP may be high enough to constitute short-term temperature abuse.
- Raw products may be exposed to short-term temperature abuse during common processing steps, e.g. grinding, CCP deviations, and processing/ scheduling problems.
- If deviation from a critical limit occurs, the processor must take corrective action that is supported by scientifically valid information.
- Previously we developed a computer-based tool for predicting pathogen behavior in raw pork, beef, and poultry during short-term temperature abuse called **THERM** (Temperature History Evaluation for Raw Meats).
- THERM v. 2, available at <http://www.meathaccp.wisc.edu/THERM/calc.aspx>, is based upon **linear interpolation** between experimentally determined pathogen lag-phase duration (LPD) and growth rate (GR) values. It uses an interval accumulation technique with entered time/temperature pairs to predict the behavior of *Salmonella* serovars, *E. coli* O157:H7, and *Staphylococcus aureus* in raw meat and poultry products.

Objective:

- This study was done to optimize THERM accuracy for predicting growth of *Salmonella* serovars, *Escherichia coli* O157:H7, and *Staphylococcus aureus* in temperature-abused raw meat and poultry.
- We compared the existing THERM v. 2 with other THERM versions utilizing different mathematical methods for determining LPD and GR values.

Materials and Methods:

Inoculum Preparation:

- Ten-strain cocktail of *Salmonella* spp. and *E. coli* O157:H7 resuspended in Butterfield's phosphate diluent (BPD).
- Five-strain cocktail of *Staphylococcus aureus* resuspended in BPD.

Preparation and Inoculation of Meat Products:

- Isothermal studies were conducted at either 5° or 10°F intervals ranging from 50° to 115°F (10 – 46.1 C) with ground beef, ground turkey, or bratwurst mix.
- Ground meat (ca. 25g) was weighed into plastic sample bags and allowed to reach temperature either in a static water bath or incubator. A thermocouple was inserted into a single sample to monitor meat temperature.
- When test temperature was reached, each bag of sample, except that containing the thermocouple, was inoculated with 100 µL of either the combined or *S. aureus* inoculum, closed, manually massaged for approximately 20 s, and returned to the test temperature as quickly as possible (< 5 min).
- Three concurrent trials were conducted for each temperature with separate inocula prepared for each trial.

Determination of Experimental LPD and GR Values

- At each sampling time, 3 bags of meat per inoculum type (one per trial) were analyzed.
- Bag contents and the everted sampling bag were aseptically transferred to a filter bag, combined with 99 mL of BPD, and stomached for 30s.
- Serial dilutions were prepared and 100 µL of each appropriate dilution was plated on each appropriate selective medium: XLD agar (Oxoid) – *Salmonella* serovars, Sorbitol MacConkey agar (Difco) – *E. coli* O157:H7, or Baird-Parker agar with tellurite egg yolk supplement (Difco) – *Staphylococcus aureus*.
- XLD and Sorbitol MacConkey agar plates were incubated at 35°C for 24 hours. Baird-Parker plates were incubated at 35°C for 48 hours.
- For each pathogen, product, and test temperature, log CFU/ sample was determined at each sampling time. Data for the 3 trials was combined and entered into DMFit software (J. Baranyi, Institute of Food Research) to yield LPD, GR, and R² values.

Best-fit Curves for LPD and GR

- The experimental LPD values were utilized to calculate **quadratic equations** (Mini-Tab), **piece-wise linear regression equations** (R-software), and **exponential decay equations** (R-software) to fit the data. Corresponding best-fit **quadratic equations** and **piece-wise linear regression equations** were constructed using GR values. Examples of equation coefficients for raw ground beef are shown in **Tables 1, 2, and 3, respectively**.

Product	Pathogen	LPD/ GR	a	b	c	R2 values
Beef	EC	LPD	0.702	-127.6	5761	0.77
		GR	0.000008	-0.000899	0.025930	0.96
	S	LPD	1.265	-226.4	9985	0.70
		GR	0.000005	-0.000407	0.009349	0.85
	SA	LPD	0.435	-86.99	4445	0.86
		GR	-0.000004	0.000987	-0.046330	0.93

Table 1: Coefficients for **quadratic equations** fitted to observed LPD and GR values for *Salmonella* serovars (SALM), *E. coli* O157:H7 (EC), and *S. aureus* (SA) in raw ground beef. Format of equation is LPD or GR = ax² + bx + c, where x is temperature in °F.

Product	Pathogen	Intercept (LPD units)	Temperature Range (°F)	Slope Range (SR) (°F)	Slope (LPD units)	R ²
Beef	EC	212.4	50 - 110 °F	SR ≤ 55°F	-178.15	0.99
				55°F ≥ SR ≤ 65 °F	-34.79	
				SR ≥ 65°F	-3.98	
	SALM	210.3	50 - 110 °F	SR ≤ 55°F	-349.62	0.99
				55°F ≥ SR ≤ 65 °F	-55.04	
				SR ≥ 65°F	-3.32	
SA	330.8	65 - 110 °F	SR ≤ 70°F	-84.03	0.96	
			SR ≥ 70°F	-6.67		

Table 2: Coefficients for **piecewise linear regression equations** fitted to experimentally determined LPD values for *Salmonella* serovars (SALM), *E. coli* O157:H7 (EC), and *S. Aureus* (SA) in raw ground beef.

Product	Pathogen	lag (min) (minutes)	lag (exp) (minutes)	T (min) (oF)	half-temp	R2 values
Beef	EC	110.6	1603.1	50	5.005	0.98
	SALM	134.6	2767.3	50	3.884	0.99
	SA	127.1	719.9	65	7.858	0.92

Table 3: Coefficients for **exponential decay equations** fitted to experimentally determined LPD values for *Salmonella* serovars (SALM), *E. coli* O157:H7 (EC), and *S. aureus* (SA) in raw ground beef. Lag (min) is the calculated minimum lag time. Lag (exp)₀ is the calculated LPD value at the lowest experimental temperature at which growth was observed. T (min) is the lowest experimentally tested growth temperature. Half-temp is the calculated half-life value in units of decreasing minutes per increase in 1°F.

Versions of the THERM Predictive Tool

- The original THERM tool (v2.0) utilized linear interpolation between experimental LPD and GR values to determine LPD and GR values for entered temperatures. New THERM versions utilized quadratic equations, piecewise linear regression equations, or exponential decay curve equations to calculate LPD or GR for each temperature entered (see **Table 4**).

Version	Method of LPD Calculation	Method of GR Calculation
2.0	Linear Interpolation	Linear Interpolation
2.3	Linear Interpolation	Quadratic Equation
2.4	Linear Interpolation	Linear Regression
3.0	Quadratic Equation	Quadratic Equation
3.2	Quadratic Equation	Linear Interpolation
3.4	Quadratic Equation	Linear Regression
4.0	Linear Regression	Linear Regression
4.2	Linear Regression	Linear Interpolation
4.3	Linear Regression	Quadratic Equation
5.2	Exponential Decay Curve	Linear Interpolation
5.3	Exponential Decay Curve	Quadratic Equation
5.4	Exponential Decay Curve	Linear Regression

Table 4: Different Versions of THERM

- Each THERM version was bounded by the minimum LPD and maximum GR determined experimentally, and a GR minimum value of zero.
- Each THERM version used an interval accumulation strategy to first calculate LPD and then, after lag phase ended, to predict the total log CFU of pathogen growth.

Validation Testing of THERM Predictive Tool Versions

- 26 different beef (12), bratwurst/ breakfast link (6), and poultry product (8) inoculation experiments were conducted.
- Experiment temperature-abuse times ranged from 180 to 900 minutes and temperatures ranged from -3.3 to 101.9°F. These conditions resulted in observed pathogen growth ranging from 0.0 to 5.4 log CFU.
- For each experiment 20 time/temperature pairs from within the known pathogen growth-temperature range were entered into each THERM version to obtain predicted growth values. Plating of samples (see above) was done to obtain observed growth values.
- A mean observed Δ log CFU value was calculated for each pathogen in each experiment.

Statistical Analysis

- Observed and predicted Δ log CFU values were **qualitatively described** as “**growth**” (>0.3 log CFU increase) and “**no growth**” (≤ 0.3 log CFU increase).
- Each **qualitative prediction** was classified as “**accurate**” when matching its corresponding observation, “**Fail Safe**” (FS) when growth was predicted but not observed, and “**Fail Dangerous**” (FD) when growth was not predicted but was observed.
- Observed values from each experiment were **quantitatively compared** to predicted values.
- Quantitative predictions** were classified as “**accurate**” if **within 0.3 log CFU** of observed values, “**FS**” if predicted growth was **>0.3 log CFU more than observed**, and “**FD**” if predicted growth was **>0.3 log CFU less than observed**.
- Logistic regression analysis (see Equations 1 and 2) was used in combination with a likelihood ratio test to determine if there was potential statistically significant difference (p<0.05) between THERM versions.

$$\begin{aligned} \text{Equation 1: } \log(\text{probability}/1-\text{probability}) &= \mu + \beta_{\text{product*pathogen}} + \beta_{\text{experiment}} + \beta_{\text{LPD}} + \beta_{\text{GR}} \\ \text{Equation 2: } \log(\text{probability}/1-\text{probability}) &= \mu + \beta_{\text{product*pathogen}} + \beta_{\text{experiment}} + \beta_{\text{THERM version}} \end{aligned}$$

- If a statistically significant difference was detected, pair wise comparisons of THERM versions were conducted.

Results and Discussion:

Table 5: Qualitative and Quantitative Accuracy of THERM versions.

THERM Version	Qualitative			Quantitative			
	(% accurate)	(% FS)	(% FD)	(% accurate)	(% FS)	(% accurate + FS)	(% FD)
2.0	88.6	8.6	2.8	67.2	21.4	88.6	11.4
2.3	87.1	8.6	4.3	61.4	28.6	90.0	10.0
2.4	88.6	7.1	4.3	62.9	25.7	88.6	11.4
3.0	84.3	7.1	8.6	52.9	31.4	84.3	15.7
3.2	84.3	7.1	8.6	60.0	22.9	82.9	17.1
3.4	84.3	7.1	8.6	58.6	25.7	84.3	15.7
4.0	87.1	8.6	4.3	67.1	20.0	87.1	12.9
4.2	88.6	5.7	5.7	67.1	18.6	85.7	14.3
4.3	85.7	8.6	5.7	60.0	27.1	87.1	12.9
5.2	82.9	12.9	4.2	60.0	25.7	85.7	14.3
5.3	81.4	12.9	5.7	51.4	35.7	87.1	12.9
5.4	82.9	12.9	4.2	61.4	25.7	87.1	12.9

- There was **no significant difference** between THERM versions in **qualitative accuracy**.
- THERM versions using **linear interpolation or regression** produced **accurate qualitative results** for **82.9 – 88.6% of predictions (Table 5)**.
- There was **no significant difference** in rates of **quantitative fail-dangerous predictions**, but **linear interpolation or regression versions** were significantly **more accurate at quantitative prediction of LPD and GR**.
- THERM versions using **linear interpolation or regression** produced **accurate or fail-safe quantitative results for 84.3 – 90% of predictions**.

Conclusions:

- All THERM versions were statistically equal at making qualitative pathogen growth predictions.
- THERM versions using linear interpolation or regression were significantly more accurate in quantitative predictions.
- Due to concerns with over-fitting with linear interpolation, we recommend the use of piecewise linear regression for both LPD and GR calculations (THERM version 4.0).

Acknowledgment:

- This project was funded by the USDA, CSREES, National Integrated Food Safety Initiative, project number 2004-51110-02165.
- The authors gratefully acknowledge the mathematical and statistical assistance of Dr. Cecile Ane (UW-Madison, Department of Statistics).