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A Thermal Process Lethality Model for Low Water Activity Food

ABSTRACT

Science-based verification of pathogen-reduction processes is an increasingly necessary action for regulatory or customer compliance and is a relatively new initiative for baked goods. Direct measure of reduction potential that uses appropriate surrogate microorganisms is effective but becomes cost prohibitive when evaluating multiple food formulas or operational conditions. Thermal predictive models are available and provide valuable tools for foods with a single key changing variable such as temperature; however, a model applied to baked goods must accommodate at least two changing variables, water activity and food temperature, to avoid erroneous results. A single surface response equation (r^2 = 0.755) was created from laboratory thermal death time measurements of one formula of grain-based breakfast cereal sampled across three points (start, middle, end) of a baking process. Four additional grain-based food formulas were sampled from the entrance and exit of ovens to verify the original equation and permitted expanded application to cookies and crackers (r^2 = 0.756). The surface response equation was combined with exposure time to generate a process inactivation model for low water activity foods. Process lethality estimated by use of the model with commercial production data was comparable to results obtained with direct measure using surrogate organism tested with the same conditions and equipment.

INTRODUCTION

Background

Reducing water activity (a_w) through baking or drying develops conditions that minimize microbiological growth *(10)* in low water activity foods (LWAF) such as cookies, crackers, breakfast cereals, and dry ready-to-eat ingredients. Accurate measurement of thermal processes distinguishes between drying and the application of heat at levels sufficient to reduce or eliminate pathogens in the raw materials so as to prevent consumer exposure to deleterious microorganisms such as *Salmonella*. The prevalence of *Salmonella enterica*

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serotypes in wheat flour has been reported to be as high as 6.13% at certain times of the year but more commonly has ranged between 0.14% and 1.32% *(15, 17, 18)*. Outbreaks from *Salmonella* have been traced to many ingredients used in LWAF formulas, including dried milk, nuts and nut butters, seeds, spices, and soy products such as soy grits and hydrolyzed vegetable protein *(8)*. Measuring process lethality ensures that target standards are met for the finished food and provides inactivation data when risks of contamination to the food stream are reviewed *(14)*.

The direct measure of process lethality using an appropriate surrogate microorganism in the target food is useful but may be cost prohibitive for LWAF because of frequent changes of food formulation, product mass, production rate, temperature settings or air flow. Multiple variables likely necessitate additional tests to ensure accurate estimates of process lethality under different conditions. Some LWAF manufacturers have used high moisture food lethality models *(13)* and the thermal death time (TDT) results from meat products for predicting lethality. However, application of this method to LWAF is not recommended, as these models are designed for foods with stable and high water activity rather than for foods with properties that change from start to finish.

Multiple publications define survival rates for *Salmonella enterica* serotypes and provide insight into which organisms may be most resistant to inactivation in LWAF. Of eight serotypes evaluated in sucrose solution, *S.* Alachua, *S.* Anatum, and *S.* Senftenberg 775W were most highly resistant to inactivation *(9)*. High inactivation rates in dry animal feed were also reported for *S.* Senftenberg 775W *(12)*, but this organism is not considered an important foodborne pathogen *(6)*. Increased resistance to inactivation as water activity decreased (a_w 0.99–0.83) was demonstrated in two studies with *S.* Typhimurium in sucrose solution *(9, 19)*.

S. Weltevreden in wheat flour, a substrate more analogous to LWAF, was also increasingly resistant to thermal inactivation as water activity was reduced from 0.50 to 0.25 *(3)*. *S.* Tennessee was more heat resistant than *S.* Thompson, *S.* Senftenberg, *S.* Cubana, *S.* Kentucky, *S.* Anatum, *S.* Typhimurium and *S.* Newington when assessed in corn flour (15% moisture) *(20)*. *S.* Tennessee has also been implicated in recent outbreaks associated with LWAF *(8)*.

In these published investigations, *Salmonella* inoculated samples were commonly exposed to heat in open laboratory ovens to mimic thermal processes and yielded non-log linear results. Repeatability of non-log linear results collected from single exposures limited the use of these findings to a process that would duplicate the original test parameters. To mitigate this issue some investigators elected to report log-linear results as D- and z-values from the rapid initial inactivation or from the slower inactivation at the end of the thermal process. Analysis of baking conditions using values derived from only the initial results, where pathogen reduction was measured at a higher rate of decline, overestimate the total process lethality achieved. The use of D- and z-values derived from only the end of a non-log linear result underestimate total process lethality. Further review of the potential influence of unmeasured variables such as pH, salt, fat, and a_w have been suggested *(3, 9, 12, 19, 20)*. Later work confirmed that heat resistance of *Salmonella* increased as water activity in the test foods was lowered *(16)*.

The use of any single D- and z-value to evaluate a commercial process should be carefully assessed, as the resulting estimates might be skewed because of long exposure times and differences in food substrates used in the laboratory analysis. An equation capable of accurately calculating the thermal inactivation of *Salmonella* in LWAF at any discrete point and reporting the result from multiple discrete points as a cumulative value would improve accuracy and provide a commercially applicable method of mathematically verifying process lethality for LWAF.

MATERIALS AND METHODS

Project organization

Three work streams of model creation, model validation and model application were undertaken to complete this project. Within each work stream, a series of tasks were performed in parallel with other project activities *(Fig. 1)*.

Model creation — collection of food samples

All food samples were collected from formulas proprietary to Kellogg Company (Battle Creek, MI); therefore, *(Table 1)* provides only the composition in terms of the major ingredients for the cereals, cookies and crackers used in the tests. Food samples were collected aseptically at the manufacturing plants, wrapped and closed with minimum headspace in heat-resistant plastic containers (#7J76, Rubbermaid, Atlanta, GA) or in double-lined plastic bags (Ziploc, SC Johnson, Racine, WI). Samples were shipped overnight to the laboratory, and food samples that were not shelf stable were mailed under frozen conditions. General appearance of the food was confirmed acceptable and the water activity (AquaLab 4TE, Decagon Devices, Pullman, WA) of each product was measured upon arrival and monitored as part of the testing process.

Model creation — thermal death times (TDTs) — preparation

Salmonella Tennessee (ATCC 10722) was selected for the TDTs because of the organism's high heat in LWAF identified in published works and the persistent prevalence of this organism in foodborne outbreaks associated with LWAF. The inoculum preparation, TDT tests and enumeration were completed at The National Food Lab, now Covance Food Solutions (Livermore, CA). The inoculum was prepared by following the procedures developed by Danyluk et al. *(5)*, with modifications to harvesting as per the procedure from Du et al. *(7)*. Additional modifications to the inoculation of the LWAF, storage, and TDT preparation, as outlined in *(Table 2)*, maintained the water activity of the food samples and

stabilized the population of *S.* Tennessee in the LWAF before the TDTs were performed. Inoculation levels averaged 8-log CFU/g, with a range of 6.5–8.5 log CFU/g.

Model creation — TDTs — inactivation

Sealed test containers *(Table 2)* prevented loss of moisture during exposure to heat. Temperatures were measured with precision fine wire thermocouples (OMEGA, Stamford, CT) inserted into an uninoculated sacrificial product sample and recorded every 6 seconds during heat treatment. After heating, samples were immediately cooled in an ice bath. Inoculated but not heat-treated product served as positive controls. Water activities of the samples were measured (AquaLab 4TE, Decagon Devices, Pullman, WA) before heat treatment and after heat treatment for each interval. Samples were collected in triplicate during six time intervals for at least four temperatures for each TDT analyses. Time intervals shortened as temperatures increased to accommodate increasing rates of inactivation (example from cereal 1 tests provided in *Table 3*).

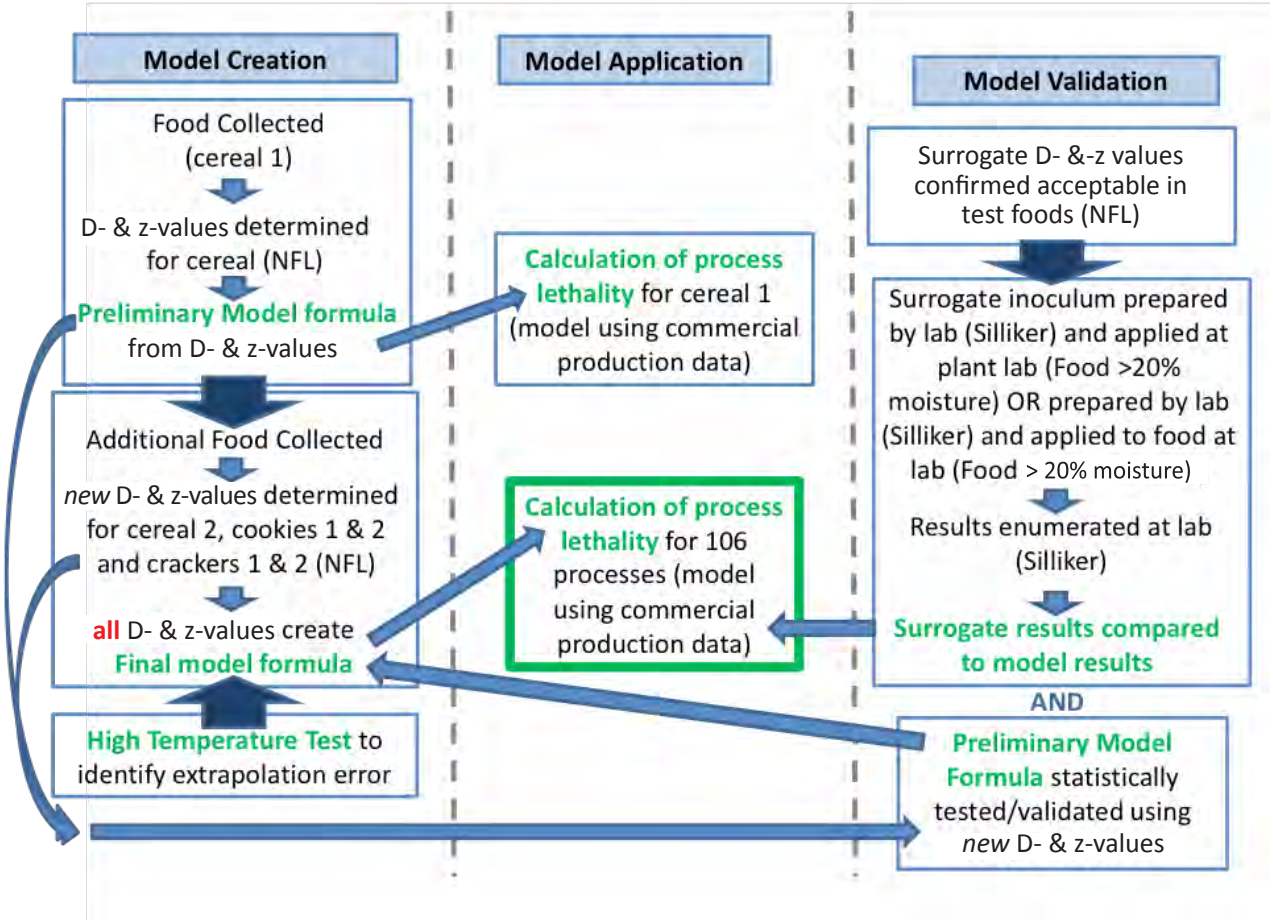

Figure 1. Project flow and relationship of tasks across three work streams

Table 1. Key components, % dry basis, of food sampled for TDTs

	% Flour	% Solutes	% Fat
Cereals	90–95	4–10	0
Cookies	35–43	31–47	14–25
Crackers	58–78	4–24	17–20

Table 2. Water activity as indicator of method of sample inoculation, drying, storage, and TDT

Sample a_w Range	Inoculation and Drying	Storage until Test	Container	Quantity	Heat Bath
< 0.40	**Step 1.** Small portion of food ground to powder and inoculated with high concentration of *Salmonella* Tennessee **Step 2.** Dried 24 h; single layer on filter paper, then dried in closed container with silica desiccant 24 h to meet the original sample a_W **Step 3.** Inoculated food blended with additional food in food processor to create sample size at > 6 log CFU/g	Closed bag, room temperature	Glass TDT tubes; heat sealed	1.5 g	Oil
0.40–0.70	**Step 1.** Inoculum homogenized with food in a sterile stomacher bag **Step 2.** Dried 24-h single layer on filter paper to achieve food a_W	Closed bag, RT ground at use		2 g	
0.71–0.90	**Step 1.** Inoculum sprayed onto ground food **Step 2.** Air dried and then additional drying with silica desiccant to achieve food a_W	Closed bag refrigerated	4" × 6" Heat-resistant pouches, vacuum sealed	< 3mm thick, spread evenly in pouch	Water
> 0.90	**Step 1.** Sprayed Inoculum onto ground food **Step 2.** Air dried; measuring until reaching previous a_W				

Model creation — TDTs — enumeration

Samples were enumerated by aseptic removal of the food to Whirl-Pak bags (Nasco, Fort Atkinson, WI) and dilution (1:10) of the food with 1% peptone (Difco, BD, Sparks, MD). After stomaching (Smasher, bioMérieux Inc., Hazelwood, MO) for 2 minutes at the "Fast" setting (approx. 550 – 660 strokes per minute), samples were serial diluted (1:10) with 1% peptone and pour-plated on Phenol Red Agar (Acumedia, Neogen, Lansing, MI) supplemented with sucrose (EMD Millipore, Merck KGaA, Darmstadt, Germany), lactose (Acumedia, Neogen, Lansing, MI), sodium thiosulfate (EMD Millipore, Merck KGaA, Darmstadt, Germany), and ferrous sulfate (JT Baker, Center Valley, PA). Plating was performed in duplicate and plates were incubated at 35°C for 48 h. Colonies with black centers were counted as the target organism.

Model creation — statistical analyses of TDTs

TDT results were evaluated for best fit to log-linear or non-log linear equations to describe the observations. Variables of temperature, a_W, fat, and salt were analyzed for their effects on log D by use of OpenBUGS version 3.2.2 rev 1063 (OpenBUGS Project Management Group). After elimination of extraneous terms, the remaining factors were used to estimate a Bayesian main-effects regression model. Each set of estimates was calculated by use of a Markov chain Monte Carlo burn-in run of 2,000 draws with two chains followed by an estimation run of 20,000 draws.

The first series of TDT observations were used to create the preliminary surface response equation. The second series of TDT observations were used to analyze and enhance the first equation and to determine if the equation was suitable for LWAF formulas of differing characteristics (*Fig. 1* Vali-

Table 3. Summary of time and temperature intervals for thermal death time of *Salmonella* Tennessee in cereal 1

Product	Temp (°C)	Time (s)
Cereal 1 – 0.84 a_w	68.33	20–150
	71.11	8–58
	73.89	5–35
	76.67	2–12
	79.44	2–7
Cereal 1 – 0.23 a_w	90.56	20–130
	93.33	10–70
	96.11	6–40
	98.89	4–20
	101.67	2–12
Cereal 1 – 0.55 a_w	76.67	15–105
	79.44	10–60
	82.22	5–30
	87.78	2–12

dation—Preliminary Model Formula). For both data sets, r^2 was assessed by use of the Pearson correlation coefficient.

Model creation — Evaluation of extrapolation error

The statistical complication of extrapolation error caused by LWAF reaching temperatures in excess of those used for laboratory TDTs was evaluated by testing high temperature survival of *Salmonella* Tennessee, in dry breakfast cereal (a_W 0.2). Food was inoculated with *S.* Tennessee, using the same techniques as previously described for *Thermal Death Times—Preparation* and the additional steps used for samples of less than 0.40 a_W *(Table 1)*. Positive control samples were tested after the drying period and immediately prior to heat treatment to confirm a concentration of 7.93 log CFU/g on inoculated-dried samples. Inoculated food samples (1.5 g) were rapidly heated in an oil bath set at 126.67°C until they reached a temperature of 121°C. Samples were immediately removed from the oil bath, cooled in an ice bath and enumerated, using the same methods as described for *Thermal Death Times—Enumeration.*

Model validation — Surrogate data collection (KIM)

Three industrial thermal processes were tested for process lethality, using *Enterococcus faecium* spp. NRRL B-2354 as the surrogate in three grain-based cereals. This microorganism is often selected for replacing *Salmonella* when validating commercial processes *(1)*. Because no published work had evaluated suitability of *E. faecium* in studying breakfast cereal, TDTs were collected by use of methods as already described for *S.* Tennessee, and it was confirmed that the surrogate was a suitable replacement for *S.* Tennessee before the facility tests were performed.

To prepare the surrogate inoculum, *E. faecium* was cultivated at the Silliker-Food Science Center (South Holland, IL; now Crete, IL) in Tryptic Soy Broth (TSB; BD, Sparks, MD), a medium that has been shown to enhance the heat resistance of this organism *(11)*, and incubated at 37°C for 24 h. After incubation, a 0.1 ml aliquot of the culture was transferred to 100 ml of TSB and incubated again at 37°C for 24 h. The broth culture was centrifuged at 7000 rpm for 20 minutes (Avanti J-E, Beckman Coulter, Brea, CA), the supernatant discarded, and the bacterial pellet suspended in 0.1% peptone to provide a final concentration of 10^{8-9} CFU/ml. Enumeration on KF agar (Difco, BD, Sparks, MD) after incubation for 24 h at 35°C verified the culture level and purity by the appearance of red or pink colonies.

The moisture content of the cereal product for the first surrogate test exceeded 20% (Computrac Max, Arizona Instrument, Chandler, AZ) at the entry to the impingement oven, which provided suitable conditions to inoculate the product at the manufacturing facility using a surrogate culture shipped overnight under refrigerated conditions *(4)*. Four pounds of food was collected at the last point prior to entering the oven. The inoculum was colored with approximately 5 drops of food-grade dye (Chefmaster-Leaf Green, Amazon.com) in the culture container from the laboratory and gently swirled by hand to incorporate the dye evenly. A 1-oz. mist sprayer (Vivaplex, Amazon.com) was used to spray a measured volume of inoculum onto the weighed food to achieve a 1:100 wt/wt rate. The inoculum

was sprayed while the food was slowly turned in a candy panning drum (DB-3500, pastrychef.com). The collection and tumbling activities were gentle (thin layers of food on open trays and slowest speed of the drum, respectively) to maintain the integrity of form in comparison to "normal" food. After tumbling, inoculated food was held in thin layers on open trays for 15 minutes before being divided into large Whirl-Pak bags that were used to carry the product to the line. The product was held in the bags for an additional 45 minutes to permit equilibration before exposure to the thermal process. Two samples (100 g) of inoculated sample food were collected, designated as positive controls and carried with all other food samples. From "normal" food running on the line at time of test, two 100-gram negative control samples were collected at the exit of the thermal process. The remaining inoculated product was released at the entrance of the heating process and scattered within the food running on the line. All food was diverted to lined containers at the exit of the thermal process, and food colored in the inoculation process was collected with use of sterile gloves into new, sterile Whirl-Pak bags, so as to have at least 100 grams of finished food in each of 12 samples.

The food selected for the second surrogate test was less than 20% moisture prior to the impingement oven and was inoculated at the third-party laboratory by use of the same methods as previously described. The food was held, and the organism was tested for a stable population and sufficient concentration by enumeration on KF agar, before shipment overnight and under refrigerated conditions to the production facility *(4)*. Five pounds of inoculated food was shipped to the manufacturing facility, where the product was heat treated and collected as already described.

The food selected for the third surrogate test was more than 20% moisture at the entry of the direct-fired oven and was suitable for inoculation at the manufacturing facility, but its structure degraded with handling. To maintain the integrity of the food, the inoculation method was modified. After establishing the weight of the target food within each square foot of the manufacturing line, the colored inoculum was sprayed evenly to the top surface of the food at the same 1:100 wt/wt ratio used in previous tests. Because of accessibility limitations and movement of the food belt, the holding time was limited to 3 minutes. After treatment in the oven, food was collected by spatula onto trays and then into twelve sample bags, as had been done in the previous tests. Two samples each of positive and negative controls were also collected.

The collected food (2 uninoculated controls, 2 inoculated controls, and 12 thermally treated samples of at least 100 grams each) was immediately shipped under refrigeration to the third party laboratory for enumeration. All remaining food was discarded. For each sample, one part (25 g) of the food was combined with 2 parts (50 g) Butterfield's phosphate buffer (BPB; Difco, BD, Sparks, MD) and homogenized by stomaching for 2 minutes (Stomacher 400, Seward Ltd., West Sussex, UK). Serial dilutions (1:10) were made using BPB and analyzed by plate count on TSA with KF overlay after incubation for 48 h at 35°C under aerobic conditions. Tests were performed in triplicate *(11)*.

Model application — Use of the Kellogg Inactivation Model (KIM)

Dwell time, water activity and food temperatures were collected from 106 commercial thermal processes, all of which were primary and secondary thermal processes consisting of direct and indirect fired ovens, impingement ovens, and extruders. The data were entered into a spreadsheet designed to calculate cumulative process lethality from the surface response equation.

Water activities (Aqualab Series 3, Decagon Devices, Pullman, WA) were measured by sampling food at the start and end of the process and, where access permitted, sampling at mid-zones. Food temperatures were collected by data logger (Scorpion 2, Reading Thermal, Sinking Spring, PA; Datatrace RF, Mesa Labs, Lakewood, CO), or direct probe (HDX-SET-RT-K-SMP-36" K-Type probe with ½" × 1/8" tip, Omega, Stamford, CT), selected on the basis of accessible points of contact and sufficient clearance within the manufacturing equipment. For extruders designed with an internal probe, temperature measurements were recorded from the equipment. Probes were confirmed to be within calibration, and models varied according to the extruder manufacturer. In all cases, the lowest temperatures of the food within the thermal process were sought to establish "worst case" parameters and provide minimum operational standards for the thermal processes. Exposure time was measured via the timing associated with a data logger or by measuring the length of time needed for food to pass from one end to the other. D-values and resulting process lethality for each time interval were calculated within the KIM spreadsheet, using the surface response equation. The cumulative process lethality was reported as the sum of the intervals. For ease of communication to a wide audience, results were expressed as log lethality rather than F value.

RESULTS

Model creation — TDTs

Food samples showed consistent water activity when tested from the same location in the manufacturing equipment and varied widely across the production processes when sampled at different production points (0.21 – 0.85 a_w). All uninoculated laboratory samples were negative for *Salmonella*.

The first TDTs, performed using cereal product 1, were collected from three different points on a commercial production line (entrance, mid-point and exit of thermal process). Two validation samples for statistical analysis of the first series of results were collected from a different formula (cereal 2) on another line at two locations (entrance and exit of thermal process). These provided a total of 5 TDT tests with 24 observations *(Table 4)*. The second series of TDTs

were generated using an additional four proprietary formulas. Two cookie formulas and one cracker formula were sampled at the entrance of the ovens. One cookie and one cracker formula were sampled at the exit of the ovens. One dough sample (cracker 2) did not meet the criteria for TDT testing because of interference by fermentation microflora, and the two remaining finished product samples (cookie 2, cracker 1) were scheduled for future analysis. The five TDT tests provided 25 observations *(Table 4)*.

Model creation — Surface response equation

With a fit of $r^2 > 0.90$, log-linear regression was selected for creating the surface response equation to calculate the D-value of any given point of a LWAF thermal process.

Step-wise regression of 5 variables (temperature, a_W, fat, salt and food type) for their effects on log D removed all but linear terms for temperature and a_W. The resulting Bayesian regression model was of the form

$$\log D_i \sim N(\mu_i, \sigma)$$
$$\mu_i = \beta_0 + \beta_1 * a_{Wi} + \beta_2 * T_i,$$

where T_i = temperature of run i measured in °C, and μ_i and σ are the mean and standard deviation of a normal distribution. The four parameters were given non-informative priors.

The first equation was written as

$$\log D = 10.09 - 3.606 \times a_W - 0.09167 \times Temp,$$

using estimates found in *Table 5*.

Model validation (preliminary model) and creation of secondary model — additional TDTs

The preliminary surface response equation had good fit ($r^2 = 0.755$). The data from the second set of TDTs remained closely correlated to the first, with comparative analysis resulting in a minor adjustment to:

$$\log D = 10.26 - 3.6647 \times a_w - 0.09042 \times Temp,$$

and a slight improvement of fit ($r^2 = 0.756$). Distribution of the residuals for the TDTs used in the surface response equation had a small (0.5) deviation from zero and revealed an acceptable bell curve.

Model creation — Evaluation of extrapolation error

In a high temperature test of breakfast cereal inoculated with *S.* Tennessee, an inoculum level of 7.93 log resulted in no recovery of *Salmonella* after the treatment. The KIM estimated a > 10 log reduction (19.8 log) using the same process parameters as those achieved in the laboratory test. When the results were compared, a 19.8 log reduction achieved in an inoculum of 7.93 log would be reported as "no recovery" from a laboratory; therefore, the KIM calculation was considered analogous to the lab test. The results from the laboratory test were used to provide the highest food temperature permitted in the KIM for the calculation of D-values by addition of a limiting factor in the model formula of:

$Temp$ >120°C, $\log D = -0.0873 \times Temp + 9.0586$.

Model validation – Surrogate comparison to KIM

Surrogate organism tests were completed in multiple thermal processes and compared to the calculated estimates from the KIM, resulting in satisfactory results *(Table 6)*. Three commercial ovens were directly measured for inactivation potential, using *Enterococcus faecium* spp. NRRL B-2354 as the surrogate. When food samples were homogenized with the surrogate inoculum before being passed through the thermal process, a close relationship between the results from direct measure and the calculated process lethality from the KIM were observed (*Table 6 — impingement ovens*). For one surrogate test, the food structure was compromised by homogenization (*Table 6 — direct fired oven*) and the inoculation method was adjusted to a spray on the top surface of the food. Because of this change in method and the need to apply the inoculum at the line, the holding time was also significantly reduced, to 3 minutes. The food dye migrated approximately 50% into the product but the location of color may not have correlated with the location of the surrogate organism on the food. When the core food temperatures, water activities and dwell time from the process were used, the KIM lethality estimates were significantly lower than enumeration of the surrogate inoculated food samples from the direct fired oven. If the inoculum remained closer to the surface of the food, results of the surrogate test likely overestimated process lethality. This comparison revealed the importance of identifying the coolest point in the food when evaluating thermal processes with any technique. These results remained a successful use of the KIM, as the calculation prevented overestimation of the inactivation achieved in the process.

Model application — Use of the Kellogg inactivation model (KIM).

The surface response equation and the formula to prevent extrapolation error combined all of the TDT results into a single predictive formula to estimate log lethality of *S.* Tennessee at numerous combinations of a_W and temperature *(Fig. 2)*. When time was incorporated, cumulative lethality from exposure of the LWAF to a thermal process was calculated.

Calculation of process lethality using the KIM was performed on 106 thermal processes (use of ovens and extruders) for various formulas of cereal, cookies, or crackers. Industrial production of LWAF most commonly had one thermal process, such as use of an oven or an extruder, but some lines contained more than one system that applied heat to the food, and each thermal system was measured as an isolated system. The first thermal process in each line consistently mitigated potential pathogens from the raw materials at levels exceeding 5 log CFU/g and, where there were subsequent (secondary) systems, there was a range of process lethality results, often significantly lower than

Table 4. D-values of *Salmonella* Tennessee for multiple grain-based foods at various water activities

Product	a_w	T (°C)	D-value Actual	z
Cereal 1	0.55	76.67	10.90	10.80
		79.44	5.90	
		82.22	3.40	
		87.78	1.00	
Cereal 1	0.23	90.56	14.10	11.50
		93.33	8.00	
		96.11	4.80	
		98.89	2.80	
		101.67	1.50	
Cereal 1	0.84	68.33	20.60	7.40
		71.11	9.80	
		73.89	4.30	
		76.67	1.80	
		79.67	0.70	
Cereal 2	0.85	68.33	27.80	6.80
		71.11	10.30	
		73.89	3.50	
		76.67	1.70	
		79.44	0.60	
Cereal 2	0.21	90.56	17.50	15.20
		93.33	12.40	
		96.67	7.00	
		100.00	4.20	
		103.33	2.60	
Cookie 1	0.27	82.22	34.50	22.20
		86.11	23.00	
		90.00	15.40	
		93.89	10.20	
		97.78	6.80	
Cookie 1	0.68	68.33	27.30	16.10
		71.11	18.30	
		73.89	12.30	
		76.67	8.30	
		79.44	5.60	
Cookie 2	0.80	62.78	15.80	8.40
		65.56	7.40	
		68.33	3.40	
		71.11	1.60	
		73.89	0.70	

Continued

Table 4. D-values of *Salmonella* Tennessee for multiple grain-based foods at various water activities (cont.)

Product	a_w	T (°C)	D-value Actual	z
Cracker 1	0.91	59.44	34.60	4.60
		61.11	16.20	
		62.78	6.90	
		64.44	3.50	
		66.11	1.20	
Cracker 2	0.25	82.22	26.90	14.00
		86.11	14.20	
		90.00	7.50	
		93.89	3.90	
		97.78	2.10	

Table 5. Bayesian estimates of regression parameter distributions

Parameter	Mean	Std. Dev.	2.5 Percentile	Median	97.5 Percentile
Beta 0	10.09	0.5343	8.995	10.11	11.18
Beta 1	-3.606	0.245	-4.092	-3.611	-3.112
Beta 2	-0.0917	0.0051	-0.1021	-0.09191	-0.08121
Sigma	2.60E-04	2.10E-04	6.55E-05	2.03E-04	7.95E-04

Table 6. Log lethality results for LWAF processes via direct measure using surrogate and calculated with the KIM

Process	KIM Estimated Lethality (log)	Organism Used for Direct Measure	Direct Measure Lethality (log)	Direct Measure Initial Load (log)
Laboratory (high temperature test)	> 10	*Salmonella* Tennessee	Not Detected (> 7 log)	> 7 log
Industrial Oven, impingement (Surrogate #1)	1.73	*Enterococcus faecium* spp. NRRL B-2354	1.7–3.08	> 7 log
Industrial Oven, impingement (Surrogate #2)	4.4	*Enterococcus faecium* spp. NRRL B-2354	3.86–5.85	> 5 log
Industrial Oven, direct fired (Surrogate #3)	0.11	*Enterococcus faecium* spp. NRRL B-2354	4.54–5.93	> 7 log

results seen with the first process *(Table 7)*. Repeating the data collection and calculations on different days and shifts indicated reliable results from the KIM.

Challenges and potential impact to the final result were realized in an early analysis when data collection was limited to probing of the food at the entrance and exit of the oven. A cookie oven with clearance that precluded the use of data loggers through the equipment resulted in a preliminary process lethality estimate that was less than expected (0.549 log) for the operational settings. After further investigation, it was determined that the last zone of the oven was turned off, producing cooler measurements for the exiting food temperatures (*Table 8 — 1st Check*). Additional data were subsequently collected from food at two access ports in hotter zones of the oven. This information provided a more accurate representation of the food temperature and improved measurement of process lethality results to satisfactory conditions (*Table 8 — Recheck*).

DISCUSSION

The aim of this investigation was to develop an alternative to surrogate organism testing for measuring the log reduction of *Salmonella* in heat processed, low-moisture foods such as breakfast cereal, cookies, or crackers. Past explorations of mathematical methods for this food category have been scant, and the results were difficult to apply to commercial situations because of non-log linear results. Previous investigators' discussions suggested controlling additional variables during TDT analyses and led to a surface response equation that provided accurate calculation of process lethality for any point of a baking step. This equation, in combination with exposure time, permitted development of an inactivation model that consistently provided process lethality calculations comparable to results of direct measure with a surrogate organism.

An advantage to this approach of measuring process lethality is the flexibility to evaluate any number of different operational settings based on measurement of three key variables associated with the exposure of the food to heat. While direct measure using surrogate organism tests would be an effective approach for consistent processes already in production, the KIM also offers rapid insight to process lethality achieved under new conditions, such as those made possible by innovation. Additionally, should the surrogate test be performed incorrectly, such as through non-homogenous distribution of the inoculum or enumeration of samples exposed to temperatures higher than those at the coldest point in the thermal process, accurate process data applied to the KIM may provide a better representation of worst case results from an operation.

Salmonella Tennessee was selected as the target pathogen to create the surface response equation because of its higher heat resistance compared with other *S. enterica* serotypes in LWAF and the prevalence of *S.* Tennessee in outbreaks associated with LWAF. Successful analysis of additional formulas from cereal, cookies and crackers permitted process lethality calculations for all of the tested LWAF formulations under a single mathematical formula in the model. These results may differ for other pathogenic microorganisms or different food formulations. Similar to the recommendation when selecting a surrogate organism for use in different foods, *S.* Tennessee should be verified as the optimum choice before this serotype is used to establish a predictive model for a new food, particularly if the composition significantly differs from that of the foods used in this investigation *(Table 2)*. The pathogen selected should provide

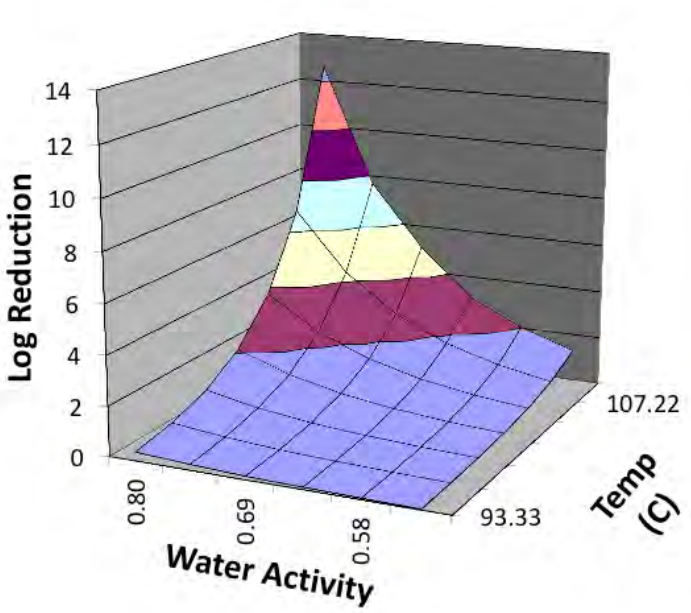

Figure 2. Influence of temperature and water activity on log reduction (CFU/g) for Salmonella *Tennessee within 1 second exposure*

Table 7. KIM log lethality calculated using water activities, exposure times, and food temperatures collected from 106 LWAF heat processes

Process		# Systems Assessed	Water Activity In	Water Activity Out	Exposure Time (Sec)	Food Temperature (°C)	KIM log Lethality
Oven (Direct/Indirect)	First/Primary	36	0.68–0.95	.04–0.7	120–1200	101–155	> 10 log
	Secondary	9	0.379–0.85	0.06–0.65	30–530	12.8–146	0–6.7
Oven (Impingement)	First/Primary	32	0.61	0.18	48	108	> 10 log
	Secondary	5	0.61–0.82	0.11–0.6	24–120	52.8–129	0–3.3
Extrusion	First/Primary	24	0.25–0.28	0.56–0.82	18–45	110–125	> 10 log

the highest level of resistance to thermal inactivation within the target food balanced with the prevalence of outbreaks for that organism within the food type. For example, *S.* Enteritidis Phage Type 30 is the targeted organism when evaluating inactivation of *Salmonella* in almonds because of the higher heat resistance and prevalence of this organism within the almond industry *(1)*.

Surrogate organism tests resulted in a range of results from the enumeration of the test samples (*Table 6* — Direct Measure Lethality), and the KIM calculated a single result from the measured set of conditions. Ensuring that measured parameters represented the worst-case conditions shielded the KIM from producing erroneous results. Similar to the situation of surrogate organism testing, it was important to evaluate individual thermal processes as unique systems, since variation in similar equipment could occur as a result of design changes, repair or replacement of parts, or internal modifications.

The data collected and entered into the KIM demonstrated an acceptable level of equivalence, compared with direct measure with a surrogate organism. Water activity and exposure time were the simplest variables to measure for all equipment evaluated, but complications from equipment design required testing of a variety of tools to accurately measure food temperatures. The tool that provided the greatest amount of information for food temperature was a data logger and probe capable of passing through the thermal process to measure the temperature profile of the food throughout its exposure to heat *(2)*. This approach provided full access to precise food temperatures for every time interval and subsequently the most visibility to the process lethality. However, the use of this tool was labor intensive, and some ovens did not have adequate clearance to permit safe passage of the tool through the equipment. In these situations, data was collected at a minimum from the entrance and exit of the oven and, when possible, at multiple access ports on the line. These individual measurements were often sufficient to demonstrate a satisfactory level of process lethality, but represented less than the full temperature profile that could be established with a data logger.

Measuring food temperature from extrusion was most efficient when the equipment contained an interior, calibrated probe just prior to the die plate that interfaced with the food. Food cooled very quickly after exiting the extruder, and sampling food after the exit did not provide satisfactory representation of the system. When extruders did not have an internal method of food temperature measurement, a safe plan of action was established to provide the opportunity to turn off the blades and insert a long, sturdy probe through the die plate and into the food to measure temperature of the food.

The first thermal process that eliminated the pathogen from the food ingredients was the key focus for establishing process lethality. Additional ovens were sometimes used as secondary heat processes to further enhance the appearance of the product, but the characteristics of the food, operational settings and production rates at these later points resulted in much lower levels of lethality than those achieved with similar equipment earlier in the production flow *(Table 7)*. Once the key thermal process was established, reporting the lowest amount of process lethality achieved from this system was the conservative approach to managing a thermal process verification program. This was particularly important for equipment that produced variable results.

A statistically verified surface response equation from TDTs collected in LWAF and integrated with time created an inactivation model that permitted calculation of process lethality in baking systems. In this case, the same inactivation model was applicable to multiple formulations of LWAF, but investigators should independently confirm that their foods and manufacturing conditions are suitable for application of this model before proceeding.

Table 8. Improved visibility to process lethality results in an oven by adding data from 2 more points of data collection (end of temperature zones 2 and 3) to the entrance and exit food temperature data

Common values to both assessments			1st Check: log reduction using entering and exiting food temperatures			Recheck: log reduction with multi-port collection of food temperatures		
	time (s)	a_w	T (°C)	D	log	T (°C)	D	log
Zone 1	1	***0.804***	***25.6***	69827.1	0	***25.6***	69827.1	0
	32.6	0.781	28	50801	0	30.7	28610.8	0
	65.2	0.759	30.4	36959	0	35.8	11722.9	0
	97.8	0.736	32.8	26888.6	0	41	4803.32	0
	130.4	0.713	35.2	19562.2	0	46.1	1968.1	0
	163	0.691	37.6	14232	0	51.2	806.405	0.001
	195.6	0.668	40	10354.1	0	56.3	330.414	0.003
	228.2	0.645	42.4	7532.89	0	61.4	135.383	0.007
	260.8	0.622	44.8	5480.37	0	66.6	55.472	0.017
Zone 2	293.4	0.6	47.2	3987.11	0	71.7	22.729	0.04
	326	0.577	49.6	2900.72	0.001	76.8	9.313	0.099
	358.6	0.554	52	2110.35	0.001	81.9	3.816	0.241
	391.2	0.532	54.4	1535.33	0.001	87	1.563	0.589
	423.8	0.509	56.8	1116.99	0.002	92.2	0.641	1.437
	456.4	0.486	59.2	812.641	0.002	97.3	0.262	3.507
	489	0.463	61.6	591.217	0.003	***102.4***	0.108	8.559
Zone 3	521.6	0.441	64	430.126	0.005	104	0.092	14.436
	554.2	0.418	66.4	312.927	0.006	105.6	0.079	21.274
	586.8	0.395	68.8	227.663	0.009	107.2	0.068	29.229
	619.4	0.373	71.2	165.63	0.012	108.8	0.059	38.484
	652	0.35	73.6	120.5	0.017	110.5	0.05	49.252
	684.6	0.327	76	87.667	0.023	112.1	0.043	61.779
	717.2	0.305	78.4	63.78	0.031	113.7	0.037	76.353
	749.8	0.282	80.8	46.402	0.043	***115.3***	0.032	93.381
Zone 4	782.4	0.259	83.2	33.758	0.059	113.4	0.058	102.804
	815	0.236	85.6	24.56	0.081	111.5	0.104	108.019
	847.6	0.214	88	17.868	0.112	109.6	0.188	110.905
	880.2	0.191	90.4	12.999	0.153	107.7	0.34	112.502
	912.8	0.168	92.8	9.457	0.211	105.8	0.615	113.386
	945.4	0.146	95.2	6.881	0.29	103.8	1.111	113.875
	978	0.123	97.6	5.006	0.398	101.9	2.007	114.146
	1011	***0.1***	***100***	3.651	0.549	***100***	3.651	114.297

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