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Predicting Pathogen Growth during Short-Term Temperature Abuse of Raw Sausage

STEVEN C. INGHAM,* BARBARA H. INGHAM, DARAND BORNEMAN, EMILIE JAUSSAUD, ERICA L. SCHOELLER, NATHAN HOFTIEZER, LAUREN SCHWARTZBURG, GREG M. BURNHAM, AND JOHN P. NORBACK

Department of Food Science, University of Wisconsin–Madison, 1605 Linden Drive, Madison, Wisconsin 53706, USA

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ABSTRACT

Lag-phase duration (LPD) and growth rate (GR) values were calculated from experimental data obtained using a previously described protocol (S. C. Ingham, M. A. Fanslau, G. M. Burnham, B. H. Ingham, J. P. Norback, and D. W. Schaffner, *J. Food Prot.* 70:1445–1456, 2007). These values were used to develop an interval accumulation-based tool designated THERM (temperature history evaluation for raw meats) for predicting growth or no growth of *Salmonella* serovars, *Escherichia coli* O157:H7, and *Staphylococcus aureus* in temperature-abused raw sausage. Data (time-temperature and pathogen log CFU per gram) were obtained from six inoculation experiments with *Salmonella, E. coli* O157:H7, and *S. aureus* in three raw pork sausage products stored under different temperature abuse conditions. The time-temperature history from each experiment was entered into THERM to predict pathogen growth. Predicted and experimental results were described as growth (>0.3 log increase in CFU) or no growth (≤0.3 log increase in CFU) and compared. The THERM tool accurately predicted growth or no growth for all 18 pathogen-experiment combinations. When compared with the observed changes in log CFU values for the nine pathogen-experiment combinations in which pathogens grew, the predicted changes in log CFU values were within 0.3 log CFU for three combinations, exceeded observed values by 0.4 to 1.5 log CFU in four combinations, and were 1.2 to 1.4 log CFU lower in two combinations. The THERM tool approach appears to be useful for predicting pathogen growth versus no growth in raw sausage during temperature abuse, although further development and testing are warranted.

Under the mandatory hazard analysis critical control point (HACCP) system for ensuring food safety *(4),* U.S. processors of raw meat and poultry products must obtain scientifically valid information to support their choice of critical limits at critical control points (CCPs). Processors also need scientifically valid information to support their choice of corrective actions taken when critical limits at a CCP are not met. Computer-based tools for predicting pathogenic bacterial growth in raw meat are potentially important sources of this scientific information. However, a major challenge to the development of predictive tools that are practical for use in the meat industry is that the tools must be capable of accurately predicting bacterial behavior under nonisothermal conditions. Even in well-controlled raw meat processing systems, meat temperature can change during steps such as grinding, mixing, and packaging. Furthermore, meat temperatures will fluctuate during deviations involving temperature abuse.

Several researchers have reported on the development and application of mathematical models of bacterial growth under nonisothermal conditions. One major conceptual and mathematical question to be addressed in developing these models is how much lag-phase duration (LPD) and growth rate (GR) change when bacteria are exposed to a changing temperature. Baranyi and Roberts *(1)* proposed the use of a single mathematical function to describe both the physiological condition of the bacteria at the time of inoculation into the test medium and the new environment encountered by the bacteria immediately after inoculation. By incorporating this function into a predictive equation, the need to separately predict LPD was removed. In a subsequent study, Baranyi et al. *(2)* used this approach to predict the growth of *Brochothrix thermosphacta* in a laboratory medium during nonisothermal conditions. Accurate predictions were obtained for some nonisothermal regimes, but less accurate predictions were obtained when the temperature fell below a minimum level. Zwietering et al. *(17)* developed separate predictions for LPD and GR of *Lactobacillus plantarum* in a laboratory medium and concluded that exposing lag-phase cells to a shift in temperature resulted in a 25% increase in LPD at the new temperature beyond the expected remaining proportion of lag phase. These authors reported a similar adaptation-related delay when growing cells were transferred to a new temperature, i.e., the cells did not instantaneously begin growing at the growth rate normally observed at the new temperature. However, these authors did propose that ignoring these adaptation-related delays was a convenient approach to prediction, particularly for situations with frequent temperature changes, and that the latter approach still had a high level of predictive accuracy. Furthermore, ignoring the adaptation times would be expected to increase the likelihood of a fail-safe prediction, i.e., it would more likely result in overprediction of growth when the temperature increased

* Author for correspondence. Tel: 608-265-4801; Fax: 608-262-6872; E-mail: scingham@wisc.edu.

over time—such as when temperature control is lost in raw meat processing.

In predicting growth of pseudomonads on fish under nonisothermal conditions, Koutsoumanis *(11)* used an integration approach to calculate the lag phase and an interval accumulation strategy to describe subsequent growth. When predictions were compared to subsequent experimental observations, actual pseudomonad levels were generally within a 95% confidence interval around the predicted value. This approach was successfully extended to predicting the growth of several bacterial groups, e.g., pseudomonads, lactic acid bacteria, and *Enterobacteriaceae,* in fresh ground pork under periodic temperature abuse *(12).* Based on studies using *Escherichia coli* in a laboratory medium, Fujikawa et al. *(9)* developed a new logistic model for predicting growth that involved a differential equation with a term accounting for lag phase. This tool was accurate for predicting growth when the temperature fluctuations were within the 30 to 35°C range, were periodic, and occurred over an 8- to 12-h duration.

In a previous article we described the development of an empirically based computer tool for predicting behavior of certain pathogens in raw meat and poultry during short-term temperature abuse *(10).* This tool, named THERM (temperature history evaluation for raw meats), and a subsequent internet-accessible version (http://www.meathaccp.wisc.edu/THERM/calc.aspx) are intended to be used by meat and poultry processors to support critical limit and corrective-action decisions. The THERM tool is similar to some of the predictive microbiological approaches described above in that it is based on an interval accumulation approach. However, to simplify the mathematics supporting the THERM tool, we treated lag phase and growth phase as distinct sequential conditions of the pathogenic cells. In order to minimize the chance of underpredicting pathogen growth, we also assumed that the pathogen cells instantaneously attain a new LPD or GR upon exposure to each new temperature.

The THERM tool uses linear interpolation of experimentally determined pathogen LPD and GR data in an interval accumulation technique to predict behavior of *Salmonella* serovars, *E. coli* O157:H7, or *Staphylococcus aureus* in raw pork, beef, or poultry based on a time-temperature history entered by the user. The THERM tool performs best in a qualitative use, i.e., predicting growth or no growth, but also provides a quantitative prediction of growth.

The three pathogens for which THERM makes predictions are significant hazards in raw meats. Performance standards for cooking lethality are based on salmonella levels being below a threshold level *(5)*; *E. coli* O157:H7 is considered an adulterant in nonintact raw beef products; and growing *S. aureus* cells can produce heat-stable enterotoxins, which would not be inactivated by cooking. *Listeria monocytogenes* is not currently considered an important hazard to be targeted in a hazard analysis for raw meat products. Rather, the focus in preventing meat-linked listeriosis is the prevention of postcooking contamination—which is outside the scope of raw meat HACCP plans.

The raw ground meats initially used to develop THERM did not contain inhibitory agents such as sodium chloride or spices. In addition, those meats contained very low levels of fat. Previously it has been shown that increased fat levels in ground beef lead to a greater LPD for *E. coli* O157:H7 *(16).* Thus, the accuracy of THERM for predicting pathogen behavior in higher-fat, salt-containing products such as raw sausage mix could not be ensured. In the present study, our objective was to expand THERM to enable prediction of pathogen behavior in pork sausage and related products that contain higher fat levels, sodium chloride, and spices.

MATERIALS AND METHODS

Developing the predictive tool: bratwurst. Fresh bratwurst (not stuffed in casings) was obtained from a local wholesale processor. The bratwurst was packaged in plastic bags (ca. 500 g per bag) and frozen at −20°C until it was thawed at 5°C for use. During the course of the study, two lots of bratwurst were used. The rationale for using multiple product lots to develop primary models of pathogen growth was previously described by Oscar *(13).* The ingredients in the bratwurst were (in order of decreasing amount) pork, water, salt, spice, and sugar.

Developing the predictive tools: raw meat microbiological and chemical analyses. At the start of each experiment, a 25-g sample of bratwurst was placed in a filter bag (15.25 by 23 cm) and diluted with 99 ml of Butterfield's phosphate diluent (BPD; Nelson Jameson, Marshfield, WI). The sample was then stomached at normal speed for 30 s using a stomacher lab blender (Fisher Scientific, Itasca, IL), appropriately diluted in BPD, plated on 3M Petrifilm Aerobic Count Plates (3M Microbiology, St. Paul, MN), and incubated at 35°C for 48 h to determine the concentration of indigenous bacteria or aerobic plate count (APC) in the bratwurst before inoculation. One sample from each lot of bratwurst was sent to a commercial laboratory for determination of pH, water activity, percent moisture, percent fat, percent protein, and percent salt (Table 1).

Developing the predictive tools: preparation of inocula. Five strains each of *Salmonella* serovars, *E. coli* O157:H7, and *S. aureus* were used in developing and testing THERM (Table 2). Each strain was prepared from frozen stock culture, with a working culture plate prepared by successively culturing twice at 35°C for 18 to 24 h in brain heart infusion broth (BHIB; Difco, Becton Dickinson, Sparks, MD), streaking to brain heart infusion agar (BHIA; Difco), incubating at 35°C for 18 to 24 h, examining for homogeneous colony morphology, and then storing the plate at 5°C. An isolated colony of each strain was transferred from its working culture plate to 9 ml of BHIB and then incubated at 35°C for 24 h. Inocula were prepared for each pathogen by combining each of the five cultured strains into a 50-ml centrifuge tube and centrifuging at 5,000 × *g* for 12 min. Two different inocula were prepared for bratwurst experiments. Previous experiments in our laboratory had shown that combining *Salmonella* and *E. coli* O157:H7 did not have a statistically significant effect ($P > 0.05$) on the growth of either pathogen, compared to that observed when using single-pathogen inocula *(3).* Therefore, the first inoculum contained *Salmonella* serovars and *E. coli* O157:H7 prepared as follows: the supernatant was decanted from each five-strain mixture, and each pellet was resuspended to 25 ml by using BPD. From both five-strain mixtures, 10 ml was transferred to another 50-ml centrifuge tube, creating 20 ml of a 10-strain, two-pathogen

TABLE 1. *Chemical and physical characteristics of commercial bratwurst mix used to develop the predictive tool and commercial bratwurst and breakfast sausage links used in experiments to test the predictive tool*

Analysis	Commercial bratwurst		Bratwurst		Breakfast links
	Lot 1	Lot 2	Beer	Standard	
pH	5.9	5.6	6.2	6.3	5.4
Water activity	0.97	0.98	0.97	0.97	0.97
% moisture	61.8	60.4	57.1	55.2	42.6
% fat	14.2	19.2	21.7	25.6	42.9
% protein	15.5	15.5	13.5	14.6	11.7
% salt	2.4	2.5	2.1	1.9	1.6

inoculum containing ca. 9 log CFU/ml. The second inoculum, containing only *S. aureus* at a level of about 9 log CFU/ml, was prepared by decanting the supernatant from the five-strain mixture and resuspending the pellet to 45 ml, using BPD. Finally, each inoculum was diluted 100-fold in BPD.

Developing the predictive tools: preparation and inoculation of meat products. Isothermal studies were conducted at either 2.8 or 5.6°C intervals (actually measured as 5 or 10°F because the U.S. meat industry uses the Fahrenheit scale) ranging from 18.4 to 46.1°C (65 to 115°F). Bratwurst (ca. 25 g) was weighed out into sample bags (7.5 by 18.5 cm) and allowed to reach the test temperature either in a static water bath (at temperatures greater than room temperature) or in an incubator (at temperatures less than or equal to room temperature). A type-K thermocouple, attached to a model SP150 data-logger (Dickson, Addison, IL), was inserted in the center of a bag of bratwurst to determine when the test temperature had been reached. When the test temperature was reached, each sample (except the one containing the thermocouple) was inoculated with 100 μl of either the combined inoculum (*Salmonella* serovars and *E. coli* O157:H7) or the *S. aureus* inoculum. Previously we had determined that pathogen growth was faster ($P < 0.05$) when the inoculum was dispersed in the ground meat than when the inoculum was localized in a ''hole'' within the meat mass *(3),* so each inoculated sample bag was closed and manually massaged for 20 s to distribute the inoculum throughout the meat mass. Bags of inoculated bratwurst were returned to the isothermal experiment temperature as quickly as possible (<5 min). Three concurrent trials were conducted for each temperature with separate inocula prepared for each trial, and enough bags of inoculated product were prepared to allow analysis of one bag for each inoculum type in each trial at every sampling time. Bags of bratwurst inoculated with *Salmonella* serovars and *E. coli* O157:H7 were exposed to the test temperature for total times ranging from 720 min at 43.3°C to 1,440 min at 21.1°C, with 6 to 15 sampling times. For bratwurst inoculated with *S. aureus,* exposure times ranged from 420 min at 46.1°C to 1,800 min at 18.4°C, with 6 to 12 sampling times.

Developing the predictive tools: determination of LPD and GR. In experiments with bratwurst mix to develop the predictive tools, three bags per inoculum type (one per trial) were

TABLE 2. *Pathogen strains used for development and testing of the predictive tools*

Species	Strain no.	Isolated from:	Source[a]
E. coli O157:H7	USDA-FSIS 380-94	Salami implicated in illness outbreak	1
E. coli O157:H7	ATCC 43894	Clinical sample	2
E. coli O157:H7	ATCC 43895	Ground beef implicated in illness outbreak	2
E. coli O157:H7	ATCC 51657	Clinical sample	2
E. coli O157:H7	ATCC 51658	Clinical sample	2
Salmonella Typhimurium	S9	Clinical sample, Wisconsin Laboratory of Hygiene	3
Salmonella Heidelberg	S13	Clinical sample, Wisconsin Laboratory of Hygiene	3
Salmonella Infantis	S20	Unknown	3
Salmonella Hadar	S21	Unknown	3
Salmonella Enteritidis	E40	Chicken ovary isolate, New York Department of Health	3
S. aureus	ATCC 12600	Clinical sample	2
S. aureus	ATCC 25923	Clinical sample	2
S. aureus	FRI-100	Cake implicated in illness outbreak	4
S. aureus	FRI-472	Turkey salad implicated in illness outbreak	4
S. aureus	FRI-1007	Genoa salami implicated in illness outbreak	4

[a] Sources: 1, Dr. John Luchansky, formerly Food Research Institute, University of Wisconsin–Madison, now at USDA, ARS, Eastern Regional Research Center, Wyndmoor, PA; 2, American Type Culture Collection, Manassas, VA; 3, Dr. Eric Johnson, Food Research Institute, University of Wisconsin–Madison; 4, Dr. Amy Wong, Food Research Institute, University of Wisconsin–Madison.

removed at each sampling time from the water bath or incubator. The outer surface of each bag was sanitized with 70% ethanol and allowed to dry. Once dry, the contents of each bag were transferred to a filter bag (15.25 by 23 cm). The original sample bag was everted to expose any inoculum still on the bag and was also placed into the filter bag. The sample and original sample bag were combined with 99 ml of BPD, stomached at normal speed for 30 s using a stomacher lab blender (Fisher), and serially diluted (in BPD). Similar sampling and initial sample homogenization were done at each sampling time in experiments to test the predictive tool (see below). For each dilution, 100 μl was spread on a single plate of the appropriate selective medium by using a sterile bent plastic spreader. The selective medium used for *Salmonella* serovars was XLD agar (Oxoid, Ogdensburg, NY), on which typical colonies have a black center and a well-defined clear-to-opaque halo. The selective medium used for *E. coli* O157:H7 was sorbitol MacConkey agar (SMAC; Difco), on which typical colonies are colorless to white and opaque. The selective medium used for *S. aureus* was Baird-Parker agar base (B-P; Difco) with tellurite egg yolk supplement (Difco). Typical *S. aureus* colonies on B-P are shiny black with a distinctive clear zone in the surrounding agar. The SMAC and XLD plates were incubated at 35°C for 24 h, and the B-P plates were incubated at 35°C for 48 h. After colony enumeration, one typical colony of each pathogen per test temperature was transferred to BHIA and grown overnight at 35°C for confirmation. Gram reaction, cell morphology, and colony morphology were observed for all isolates. Presumptive *Salmonella* serovars were confirmed using the oxidase test (DrySlide kit; Fisher Scientific) and API 20E biochemical characterization (bioMerieux, Hazelwood, MO), and an oxidase test and latex agglutination test (Oxoid) were used to confirm presumptive *E. coli* O157:H7. A latex agglutination test (Oxoid) was performed for presumptive *S. aureus* colonies.

For each pathogen and test temperature, the log CFU per sample was determined at each sampling time for each of the three trials. Three data treatment approaches were used in entering these sampling time and log CFU per sample data for each pathogen and test temperature into the DMFit 2.0 program (J. Baranyi, Institute of Food Research, Norwich Research Park, Norwich, UK). These approaches were (i) to enter all data from the three trials into DMFit 2.0 at once to obtain a single best-fit growth curve, with an estimated LPD, GR, and corresponding R^2 value (hereafter referred to as treatment a); (ii) to enter data for one trial at a time, obtain an LPD and GR value for each trial, and then calculate mean LPD and GR values and standard deviation (hereafter referred to as treatment b); and (iii) calculate average log CFU per sample values for each sampling time, enter the average value, and obtain a single curve with LPD, GR, and R^2 values (hereafter referred to as treatment c). The most conservative values, i.e., the smallest LPD and the largest GR, were then used in THERM.

The THERM predictive tool. THERM is a tool which uses a sequence of time-temperature combinations to predict the extent of pathogen growth. When entered temperatures correspond to temperatures tested in experiments, the LPD and GR values directly determined from experimental data are used to predict growth. When entered temperatures are different from those used in experiments, the LPD and GR values are calculated using linear interpolation between values for the two experimental temperatures closest to the entered temperature. Using the calculated LPD and GR values, THERM uses an interval accumulation strategy to calculate first the time elapsing before the pathogen would begin growing and then the amount of growth that would occur. An interval was defined as the difference in time values between two entered time-temperature data pairs. The percent LPD elapsing in each time interval (constant temperature assumed) was estimated by dividing the interval time by the LPD for the final temperature in the interval and multiplying the resulting value by 100. The percent LPD contributed by each interval was accumulated until 100% of the time in lag phase had elapsed (equation 1).

$$\text{Total \% LPD} = \sum_{i=1}^{N} \frac{\text{interval time}}{\text{LPD}_i} \times 100 \qquad \text{(A)}$$

After calculations had determined that lag phase was complete, interval accumulation was used to estimate subsequent growth, in log CFU. The log CFU of growth was computed by multiplying GR (log CFU per minute) either by the time (minutes) remaining in the interval during which lag phase ended or by the total time of the interval (for all intervals thereafter) (equation 2).

$$\text{Total growth} = \sum_{i=1}^{N} \text{GR for interval}_i \times \text{interval time}_i$$

Temperature was assumed to be constant throughout each interval and was the final temperature occurring in the interval.

Testing predictive tool performance in commercial-product inoculation studies. The accuracy of the THERM predictive tool was tested using data from six different inoculation experiments. For each experiment, multiple sausages were inoculated with pathogen(s) and subjected to a temperature abuse regime. Uninoculated sausages were used to obtain product time-temperature histories during the temperature abuse regime. Time-temperature data were then entered in THERM to obtain predictions of pathogen growth. Samples of product were also analyzed to determine the actual extent of pathogen growth. Comparisons were then made between observed pathogen growth and pathogen growth predicted by THERM.

Inoculation studies were done with *Salmonella* serovars, *E. coli* O157:H7, and *S. aureus* in one commercial brand each of beer bratwurst (for which the recipe includes beer), standard bratwurst, and breakfast link sausage. Each sausage product was obtained from a local retail store. A representative sample of each product was sent to a commercial laboratory for chemical and physical analysis (Table 1). Inocula were prepared as described previously, with dilutions made to create a suspension containing ca. 4 log CFU/ml. Three sets of each inoculum were independently prepared for each experiment, and the sample unit for each experiment was a single sausage per sampling time. For each experiment, 19 sample units were prepared (9 units inoculated with *Salmonella* serovars and *E. coli* O157:H7, 9 units inoculated with *S. aureus,* and 1 unit containing the temperature probe attached to a data logger as described earlier). To inoculate a sausage, the sausage was slit open lengthwise using an ethanol- and flame-sanitized knife and placed in a sterile sampling bag (15 by 23 cm). Then, 1.0 ml of either the *Salmonella* serovars and *E. coli* O157:H7 inoculum or the *S. aureus* inoculum was pipetted into the exposed raw sausage, the sample bag was closed, and the sausage was manually massaged for 30 s to disperse the inoculum. The inoculated samples were then stored overnight at 5°C to simulate the common industry practice of refrigerating raw products before and after processing. After the refrigerated storage period, the samples were placed into an incubator. The incubator temperature was adjusted to produce four different time-temperature profiles intended to be similar to what may occur during processing or distribution. In the first situation, beer bratwurst and breakfast sausage were held in a 25°C incubator for 3 h and then refrigerated. Three samples each per inoculum were analyzed at the start

TABLE 3. *Lag-phase duration (LPD) derived using DMFit for* Salmonella *serovars,* E. coli *O157:H7, and* S. aureus *in raw bratwurst*

	Salmonella serovars			*E. coli* O157:H7			*S. aureus*		
Temp (°C/°F)	LPD (h)[a]	Data treatment	Variability[b]	LPD (h)	Data treatment	Variability	LPD (h)	Data treatment	Variability
46.1/115	NR[c]			NR			1.6	a	0.80
43.3/110	3.7	a	0.81	3.5	a	0.75	2.4	a	0.97
40.6/105	4.2	a	0.75	3.0	c	0.94	1.8	a	0.91
37.8/100	2.6	a	0.90	4.1	a	0.93	1.8	b	0.1 h
35.0/95	5.4	a	0.91	5.7	c	0.99	1.9	a	0.99
32.2/90	4.5	b	0.7 h	3.4	a	0.98	2.6	c	0.99
29.5/85	7.4	c	0.96	4.7	c	0.97	2.7	c	0.97
26.7/80	4.5	c	0.99	4.5	a	0.98	3.4	b	0.7 h
23.9/75	11.0	c	0.65	8.5	c	0.96	5.3	b	Range, 0.5 h
21.1/70	13.1	c	0.86	11.1	b	0.4 h	6.1	c	0.96
18.4/65	ND[d]		ND	ND		ND	8.5	c	0.99

[a] Values shown are lowest LPD values obtained when data from three concurrent trials were subjected to treatments a, b, or c as explained in the text.

[b] Value for variability is either R^2 (data treatments a and c), standard deviation (data treatment b, with $n = 3$), or range (data treatment b with $n = 2$). Indigenous microbial load before inoculation averaged 3.7 log CFU/g.

[c] NR, no LPD value provided because of poor curve fit.

[d] ND, not determined (no experiment conducted).

of the experiment, at the end of the 25°C incubation, and after 4 h of refrigeration. In the second situation, beer bratwurst and breakfast sausage were held in a 35°C incubator for 4 h and then refrigerated, with samples analyzed at the start, after the 35°C incubation, and after 4 h of refrigeration. For the third and fourth situations with standard bratwurst, the 25 and 35°C incubations were extended to 7 and 8 h, respectively, before the product was refrigerated. Samples were analyzed at the start of each experiment, immediately before the product was moved to refrigerated storage, and after the product had been refrigerated 4 h.

The time-temperature output obtained from the data logger in each inoculation experiment was evaluated, and the portion of time for which the product internal temperature was at or above the lowest observed growth temperature for the pathogen of interest was divided into 20 equal intervals. These time-temperature pairs were entered in THERM.

Microbiological analyses to enumerate inoculum organisms were conducted as described earlier. After colony enumeration, one typical colony of each pathogen per experiment was transferred to BHIA and grown 24 h at 35°C for confirmation (described above).

Statistical analyses. For each product analyzed in each experiment done to test THERM accuracy, a mean ($n = 3$) log CFU value was calculated for each pathogen at each sampling time. Then, the mean log CFU value at the final sampling time was compared to the mean log CFU value for the start of the experiment to obtain an observed change in log CFU value. Time-temperature data from each experiment were entered into THERM to obtain predicted changes in log CFU values. The predicted and observed changes in log CFU values were qualitatively described either as growth (>0.3 log CFU increase) or no growth (≤0.3 log CFU increase). A THERM prediction was classified as accurate when it was the same as the observed result, e.g., growth was predicted and observed; it was classified as ''fail-safe'' if growth was predicted but not observed in the experiment; and it was classified as ''fail-dangerous'' if no growth was predicted but growth was observed in the experiment. The paired *t* test (Minitab Release 12.1, Minitab, Inc., State College, PA) was used to compare LPD and GR values for a given pathogen in raw bratwurst mix to those obtained previously in raw ground pork *(10).* A significance level of 0.05 was used.

RESULTS

Earlier experiments in our laboratory showed that the level of indigenous microorganisms had a significant effect on LPD values for *S. aureus (3)* but not for *Salmonella* serovars and *E. coli* O157:H7. Larger *S. aureus* LPD values resulted when there were greater numbers of indigenous organisms. Therefore, in order to develop a conservative predictive tool, all experiments used to develop the predictive tool for bratwurst were done with product that had what we considered to be a low level of background organisms, i.e., a mean of 3.7 log CFU/g ($n = 15$; standard deviation, 0.4). Indigenous microorganism counts were obtained using a 35°C incubation temperature. Higher plate count values might have been obtained if an incubation temperature more suitable for growth of psychrotrophic bacteria had been used, although the longer incubation time required for such an analysis discourages its use by meat processors and regulators.

Using the three data treatment approaches and the DMFit 2.0 program, we were able to determine conservative LPD and GR values for pathogens in bratwurst, based on a best-fit microbial growth curve(s) produced by the software (values shown in Tables 3 and 4). For each data treatment approach, a curve with a high R^2 or small standard deviation and a curve with a low R^2 or large standard deviation are shown in Figure 1. Variability was too high to obtain useful LPD and GR values for *E. coli* O157:H7 at 46.1°C (115°F) and *Salmonella* serovars at 46.1°C (115°F). *S. aureus* was the only organism studied at 18.4°C (65°F). As expected, LPD generally decreased and GR generally increased as temperature approached an optimum for growth. When data treatments a and c (described in ''Developing the prediction tools: determination of LPD and

TABLE 4. *Growth rates (GR) derived using DMFit for* Salmonella *serovars,* E. coli *O157:H7, and* S. aureus *in raw bratwurst*

	Salmonella serovars			*E. coli* O157:H7			*S. aureus*		
Temp (°C/°F)	GR[a] (log CFU/h)	Data treatment	Variability[b]	GR (log CFU/h)	Data treatment	Variability	GR (log CFU/h)	Data treatment	Variability
46.1/115	NR[c]			NR			0.46	c	0.96
43.3/110	0.78	a	0.81	0.72	a	0.75	1.44	a	0.97
40.6/105	0.59	c	0.84	0.40	c	0.94	1.02	b	0.14 log CFU/h
37.8/100	0.43	a	0.90	0.53	a	0.93	0.84	a	0.94
35.0/95	0.66	c	0.96	0.60	b	0.26 log CFU/h	0.74	c	0.99
32.2/90	0.38	b	0.09 log CFU/h	0.38	b	0.14 log CFU/h	0.58	b	0.090 log CFU/h
29.5/85	0.46	b	0.09 log CFU/h	0.50	b	0.25 log CFU/h	0.60	a	0.95
26.7/80	0.30	c	0.99	0.30	a	0.98	0.43	a	0.99
23.9/75	0.34	a	0.75	0.37	a	0.94	0.25	a	0.92
21.1/70	0.14	a	0.63	0.16	a	0.87	0.16	c	0.96
18.4/65	ND[d]		ND	ND		ND	0.13	b	0.032 log CFU/h

[a] Values shown are lowest GR obtained from three concurrent trials with three data treatments (a, b, and c) as explained in the text.
[b] Value for variability is either R^2 (data treatments a and c), standard deviation (data treatment b with $n = 3$), or range (data treatment b with $n = 2$). Indigenous microbial load before inoculation averaged 3.7 log CFU/g.
[c] NR, no LPD value provided because of poor curve fit.
[d] ND, not determined (no experiment conducted).

GR'' above) produced the most conservative LPD value, the R^2 values ranged from 0.65 to 0.99, with 19 of 25 values of $\geq$0.90. When data treatment b produced the most conservative LPD value, the corresponding standard deviation ranged from 0.1 to 0.7 h. Data treatments a or c yielded the largest GR values for 21 of 30 of meat-pathogen-temperature combinations with R^2 values ranging from 0.63 to 0.99, and 15 of 21 R^2 values of $\geq$0.90. For the six meat-pathogen-temperature combinations for which data treatment b yielded the largest GR value, standard deviations ranged from 0.032 to 0.26 log CFU per h.

Among the commercial products used to test THERM, the breakfast links had a pH lower than that of the bratwurst used in THERM development, while the beer and standard bratwursts had higher pH values (Table 1). The breakfast links, beer bratwurst, and standard bratwurst all contained less salt and water than the bratwurst used to develop THERM but had very similar water activity values (0.97 to 0.98). Neither *Salmonella* serovars nor *E. coli* O157:H7 grew in the beer bratwurst stored at either 25 or 35°C (Table 5). Similarly, neither of these two pathogens grew in breakfast sausage stored at 25°C. In standard bratwurst stored at 25°C, *Salmonella* serovars and *E. coli* O157:H7 increased by 0.6 and 0.4 log CFU, respectively. However, when standard bratwurst or breakfast links were stored at 35°C, growth of these two pathogens ranged from 0.7 to 3.8 log CFU. *S. aureus* grew well in breakfast links and standard bratwurst stored at 35°C but did not grow in beer bratwurst stored for a shorter time at 35°C. *S. aureus* did not grow in the beer bratwurst and breakfast links stored at 25°C and grew by 0.8 log CFU in the standard bratwurst during 25°C storage.

The greatest observed change in log CFU value was 5.4 for *S. aureus* in breakfast sausage stored at 35°C, while the greatest predicted change in log CFU values for THERM was 5.5 for *S. aureus* in the same experiment (Table 5). Using the criteria of growth being defined as a change in log CFU of >0.3 (more than one doubling) and of no growth being defined as a change in log CFU of $\leq$0.3, we qualitatively evaluated the predicted and observed changes in log CFU values, i.e., described predictions and observations as either ''growth'' or ''no growth.'' The THERM tool predicted pathogen growth in all nine pathogen-experiment combinations when it was observed experimentally. The THERM tool also accurately predicted no growth in nine combinations.

When compared to observed changes in log CFU values for the nine pathogen-experiment combinations in which pathogens grew, the predicted values of change in log CFU were within 0.3 log CFU for three combinations, exceeded observed values by 0.4 to 1.5 log CFU in four combinations, and were 1.2 and 1.4 log CFU lower in two combinations. Clearly, for the three sausage products tested, the THERM tool performed better when making qualitative predictions than quantitative predictions.

DISCUSSION

In earlier studies *(3),* we determined that growth of *Salmonella* serovars and *E. coli* O157:H7 as part of a two-species inoculum was not significantly different ($P > 0.05$) from that of these two species when inoculated individually. Specifically, raw ground pork was inoculated separately with *Salmonella* serovars or *E. coli* O157:H7, or with these two pathogens together. Inoculated pork was held at 32.2°C

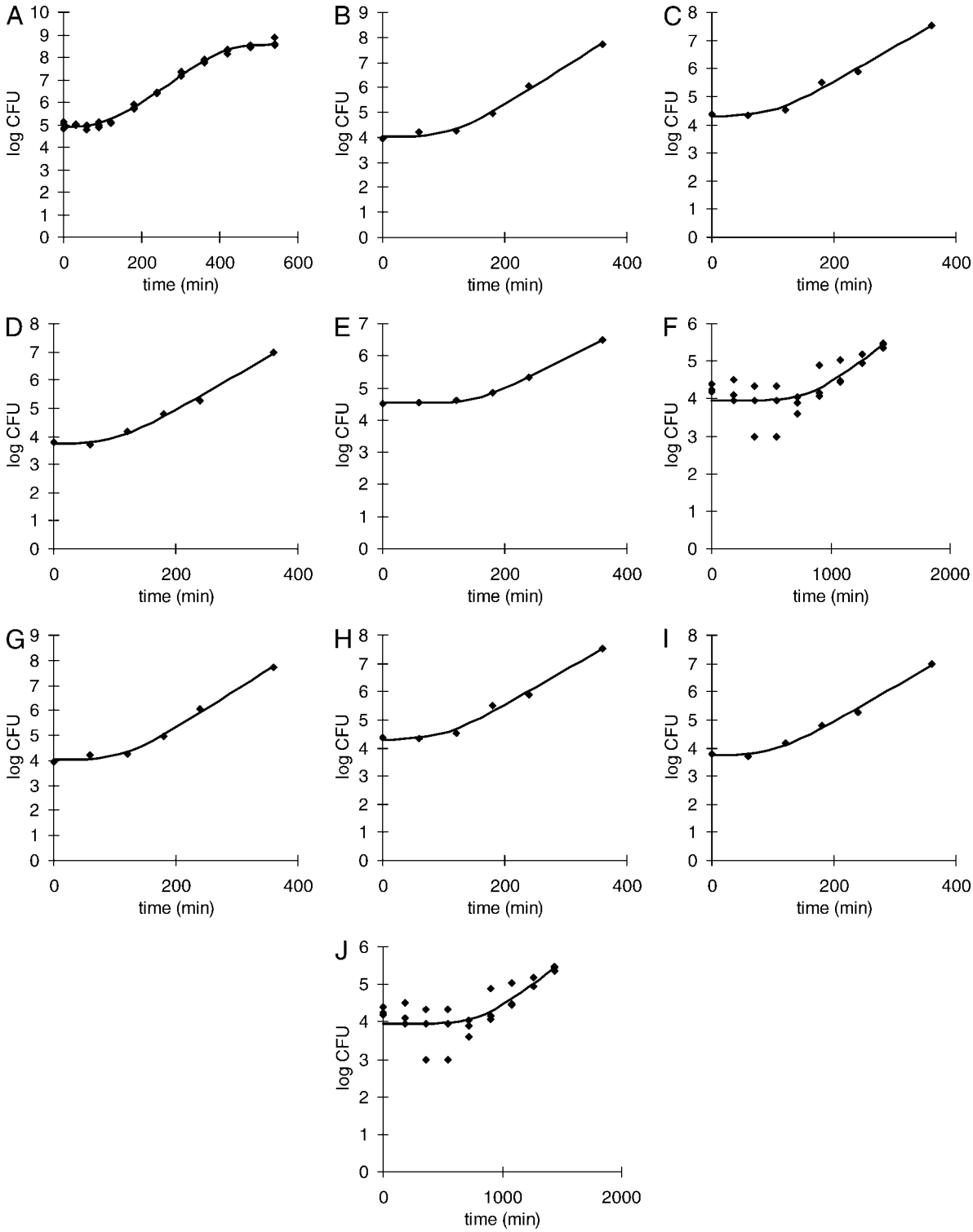

FIGURE 1. *DMFit 2.0 curves with high and low goodness of fit for pathogenic bacteria in raw bratwurst during isothermal storage. Three data treatment approaches were used in entering the sampling time and log CFU per sample data: (i) enter all data from the three trials into DMFit 2.0 at once to obtain a best-fit growth curve, with an estimated LPD (LPD), growth rate (GR), and corresponding* R^2 *value (treatment a); (ii) enter data for one trial at a time, obtain an LPD and GR value for each trial, and then calculate mean LPD and GR values and standard deviation (treatment b); and (iii) calculate average log CFU per sample values for each sampling time, enter the average value, and obtain single LPD, GR, and* R^2 *values (treatment c). Shown are curves for* S. aureus *at 35°C, with data treatment a,* $R^2 = 0.99$ *(A);* S. aureus *at 37.8°C, with data treatment b, LPD standard deviation of 0.1 h (B through D);* S. aureus *at 32.2°C, data treatment c,* $R^2 = 0.99$ *(E);* Salmonella *at 21.1°C, data treatment a,* $R^2 = 0.63$ *(F);* Salmonella *at 32.2°C, data treatment b, LPD standard deviation of 0.7 h (G through I); and* Salmonella *at 23.9°C, data treatment c,* $R^2 = 0.65$ *(J).*

TABLE 5. *Observed and predicted growth of* Salmonella *serovars,* E. coli *O157:H7, and* S. aureus *in beer bratwurst, standard bratwurst, and breakfast links during storage at abusive temperatures*[a]

Product	Temp abuse conditions		Mean log CFU/g (SD) (n = 3)							
			Salmonella serovars			*E. coli* O157:H7			*S. aureus*	
	Temp (°C)	Time (h)	T_0	$T_{i\text{-obs}}$	$T_{i\text{-pred}}$	T_0	$T_{i\text{-obs}}$	$T_{i\text{-pred}}$	T_0	$T_{i\text{-obs}}$	$T_{i\text{-pred}}$
Beer bratwurst	25	3	3.6 (n = 2)	3.5 (0.2)	3.6	3.8 (0.1)	3.9 (0.1)	3.8	3.8 (0.1)	3.8 (0.1)	3.8
	35	4	3.6 (0.1)	3.5 (0.1)	3.6	3.6 (0.3)	3.7 (0.1)	3.6	3.9 (0.1)	3.8 (0.1)	3.9
Standard bratwurst	25	7	3.7 (0.1)	4.3 (0.2)	4.3	3.7 (0.1)	4.1 (0.2)	4.5	3.8 (0.1)	4.6 (0.3)	5.4
	35	8	3.7 (0.1)	5.4 (0.3)	5.8	3.8 (0.1)	4.5 (0.8)	4.8	3.7 (0.1)	7.4 (0.1)	8.9
Breakfast links	25	3	3.4 (0.2)	2.7 (0.7)	3.4	3.8 (0.1)	3.7 (0.1)	3.8	2.9 (0.1)	3.1 (0.2)	3.0
	35	4	3.4 (0.3)	7.2 (1.0)	5.8	3.7 (0.1)	6.9 (0.2)	5.7	3.0 (0.1)	8.4 (0.1)	8.7

[a] T_0, start of temperature abuse; $T_{i\text{-obs}}$, duration of temperature abuse plus 4 h of refrigeration; $T_{i\text{-pred}}$, change in log CFU per gram (predicted by THERM) added to the T_0 log CFU per gram value.

for 6 h, and the growth of each pathogen over the storage period was determined in three independent trials. *Salmonella* serovars when inoculated singly or in combination with *E. coli* O157:H7 grew by 2.6 log CFU (standard deviation, 0.2) or 2.8 log CFU (standard deviation, 0.2), respectively. Corresponding values for *E. coli* O157:H7 were 3.0 (standard deviation, 0.2) or 2.8 (standard deviation, 0.2).

In our earlier article describing THERM *(10),* we pointed out some potential shortcomings of using multiple-strain inocula. However, we believe that the benefit of using multiple strains, namely, increasing the likelihood of including a rapidly growing strain and creating a worst-case situation for pathogen growth in pork sausage, outweighed these drawbacks.

By developing THERM with bratwurst, we accounted for the presence of some ingredients, e.g., fat, sodium chloride, and spices, to which pathogens or competing microorganisms may be exposed during temperature abuse of raw meat products. When the growth substrate for *Salmonella* serovars, *E. coli* O157:H7, and *S. aureus* was ground pork, beef, or turkey with no added salt or spices, *S. aureus* generally had the longest LPD. *S. aureus* had the longest LPD in 29 of a total of 32 combinations of temperature and ground meat type *(10).* However, the composition of bratwurst favored the initiation of *S. aureus* growth over that of *Salmonella* serovars and *E. coli* O157:H7. Among the nine temperatures for which LPD values in bratwurst were determined for all three pathogens (Table 3), *S. aureus* always had the smallest LPD value, i.e., began growing soonest. A similar trend was seen for GR. In ground pork, beef, and turkey with no added ingredients, *S. aureus* had the lowest GR value in 23 of 32 temperature-meat type combinations *(10).* By comparison, in bratwurst *S. aureus* had the most rapid growth among the three pathogens in seven of nine temperatures (Table 4). These results clearly show that the addition of salt and spices to ground meat is more inhibitory to *Salmonella* serovars and *E. coli* O157:H7 than to *S. aureus.*

Comparisons of LPD and GR values determined in raw ground pork with those determined in raw bratwurst mix showed that LPD was higher and GR was lower in bratwurst for *Salmonella* serovars and *E. coli* O157:H7. These differences were statistically significant for *Salmonella* serovars LPD ($P = 0.003$) and GR ($P = 0.016$) and *E. coli* O157:H7 LPD ($P = 0.001$), but not for *E. coli* O157:H7 GR ($P = 0.131$). When LPD values for *S. aureus* were compared between bratwurst (Table 3) and ground pork *(10),* the values were lower in bratwurst for all but two of the 10 temperatures tested. GR for *S. aureus* was higher in bratwurst (Table 3) than in ground pork *(10)* for 7 of the 10 temperatures tested. However, these differences were not statistically significant ($P = 0.189$ for LPD, and $P = 0.219$ for GR). Clearly, the inclusion of salt and spices and the higher fat content of bratwurst mix had no significant inhibitory effect on *S. aureus.*

The LPD values for *Salmonella* serovars in bratwurst (this study) were considerably smaller than those reported for *Salmonella* Typhimurium DT104 in chicken frankfurt-

ers. Likewise, the GR values for *Salmonella* serovars in bratwurst were considerably larger than reported for the *Salmonella* Typhimurium DT104 in chicken frankfurters *(13).* These differences could have resulted from additional antimicrobial ingredients in the frankfurters (potassium lactate and sodium diacetate), inherent differences in growth characteristics between at least one of the strains we used and the single strain used in the frankfurter study, or differences in competing microorganisms in the two products.

As developed, THERM will calculate potential pathogen growth in raw sausage only over the arbitrary temperature range of 21.1 to 43.3°C (*Salmonella* serovars and *E. coli* O157:H7) or 18.4 to 46.1°C (*S. aureus*). Some pathogenic *E. coli* strains may be capable of growth at temperatures above or below these limits *(14),* although the ability of hemorrhagic *E. coli* to grow at 8°C in ground beef has been reported to decrease when high levels of background organisms are present *(15).* Growth at temperatures below 21.1 or 18.4°C (THERM lower limits) would likely have little effect on the accuracy of THERM predictions because of the long LPD expected at such low temperatures. However, THERM growth predictions could be erroneously low if growth occurred at temperatures above the 43.3 or 46.1°C upper limit.

For some tested temperatures, the experimentally derived LPD and GR values appeared to be inaccurate in comparison to values at similar temperatures. For example, the LPD of 7.4 h for *Salmonella* serovars in bratwurst at 29.5°C (85°F) was higher than the LPD value of 4.5 h at 26.7°C (80°F). Seemingly aberrant values such as this are likely the result of experimental variability and may adversely affect the calculation of other LPD and GR values by linear interpolation.

An important feature of the THERM tool is that it was developed using multistrain inocula in actual nonsterile food, enhancing its applicability to industry use. Many other nonisothermal predictive models have been developed using a single bacterial strain in sterile laboratory medium *(2, 9, 17),* limiting their usefulness to industry. A notable exception is the work of Koutsoumanis et al. *(12),* who studied the growth of several different bacterial groups, e.g., *Enterobacteriaceae,* in commercial ground pork stored under periodic temperature abuse regimes. However, these authors did not evaluate growth of pathogens.

One major constraint in predicting growth of bacteria under nonisothermal conditions is understanding how the physiological condition of cells changes as the temperature changes. Baranyi and Roberts *(1)* created one mathematical solution to this problem with an ''adjustment factor.'' In developing THERM, we indirectly considered the physiological condition of the pathogen cells at each successive temperature by calculating the percentage of LPD that had previously elapsed. We chose not to add an adaptation time to the LPD or to the attainment of the predicted growth rate following a temperature shift. This approach is purposely conservative because it is more likely to overpredict growth than to underpredict it.

Another challenge in developing nonisothermal predictive tools is that of choosing a wide enough range of realistic time-temperature regimes for testing the tool. We chose to use a time-temperature regime simulating a product warming up during processing or delivery, and then being returned to proper refrigerated conditions—conditions commonly encountered in the meat industry. Koutsoumanis *(11)* also used this time-temperature regime in testing his predictive model. Other regimes tested include single upshifts in temperature *(17),* a decrease in temperature followed by a steady increase *(2),* periodic fluctuations in temperature *(1, 9, 11, 12),* and a steady decrease in temperature *(2).* A wider range of time-temperature regimes should be used in further testing of THERM.

Unlike many predictive models, THERM does not consider a maximum population density. As a result, for longer periods of temperature abuse, THERM is likely to predict unrealistically high levels of growth. However, we feel that this theoretical shortcoming in THERM is not germane in the applications for which THERM is intended to be used. For example, it is not important whether THERM predicts 7 or 9 log CFU of *S. aureus* growth, because either level of growth would be strong evidence that the sausage is unsafe to eat.

The U.S. Department of Agriculture (USDA) currently will not accept predictions from computer-based predictive models as the sole supporting information for establishing a critical limit or planning a corrective action in the HACCP system *(7).* Processors must therefore obtain additional information, such as indicator bacteria test results from samples during the actual process or a process simulation, to fully support critical limit and corrective-action decision making. This additional information would likely provide an additional guarantee of product safety.

Ideally, predictive tools such as THERM would be part of a comprehensive assessment of the risk of pathogen growth in raw meats. Essentially a risk assessment of this sort involves a probability estimate for the targeted hazard, i.e., pathogen growth, and an assessment of the severity of the hazard when it does occur. The probability of the hazard occurring and the assessed severity of the hazard are then used in developing strategies for risk management and risk communication.

In raw meat processing, the risk of pathogen growth is dependent on the likelihood of the pathogen being present and the time-temperature history of the product. It is USDA policy to assume that pathogenic bacteria are present in raw meats and poultry. This policy is manifested in the ''safe handling'' label mandated for all packages of inspected product and in HACCP plan reassessments that were mandated for processors of raw beef product in 2002 *(6)* and 2007 *(8).* Tools such as THERM evaluate time-temperature history and provide either a binary (growth versus no growth) prediction or a quantitative (change in log CFU) prediction of pathogen growth in the product. For a more correct hazard probability estimate, tools are needed that forecast the probability, e.g., 90%, of pathogen growth resulting from a particular time-temperature history.

The severity of the hazard resulting from pathogen growth in raw meats is debatable. Current USDA performance standards for cooking of meat and poultry products

are based on the assumption that very high levels of salmonellae are present *(5)*. It could be argued that, aside from the situation of raw products recontaminating ready-to-eat products, there is very little risk of illness resulting from pathogen growth in raw meats as long as the raw product is later sufficiently cooked. Following this logic, one could use a higher threshold level for growth versus no growth in THERM than the 0.3-log increase used in the present study. However, the processor making raw-meat and poultry products cannot rely on purchasers of these products to properly handle and cook them. Further, because there is a probability that some unknown proportion of purchasers will mishandle or undercook raw meat or poultry, predicting the severity of the hazard resulting from pathogen growth in raw meats or poultry is fraught with uncertainty. Despite these difficulties, THERM is an important research-based tool for use in raw meat and poultry safety systems.

In conclusion, THERM provides accurate growth/no growth predictions of pathogen growth in raw sausage. This tool will be useful to sausage processors who must validate critical limits or support corrective actions taken after a deviation. Ongoing experiments with additional raw sausage products and temperature conditions will further validate this tool.

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