Journal of Food Protection, Vol. 72, No. 10, 2009, Pages 2114–2124

Predicting Behavior of *Staphylococcus aureus, Salmonella* Serovars, and *Escherichia coli* O157:H7 in Pork Products during Single and Repeated Temperature Abuse Periods

STEVEN C. INGHAM,[1] SONG VANG,[2] BEN LEVEY,[2] LISA FAHEY,[2] JOHN P. NORBACK,[2] MELODY A. FANSLAU,[3] ANDRE G. SENECAL,[4] GREG M. BURNHAM,[4] AND BARBARA H. INGHAM[2]*

[1]*Wisconsin Department of Agriculture, Trade & Consumer Protection, Division of Food Safety, Madison, Wisconsin 53708;* [2]*Department of Food Science, University of Wisconsin–Madison, Madison, Wisconsin 53706;* [3]*Fair Oaks Farms, Pleasant Prairie, Wisconsin 53158; and* [4]*U.S. Army Natick Soldier Research, Development & Engineering Center, Combat Feeding Directorate, Natick, Massachusetts 01760, USA*

MS 09-051: Received 3 February 2009/Accepted 16 May 2009

ABSTRACT

Tools for predicting growth of *Staphylococcus aureus, Salmonella,* and *Escherichia coli* O157:H7 (THERM; temperature history evaluation for raw meats) have been developed using ground pork and sausage. THERM tools have been tested with three types of pork sausage but not with other pork products or during sequential temperature abuse periods. We conducted inoculation studies (five strains each of *S. aureus* and/or *Salmonella* plus *E. coli* O157:H7) with simulated cooling of warm sausages, in-process warming of bratwurst, isothermal temperature abuse of pork frankfurter batter, and two sequential periods of 13, 15.6, or 21.1°C temperature abuse of breakfast sausage, natural (additive-free) chops, and enhanced (phosphate solution–injected) loins. In sequential temperature abuse studies, a temperature abuse period (≤24 h) occurred before and after either refrigeration (5°C for 24 h), or freezing (−20°C for 24 h) and thawing (24 h at 5°C). Pathogen growth predictions from THERM developed using ground pork and sausage were compared with experimental results of 0 to 3.0 log CFU of growth. Across all temperature abuse conditions, qualitative predictions (growth versus no growth) made using the pork tool ($n = 133$) and the sausage tool ($n = 115$) were accurate (51 and 50%, respectively), fail-safe (44 and 50%), or fail-dangerous (5 and 0%). Quantitative predictions from the two tools were accurate (29 and 22%, respectively), fail-safe (59 and 73%), or fail-dangerous (12 and 5%). Pathogen growth was greater during the second sequential temperature abuse period but not significantly so ($P > 0.05$). Both THERM tools provide useful qualitative predictions of pathogen growth in pork products during isolated or sequential temperature abuse events.

The U.S. meat and poultry industry operates under regulations that mandate the use of a hazard analysis critical control point (HACCP) system for ensuring food safety *(16)*. Under this system, processors of raw meat and poultry products must obtain scientifically valid information to support corrective actions taken when critical limits at a processing critical control point are exceeded, i.e., when there is a process deviation. The authors' experiences suggest that a common meat processing deviation is loss of temperature control during the receipt, storage, or processing of raw meat and poultry products and that although processors may take rapid corrective action to regain temperature control of products when these deviations occur, the affected products later may experience another period of temperature abuse.

Scientifically based computer tools for predicting pathogen growth in raw meat are potentially important sources of information that processors and regulators need to evaluate meat processing deviations. Such tools must be capable of accurately predicting bacterial behavior under dynamic temperature conditions, a situation that complicates tool development. Meat temperatures may fluctuate widely during temperature abuse deviations, and even in well controlled raw meat processing systems, meat temperature can change during steps such as grinding, mixing, or packaging. Several researchers have reported on the development and application of mathematical bacterial growth models for dynamic conditions *(3, 4, 7, 12, 13, 22)*. One major question to be addressed in developing these models is the extent to which bacterial lag-phase duration (LPD) and growth rate (GR) change with changing temperature. Baranyi and Roberts *(3)* presented 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. Incorporating this function into a predictive equation eliminated the need to separately predict LPD. In a later study, Baranyi et al. *(4)* used this approach for predicting growth of *Brochothrix thermosphacta* in a laboratory medium under nonisothermal conditions. Accurate predictions were obtained for some nonisothermal regimes, but less accurate predictions were obtained when the temperature fell below a minimum level. Working with *Lactobacillus plantarum* in a laboratory medium, Zwietering et al. *(22)* developed separate predictions for LPD and GR and concluded that exposure of lag phase cells to a shift in temperature resulted in an LPD at the

* Author for correspondence. Tel: 608-263-7383; Fax: 608-262-6872; E-mail: bhingham@wisc.edu.

new temperature that was 25% longer than the expected remaining proportion of the lag phase. These authors also reported an adaptation-related delay when growing cells were exposed to a new temperature, i.e., the cells did not immediately have the GR normally observed at the new temperature. These authors suggested, however, that ignoring adaptation-related delays was a simple approach to prediction, particularly for situations with frequent temperature changes, and would still provide a high level of predictive accuracy. Zwietering et al. *(22)* suggested that ignoring the adaptation times would be expected to increase the likelihood of a fail-safe prediction, i.e., overprediction of growth, when the temperature increased over time, such as when temperature control is lost during the processing of raw meat.

Koutsoumanis *(12)* used a numerical integration approach to predict the LPD for pseudomonads on fish under nonisothermal conditions and then used an interval accumulation strategy to predict subsequent growth. 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 subjected to periodic temperature abuse *(13)*. Fujikawa et al. *(7)* studied *Escherichia coli* growth in a laboratory medium and developed a logistic growth prediction model that included a differential equation with a lag phase term. These authors were able to accurately predict growth when the temperature periodically fluctuated within the 27.6 to 36.0°C range over a period of 8 to 12 h.

In previous articles, 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 *(8, 9)*. 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 for use by meat and poultry processors to support corrective action decisions. The THERM tool uses linear interpolation of experimentally determined pathogen LPD and GR data in an interval accumulation technique to predict behavior of *Staphylococcus aureus, Salmonella* serovars, or *E. coli* O157:H7 in raw pork, beef, poultry, or sausage based on the time and temperature history entered by the user. The THERM tool performs best when used for qualitative evaluations, i.e., to predict growth or no growth, but also provides a numerical estimate of growth. To simplify the mathematics supporting the THERM tool, we treated lag phase and growth phase as distinct sequential conditions of the pathogen cells. We also assumed that the pathogen cells instantaneously attained a new LPD or GR upon exposure to each new temperature, thereby minimizing the likelihood of underpredicting pathogen growth.

The three pathogens for which THERM makes predictions are significant hazards in raw meats. Growing *S. aureus* cells can produce heat-stable enterotoxin(s), which would not be inactivated by cooking. Government-mandated performance standards for achieving lethality during cooking are based on *Salmonella* levels below a threshold level *(17)*. *E. coli* O157:H7 is considered an adulterant in nonintact raw beef products, and although this pathogen does not generally colonize swine, it could contaminate pork products via beef-to-pork cross-contamination in some meat processing plants. *Listeria monocytogenes* is not presently considered an important hazard identified in a hazard analysis for raw meat products. Rather, the current focus for preventing meat-linked listeriosis is preventing postcooking contamination, which is outside the scope of raw meat HACCP plans.

In our previous work, we conducted inoculation experiments to test the qualitative and quantitative predictive accuracy of THERM. These experiments were done with coarse-ground beef, various types of raw poultry products, two types of bratwurst, and breakfast sausage. However, no experiments to test THERM accuracy were done with other ground or intact pork products, such as enhanced (phosphate solution–injected) whole muscle products. The inoculation experiments also involved only a limited range of temperature abuse conditions. None of the experiments involved sequential periods of temperature abuse, in which the product was temperature abused, chilled to temperatures too low to allow pathogen growth, and then temperature abused a second time. In this situation, it is unclear whether the LPD during the second temperature abuse period would be the same as that during the preceding temperature abuse period. In the present study, we used experiments with various pork products and single and sequential temperature abuse conditions to test the accuracy of two THERM tools that were based on ground pork and pork sausage. The two tools were used to qualitatively and quantitatively predict behavior of *S. aureus*, *Salmonella* serovars, and *E. coli* O157:H7 under experimental conditions, and the predictions were compared with experimentally obtained values.

MATERIALS AND METHODS

Pork products. Data on pathogen responses to temperature abuse were used for comparison to pathogen responses predicted by THERM tools and were obtained in isothermal and dynamic temperature abuse trials conducted in 2004 through 2006 (experiments A through F) *(6)* and in single and sequential isothermal trials conducted in 2008 (experiments G through L). The dynamic temperature abuse trials were based on actual industry deviations encountered by the senior authors. The sequential temperature abuse trials were based on industry corrective actions and the range of mildly abusive temperatures encountered by the senior authors. The pork products and the temperature abuse conditions to which these products were exposed are listed in Table 1. For experiment A, frozen pork bratwursts (ca. 110 g each) were obtained from a local retail store and kept at −20°C until they were thawed at 5°C before use. For experiments B through E, raw hot-boned pork sausage and spiced pork sausage batters were obtained from a local processor and stored at 5°C until used. For experiment F, raw frankfurters (ca. 45 g each) were obtained from an out-of-state processor and stored at −20°C until thawed at 5°C before use. A representative sample of each of these products was sent to commercial laboratories (Silliker Laboratories of Wisconsin, Madison; and Marshfield Clinic Food Safety Services, Marshfield, WI) for pH, water activity, % moisture, % fat, % protein, and % salt using standard

TABLE 1. *Temperature abuse conditions to which inoculated pork products were exposed*

Expt	Product	Preliminary conditions[a]	Temp abuse conditions		
			Start temp (°C)	End temp (°C)	Time(h)
A	Pork bratwurst	None	9	28	4
B	Spiced pork sausage	None	20	5	7 (initial cooling rapid[b])
C	Spiced pork sausage	None	21	5	7 (initial cooling slow[c])
D	Hot-boned pork sausage	None	36	5	7 (initial cooling rapid[d])
E	Hot-boned pork sausage	None	36	8	7 (initial cooling slow[e])
F	Pork frankfurter	None	18	18	15
G-1	Sausage patty	None	13	13	24
G-2	Sausage patty	5°C, 24 h	13	13	24
G-3	Sausage patty	−20°C, 24 h→5°C, 24 h	13	13	24
H-1	Enhanced pork loin	None	13	13	24
H-2	Enhanced pork loin	5°C, 24 h	13	13	24
H-3	Enhanced pork loin	−20°C, 24 h→5°C, 24 h	13	13	24
I-1	Sausage patty	None	15.6	15.6	24
I-2	Sausage patty	5°C, 24 h	15.6	15.6	24
I-3	Sausage patty	−20°C, 24 h→5°C, 24 h	15.6	15.6	24
J-1	Sausage patty	None	21.1	21.1	11
J-2	Sausage patty	5°C, 24 h	21.1	21.1	11
J-3	Sausage patty	−20°C, 24 h→5°C, 24 h	21.1	21.1	11
K-1	Enhanced pork loin	None	21.1	21.1	24
K-2	Enhanced pork loin	5°C, 24 h	21.1	21.1	24
K-3	Enhanced pork loin	−20°C, 24 h→5°C, 24 h	21.1	21.1	24
L-1	Natural pork chop	None	21.1	21.1	24
L-2	Natural pork chop	5°C, 24 h	21.1	21.1	24
L-3	Natural pork chop	−20°C, 24 h→5°C, 24 h	21.1	21.1	24

[a] Conditions to which product was exposed before the temperature abuse conditions.
[b] Initial cooling from 20 to 10°C in 110 min.
[c] Initial cooling from 20 to 10°C in 170 min.
[d] Initial cooling from 35 to 10°C in 200 min.
[e] Initial cooling from 35 to 10°C in 330 min.

meat product analytical methods (Table 2). The hot-boned pork sausage and the spicy pork sausage batters (composed of pork, salt, and spices) in experiments B through F were packed into 50-ml sterile plastic centrifuge tubes (25 g per tube; Falcon brand, Fisher Scientific, Itasca, IL) and then inoculated. Thawed frankfurters in experiment F were cut in half perpendicular to the long axis of the frankfurter and then each half-frankfurter was placed in a sterile plastic centrifuge tube for inoculation and temperature abuse. In experiment A, the casing was removed from each full-size bratwurst, and the remaining bratwurst batter was placed into a filter sample bag (15.25 by 23 cm; Nasco, Fort Atkinson, WI) for inoculation and temperature abuse.

In experiments G through L, frozen pork sausage patties (ca. 38 g each), refrigerated vacuum-packaged enhanced pork loin (contained injected phosphate solution), and refrigerated natural (no additives) boneless center-cut pork chops were obtained from a local retail store. All three products were stored at −20°C until thawed at 5°C before use. A representative sample of each product was analyzed as described previously (Table 2). Individual entire sausage patties were used for inoculation, and each patty was placed in a sample bag (7.5 by 18.5 cm; Nasco). The pork loins and pork chops were thawed, trimmed of excess fat with a sanitized knife, and cut perpendicular to the long dimension of the loin into slices (loins) 5 cm wide or into pieces (chops, 3.8 by 3.8 cm) with a depth equaling that of the chop. Each piece of pork loin or pork chop was placed in a plastic petri dish (Fisher) for inoculation. The containers holding products were loosely closed during subsequent temperature abuse.

Preparation of inocula. Five strains each of *S. aureus, Salmonella* serovars, and *E. coli* O157:H7 were used to inoculate the pork products (Table 3). Each strain was prepared from stock

TABLE 2. *Proximate composition of raw pork products subjected to single and repeated temperature abuse periods*

Product	% moisture	% protein	% fat	% salt	pH	Water activity
Spiced pork sausage	44.1	11.7	41.6	1.9	6.2	0.98
Hot-boned pork sausage	53.3	13.5	31.3	1.5	6.1	0.98
Pork frankfurter	64.2	12.5	16.9	2.2	6.2	0.98
Pork bratwurst	53.7	14.0	25.2	2.1	6.5	0.97
Sausage patty	57.1	15.4	22.9	1.6	6.5	0.98
Natural pork chop	71.1	22.5	5.5	0.8	5.8	0.99
Enhanced pork loin	75.5	19.4	2.2	1.1	6.1	0.98

TABLE 3. *Pathogen strains used for development and testing of the predictive tools*

Species	Strain no.	Isolated from:	Source[a]
Escherichia 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
Staphylococcus 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] 1, Dr. John Luchansky (formerly Food Research Institute, University of Wisconsin–Madison; now at U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA); 2, American Type Culture Collection (Manassas, VA); 3, Dr. Eric Johnson (Food Research Institute); 4, Dr. Amy Wong (Food Research Institute).

culture frozen (−20°C) in brain heart infusion broth (BHIB; Difco, Becton Dickinson, Sparks, MD) plus 10% glycerol (Fisher). A working culture was prepared by successively culturing the strains twice at 35°C under static conditions for 18 to 24 h in BHIB, and then the culture was streaked to BHI agar (Difco, Becton Dickinson), incubated at 35°C for 18 to 24 h, and examined for homogeneous colony morphology; the plate was stored at 5°C. To begin preparing inocula, an isolated colony of each strain was transferred from its working culture plate to 9 ml of BHIB and then incubated statically 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 and resuspending the pellet in Butterfield's phosphate diluent (BPD; Nelson Jameson, Marshfield, WI).

In experiments B through F involving hot-boned pork sausage, spicy pork sausage, and pork frankfurter batters, all three pathogen species were combined in a single inoculum by mixing 10 ml of each separate five-strain mixture. Two different inocula were prepared for experiment A with bratwurst and for experiments G through L with sausage, enhanced pork loin, and natural pork chop. Experiments in our laboratory revealed that combining *Salmonella* and *E. coli* O157:H7 did not have a significant effect ($P > 0.05$) on the growth of either pathogen in ground pork compared with that observed when using single-pathogen inocula. However, the growth of *S. aureus* was significantly slower when this pathogen was included along with the other two pathogens *(6)*. 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 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 inoculum containing ca. 9 log CFU/ml. The second inoculum, containing only *S. aureus* at 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. Each inoculum was then diluted 100-fold in BPD.

Inoculation of pork products. In experiments B through F, the hot-boned pork sausage, spiced pork sausage, and pork frankfurter batters were inoculated by transferring 100 μl of inoculum (ca. 7 log CFU) into a small hole (3 to 4 mm in diameter and 2 cm deep) within the sausage or frankfurter batter in each tube for an initial inoculation level of ca. 5.6 log CFU/g. The hole was formed using a sterile bent plastic spreader. This inoculation procedure was intended to simulate spot contamination of a product. The pork bratwurst in experiment A (about 110 g in each sample bag) was inoculated by transferring 400 μl of inoculum (ca. 7.6 log CFU) into the meat and then manually massaging the bagged product for about 20 s to disperse cells throughout the meat (initial inoculation level of 5.6 log CFU/g). This inoculation method simulated pathogen contaminants being mixed into the meat during processing. The individual pork sausage patties, pork loin pieces, and pork chop pieces in experiments G through L were inoculated by transferring 100 μl of inoculum to the surface of the patty or piece, spreading the inoculum over the surface of the meat with a sterile plastic spreader, and then allowing microbial attachment to occur for 30 min at room temperature (21°C). This procedure simulated localized contamination and yielded initial inoculation levels of 4.4 log CFU/g, ca. 4.7 log CFU/cm^2, and 4.8 log CFU/cm^2 for pork sausage patties, loin pieces, and chop pieces, respectively. Each inoculated pork loin and pork chop piece was then transferred to a sample bag (Nasco) for temperature abuse treatments.

Temperature abuse of inoculated pork products. The spiced pork sausage and hot-boned pork sausage batter samples in experiments B through E were prewarmed to 21.1 and 37.8°C, respectively, inoculated, placed in a 21 or 35°C incubator, and then exposed to two different cooling regimes (different rates of initial cooling) for each product, which were created by gradually decreasing the incubator temperature. For experiments B and C, the product cooled from 20 to 10°C in 110 or 170 min, respectively, and in experiments D and E, the product cooled from 35 to 10°C in 200 or 330 min, respectively (see Fig. 1). Cooling was accomplished during 7 h, with a final product temperature of 5 to 8°C. For each experiment, a type K thermocouple attached to a data logger (model SP150, Dickson,

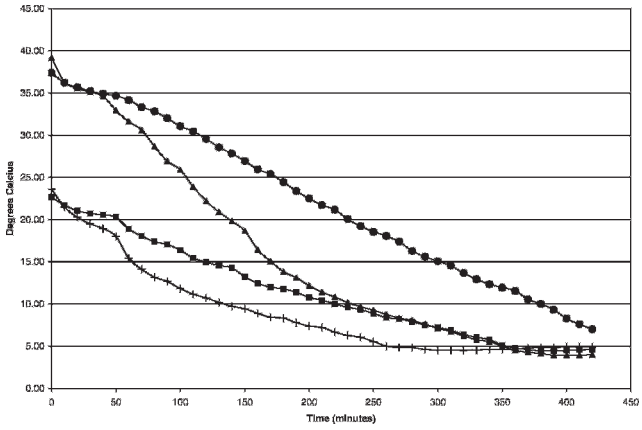

FIGURE 1. *Time and temperature history of inoculated spiced pork sausage and hot-boned pork sausage during cooling (experiments B through E). +, Spiced pork sausage cooled at a rapid rate (experiment B); ■, spiced pork sausage cooled at a slower rate (experiment C); ▲, hot-boned pork sausage cooled at a rapid rate (experiment D); ●, hot-boned pork sausage cooled at a slower rate (experiment E).*

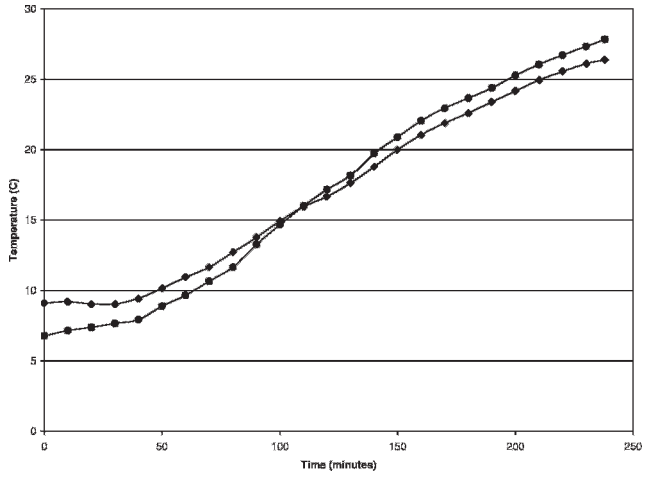

FIGURE 2. *Time and temperature history of inoculated pork bratwurst during warming (experiment A). Each line represents an individual trial.*

Addison, IL) was inserted into an uninoculated sample to record the product temperature at 5-min intervals.

The pork frankfurter batter in experiment F was prewarmed to 18°C, inoculated, and then held at this temperature for 15 h. A thermocouple and data logger were used with one uninoculated frankfurter to record product temperature.

Bratwurst batter in experiment A was removed from refrigeration immediately before inoculation, inoculated, and then rechilled at 5°C for 45 min. The inoculated bratwurst was then placed in an incubator where the temperature was gradually increased from 7 to 9°C to 26 to 28°C over 4 h. Two trials were conducted in two separate incubators, and the temperature of an uninoculated bag of bratwurst was recorded in each trial using a thermocouple and data logger (see Fig. 2).

Inoculated pork sausage patties and pieces of pork loin or pork chop (experiments G through L) were stored at 13, 15.6, or 21.1°C for 24 h, except that pork sausage patties inoculated with *S. aureus* were stored at 21.1°C for only 11 h.

Sampling of inoculated pork products. Inoculated meat samples were prepared in sufficient quantity to allow destructive sampling at periodic intervals during each temperature abuse experiment. For experiments G through L, approximately one-third of the samples were analyzed during the first period of temperature abuse (part 1 for each experiment, e.g., experiment G-1). At the end of this temperature abuse period, half of the remaining samples were stored at 5°C for 24 h and then subjected to the same temperature abuse as before (part 2), and the other half were frozen at −20°C for 24 h, thawed at 5°C for 24 h, and then temperature abused as before (part 3). For these experiments, chamber temperature was monitored.

Enumeration of pathogens in pork product samples. For experiments G through L, one sample bag per inoculum type was removed at each sampling time from the water bath or incubator. The outer surface of each bag was dried, sanitized with 70% ethanol, and allowed to dry, and the upper 1.25 cm of the sample bag (the part containing the wire closure) was cut off with sanitized scissors. The contents of each bag were then 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 2 min in a stomacher lab blender (Fisher), and serially diluted in BPD. For each dilution plated, 100 μl was spread with a sterile bent plastic spreader on a single plate of the appropriate selective medium. The selective medium used for *S. aureus* was Baird-Parker agar base (B-P; Difco, Becton Dickinson) with tellurite egg yolk supplement (Difco, Becton Dickinson). Typical *S. aureus* colonies on B-P are shiny black with a distinctive clear zone in the surrounding agar. The selective medium used for *Salmonella* serovars was xylose lysine deoxycholate agar (XLD; 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, Becton Dickinson) on which typical colonies are colorless to white and opaque. 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. Samples for experiment A were analyzed in the same manner except that this comminuted product underwent only a single homogenization by stomaching for 30 s, which was done in the original sample bag, i.e., there was no transfer to a second bag for homogenization. Samples in experiments B through G were in centrifuge tubes, and the contents of each tube were aseptically transferred to a sample bag. These comminuted products also were homogenized for 30 s with the stomacher. The remainder of the enumeration procedure was the same as described above.

Number of trials conducted. For each pathogen and experiment, the log CFU was determined at each sampling time for each sample. A trial was defined as a series of samples for a given combination of experiment, pork product, and pathogen. The overall evaluation of whether growth occurred in a trial was based on the results for the last sample taken. One trial each was conducted for each pathogen in experiments A through F. In experiment G, a single trial was conducted with *S. aureus,* and five trials were conducted with *Salmonella* and *E. coli* O157:H7. In experiments H through J and L, two trials were conducted for each pathogen, and in experiment K, three trials for *Salmonella* and *E. coli* O157:H7 and two trials for *S. aureus* were conducted.

In the experiments involving sequential temperature abuse (experiments G through L) four uninoculated samples were analyzed at the start of the experiment for aerobic mesophilic

bacteria levels using Petrifilm aerobic count plates (3 M Microbiology, St. Paul, MN) that were incubated for 48 h at 35°C.

The THERM predictive tool. THERM is a tool that utilizes a sequence of time and temperature combinations, entered by the user, to predict the extent of pathogen growth. Small-scale isothermal inoculation experiments were previously conducted to determine LPD and GR values for a range of temperatures in ground pork *(8)* and pork sausage containing salt but no nitrite *(9)*; tabulated values are available in these articles. When temperatures entered by the user 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. Based on 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 is defined as the difference in time values between two entered time and temperature data pairs. The % LPD elapsing in each time interval (constant temperature assumed) is estimated by dividing the interval time by the LPD for the final temperature in the interval and multiplying the resulting value by 100. The % LPD contributed by each interval is accumulated until 100% of the time in lag phase has elapsed (equation 1):

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

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

$$\text{Total growth} = \sum_{i=1}^{N} \text{GR for interval}_i \times \text{interval time}_i \qquad (2)$$

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

Entering experimental data into the THERM tool. For isothermal experiments (F through L), the product temperature (experiment F) or storage temperature (experiments G through L) was entered, and the entire storage period was treated as a single interval. For dynamic temperature abuse experiments (A through E), 20 evenly spaced temperature intervals were determined from the product temperatures recorded on the data logger during the storage period, and the corresponding temperature and time values were entered into THERM. When the temperature for the interval was lower than the lowest temperature for which LPD and GR values were experimentally determined, the lowest temperature for which LPD and GR were determined was used as a default temperature. For the THERM tool with ground pork, the default temperatures were 15.6°C for *S. aureus* and 10.0°C for *Salmonella* and *E. coli* O157:H7. For the THERM sausage tool, the default temperatures were 18.4°C for *S. aureus* and 21.1°C for *Salmonella* and *E. coli* O157:H7.

For the isothermal experiments (F through L), the appropriate pork product pH, % water phase salt, and temperature values were entered into the Pathogen Modeling Program (PMP 7.0; U.S. Department of Agriculture [USDA], Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA) for predicting aerobic growth of *S. aureus, Salmonella,* and *E. coli* O157:H7 in broth culture. Predictions of aerobic growth were used instead of predictions for anaerobic growth because more rapid growth was predicted for aerobic conditions. Similarly, when the PMP 7.0 tool allowed entry of a sodium nitrite level, a 0 value was entered as a worst case, even though a cured product was used in experiment F.

Data analysis. The THERM tools delivered a quantitative prediction, i.e., a Δ log CFU value (Table 4). The quantitative predictions were compared with the corresponding experimentally obtained values. To obtain qualitative THERM predictions, the predicted Δ log CFU value (change predicted at the final entered time point) was described as growth when it was >0.3 and no growth when it was ≤0.3. The same qualitative descriptions were used for observed experimental values to allow for comparisons of observations and predictions.

A qualitative prediction was considered accurate when it was the same as the observed result, fail-safe when growth was predicted but not observed, and fail-dangerous when no growth was predicted but growth was observed. A quantitative prediction was classified as accurate when it was within ±0.3 log CFU of the observed Δ log CFU value, fail-safe when it was >0.3 log CFU higher than the observed value, and fail-dangerous when it was >0.3 log CFU lower than the observed value.

A paired *t* test (Minitab version 14, Minitab, Inc., State College, PA) was used to compare the observed Δ log CFU values with the quantitative growth predictions made by the two THERM versions and PMP 7.0 for each pathogen (separately) across all products combined. A two-way analysis of variance was used to compare the Δ log CFU value in each stage of sequential temperature abuse for each pathogen (separately) across all products.

RESULTS AND DISCUSSION

Observed and predicted growth values for each experiment are shown in Table 4. Comparisons were made between the qualitative predictions made by the THERM sausage tool and qualitative descriptions of observed results for 115 trials. A total of 50% of predictions were accurate, 50% were fail-safe, and none were fail-dangerous. For the 39 trials in which pathogen growth was observed (Δ log CFU > 0.3), predictions made by the THERM sausage tool were 100% accurate. The THERM pork tool performed similarly when qualitatively predicting growth for 133 trials, with a 51% accuracy rate; 44% of predictions were fail-safe, and only 5% of predictions were fail-dangerous. For trials in which growth was observed, the THERM pork tool made accurate qualitative predictions in 87.5% of trials and fail-dangerous predictions in 12.5% of trials. The 0.3 log CFU value was chosen as the Δ log CFU boundary between growth and no growth because it represents a single bacterial doubling. However, trial-to-trial variation occasionally exceeded 0.3 log CFU. An alternative approach would be to define, based on experimental data, a no-growth confidence interval around a Δ log CFU value of 0. Such an approach may lead to greater accuracy for qualitative predictions.

For quantitative predictions, the THERM sausage tool had accuracy, fail-safe, and fail-dangerous rates of 22, 73, and 5%, respectively, for the 115 trials. When only the 39

TABLE 4. *Observed and predicted growth for* S. aureus, Salmonella *serovars, and* E. coli *O157:H7 in pork products during temperature abuse*[a]

Expt, product	Pathogen	Observed growth[b]		Predicted growth[c]							
				THERM sausage				THERM pork			
					No. of predictions				No. of predictions			PMP 7.0 (Δ log CFU)
		Result (Δ log CFU)	*n*	Result (Δ log CFU)	ACC	F-S	F-D	Result (Δ log CFU)	ACC	F-S	F-D	
A, pork bratwurst	*S. aureus*	≤+0.3	1	0	1	0	0	0	1	0	0	ND[d]
	Salmonella	≤+0.3	1	0	1	0	0	0	1	0	0	ND
	E. coli	≤+0.3	1	0	1	0	0	0	1	0	0	ND
B, spiced pork sausage	*S. aureus*	≤+0.3	1	0	1	0	0	0	1	0	0	ND
	Salmonella	≤+0.3	1	0	1	0	0	0	1	0	0	ND
	E. coli	≤+0.3	1	0	1	0	0	0	1	0	0	ND
C, spiced pork sausage	*S. aureus*	≤+0.3	1	0	1	0	0	0	1	0	0	ND
	Salmonella	≤+0.3	1	0	1	0	0	0	1	0	0	ND
	E. coli	≤+0.3	1	0	1	0	0	0	1	0	0	ND
D, hot-boned pork sausage	*S. aureus*	≤+0.3	1	+0.3	1	0	0	0	1	0	0	ND
	Salmonella	≤+0.3	1	0	1	0	0	0	1	0	0	ND
	E. coli	≤+0.3	1	0	1	0	0	+0.2	1	0	0	ND
E, hot-boned pork sausage	*S. aureus*	≤+0.3	1	+0.4	0	1	0	0	1	0	0	ND
	Salmonella	≤+0.3	1	0	1	0	0	+0.8	0	1	0	ND
	E. coli	≤+0.3	1	0	1	0	0	+0.9	0	1	0	ND
F, pork frankfurter	*S. aureus*	≤+0.3	1	+0.9	0	1	0	0	1	0	0	+1.4
	Salmonella	≤+0.3	1	+0.3	1	0	0	+3.0	0	1	0	+2.1
	E. coli	≤+0.3	1	+0.6	0	1	0	+1.3	0	1	0	0
G-1, sausage patty	*S. aureus*	−0.2	1	+1.7	0	1	0	0	1	0	0	0
	Salmonella	−0.6 (±0.4)	5	+1.5	0	5	0	+0.5	0	5	0	0
	E. coli	−0.4 (±0.1)	5	+2.1	0	5	0	+0.4	0	5	0	0
G-2, sausage patty	*S. aureus*	+1.5	1	+1.7	1	0	0	0	0	0	1	0
	Salmonella	0 (±0.5)	5	+1.5	2	3	0	+0.5	2	3	0	0
	E. coli	+0.7 (±0.4)	5	+2.1	4	1	0	+0.4	4	1	0	0
G-3, sausage patty	*S. aureus*	+0.4	1	+1.7	1	0	0	0	0	0	1	0
	Salmonella	−0.1 (±0.2)	4	+1.5	0	4	0	+0.5	0	4	0	0
	E. coli	+0.2 (±0.7)	5	+2.1	2	3	0	+0.4	2	3	0	0
H-1, enhanced pork loin	*S. aureus*	−0.2 to +0.3	2	+1.7	0	2	0	0	2	0	0	0
	Salmonella	−0.8 to 0	2	+1.5	0	2	0	+0.5	0	2	0	0
	E. coli	0	2	+2.1	0	2	0	+0.4	0	2	0	0
H-2, enhanced pork loin	*S. aureus*	0 to +0.2	2	+1.7	0	2	0	0	2	0	0	0
	Salmonella	0 to +0.1	2	+1.5	0	2	0	+0.5	0	2	0	0
	E. coli	+0.3 to +1.1	2	+2.1	1	1	0	+0.4	1	1	0	0
H-3, enhanced pork loin	*S. aureus*	+0.2	2	+1.7	0	2	0	0	2	0	0	0
	Salmonella	−0.8 to 0	2	+1.5	0	2	0	+0.5	0	2	0	0
	E. coli	−0.2 to +0.6	2	+2.1	1	1	0	+0.4	1	1	0	0

TABLE 4. *Continued*

Expt, product	Pathogen	Observed growth[b]		Predicted growth[c]							
				THERM sausage				THERM pork				
					No. of predictions				No. of predictions			PMP 7.0
		Result (Δ log CFU)	*n*	Result (Δ log CFU)	ACC	F-S	F-D	Result (Δ log CFU)	ACC	F-S	F-D	(Δ log CFU)
I-1, sausage patty	*S. aureus*	0 to +0.1	2	+1.7	0	2	0	+0.2	2	0	0	+0.3
	Salmonella	−0.5 to −0.4	2	+1.5	2	0	0	+1.8	2	0	0	+0.9
	E. coli	0 to +0.1	2	+2.1	0	2	0	+2.1	0	2	0	0
I-2, sausage patty	*S. aureus*	+0.6 to +1.3	2	+1.7	2	0	0	+0.2	0	0	2	+0.3
	Salmonella	−0.8 to +1.1	2	+1.5	1	1	0	+1.8	1	1	0	+0.9
	E. coli	−0.2 to +1.4	2	+2.1	1	1	0	+2.1	1	1	0	0
I-3, sausage patty	*S. aureus*	+0.5 to +0.7	2	+1.7	2	0	0	+0.2	0	0	2	+0.3
	Salmonella	−0.5 to +1.3	2	+1.5	1	1	0	+1.8	1	1	0	+0.9
	E. coli	+0.6 to +2.9	2	+2.1	2	0	0	+2.1	2	0	0	0
J-1, sausage patty	*S. aureus*	+0.5 to +0.9	2	+0.7	2	0	0	+0.7	2	0	0	+1.9
J-2, sausage patty	*S. aureus*	+0.8 to +1.2	2	+0.7	2	0	0	+0.7	2	0	0	+1.9
J-3, sausage patty	*S. aureus*	+1.1 to +3.0	2	+0.7	2	0	0	+0.7	2	0	0	+1.9
K-1, enhanced pork loin	*S. aureus*	−0.1 to +0.5	2	+2.8	1	1	0	+2.6	1	1	0	+2.9
	Salmonella	+0.2 (±0.2)	3	+1.5	1	2	0	+6.4	1	2	0	+6.1
	E. coli	+1.0 (±0.2)	3	+2.1	3	0	0	+3.5	3	0	0	+3.6
K-2, enhanced pork loin	*S. aureus*	−0.5 to −0.4	2	+2.8	0	2	0	+2.6	0	2	0	+2.9
	Salmonella	+2.1 (±0.8)	3	+1.5	3	0	0	+6.4	3	0	0	+6.1
	E. coli	+0.1 (±0.7)	3	+2.1	1	2	0	+3.5	1	2	0	+3.6
K-3, enhanced pork loin	*S. aureus*	+0.2	1	+2.8	0	1	0	+2.6	0	1	0	+2.9
	Salmonella	+0.2 (±0.7)	3	+1.5	2	1	0	+6.4	2	1	0	+6.1
	E. coli	+0.5 (±0.3)	3	+2.1	2	1	0	+3.5	2	1	0	+3.6
L-1, natural pork chop	*S. aureus*	+0.3 to +0.6	2	NA[e]	NA	NA	NA	+2.6	1	1	0	+2.5
	Salmonella	+1.8	2	NA	NA	NA	NA	+6.4	2	0	0	+5.3
	E. coli	+1.1 to +1.7	2	NA	NA	NA	NA	+3.5	2	0	0	+3.6
L-2, natural pork chop	*S. aureus*	−0.1 to 0	2	NA	NA	NA	NA	+2.6	0	2	0	+2.5
	Salmonella	−1.3 to +0.8	2	NA	NA	NA	NA	+6.4	1	1	0	+5.3
	E. coli	−0.1 to +0.2	2	NA	NA	NA	NA	+3.5	0	2	0	+3.6
L-3, natural pork chop	*S. aureus*	−0.4 to −0.1	2	NA	NA	NA	NA	+2.6	0	2	0	+2.5
	Salmonella	+1.3 to +2.6	2	NA	NA	NA	NA	+6.4	2	0	0	+5.3
	E. coli	+0.1 to +0.6	2	NA	NA	NA	NA	+3.5	1	1	0	+3.6

[a] Refer to Table 1 for experimental conditions.

[b] *n*, number of runs for each trial. Results are given as single values when $n = 1$, as the range when $n = 2$, and as the mean (±SD) when $n > 2$.

[c] Predictions were generated using the THERM sausage tool (raw pork sausage, salt, no nitrite), THERM pork tool (raw ground pork), or PMP 7.0, where appropriate. Growth predictions: ACC, accurate (growth of >0.3 log CFU was predicted and observed, or no growth was predicted and observed); F-S, fail-safe (growth predicted but no growth observed; F-D, fail-dangerous (no growth predicted but growth observed).

[d] ND, not determined; appropriate only for isothermal temperatures.

[e] NA, not applicable.

trials with observed >0.3 Δ log CFU were considered, the THERM sausage tool made quantitative predictions with accuracy, fail-safe, and fail-dangerous rates of 15, 69, and 16%, respectively. The THERM pork tool had accuracy, fail-safe, and fail-dangerous rates of 29, 59, and 12%, respectively, for the 133 trials overall and 17, 52, and 31%, respectively, for trials in which pathogen growth was observed. Residual plots (not shown) clearly indicated the fail-safe tendency of both tools, particularly as the duration of temperature abuse increased. These results are consistent with our earlier findings that THERM tools are more likely to produce accurate qualitative predictions than accurate quantitative predictions *(8, 9)*.

The products used in this study represented a wide range of % fat and % salt. Both of these components generally inhibit growth of bacteria, although *S. aureus* is far less affected by salt in sausage than are *E. coli* O157:H7 and *Salmonella (9)*. However, a comparison of *S. aureus* behavior across experiments J-1, K-1, and L-1; J-2, K-2, and L-2; and J-3, K-3, and L-3 clearly shows greater growth in the sausage patty (22.9% fat, 1.6% salt) than in the lower fat, lower salt natural pork chop and the enhanced pork loin. No other composition-related differences in pathogen growth were observed in the other experiments for which such comparisons were possible (experiments G and H, K and L).

In experiments G through L, initial aerobic bacterial levels averaged 3.8 ($n = 7$), 3.5 ($n = 6$), and 4.4 ($n = 4$) log CFU/g or log CFU/cm^2 for pork sausage patty, enhanced pork loin, and natural pork chop, respectively. However, the standard deviations for these means were 1.1, 0.7, and 1.7 log CFU/g or log CFU/cm^2, respectively, and single samples had levels ranging from 2.5 to 6.2 log CFU/g or log CFU/cm^2. The level of indigenous bacteria reportedly can affect the amount of pathogen growth on raw meats *(15, 21)*, and some of the variability in pathogen growth observed in the present study may be attributed to the different levels of indigenous bacteria.

Statistical analyses were done to compare the observed Δ log CFU values to quantitative predictions made by both versions of THERM. Across all experiments, the THERM sausage tool predicted significantly more growth of each pathogen than was observed ($P < 0.05$). Similarly, the THERM pork tool predicted significantly more growth of *Salmonella* and *E. coli* O157:H7 than was observed. The changes in *S. aureus* levels predicted by the THERM pork tool were, on average, higher than those observed, but the difference was not significant. For experiments with isothermal temperature abuse (experiments F through L), the observed Δ log CFU values were compared with the predicted pathogen growth in a microbiological laboratory medium with the same pH and % water phase salt as the pork products studied here (PMP 7.0). For *S. aureus* and *Salmonella,* the PMP 7.0 predicted significantly more growth than was observed ($P < 0.05$). For *E. coli* O157:H7, PMP 7.0 predictions were consistently higher than observed values but the differences were not significant. These results suggest that if adapted to an interval accumulation approach, the PMP 7.0 tools also could be safely used as predictors of pathogen growth on pork during dynamic temperature abuse conditions. This approach was successfully used with an earlier version of the PMP tools for evaluating the potential for *S. aureus* growth during slow cooking of hams *(11)*.

The conservative tendency of the THERM and PMP 7.0 tools stands in contrast to an earlier predictive model based on isothermal inoculation studies using pure culture in a laboratory medium. In a 1995 publication, Sutherland et al. *(14)* developed a model for predicting growth of *E. coli* O157 on meat and poultry products and other foods. The model was based upon the modified Gompertz equation. The data upon which the model was based were generated in inoculation studies using laboratory media with sodium chloride concentrations of 0.5 to 6.5% (wt/vol), pH values of 4.0 to 7.0, and incubation temperatures of 10 to 30°C. The model was then used to obtain predicted *E. coli* O157:H7 growth values at pH 5.5 to 6.9, with 0.5 to 1.5% sodium chloride, and storage temperatures of 8 to 40°C for each product in five different published inoculation studies. A plot of predicted versus observed values revealed that none of the 30 predicted values exceeded the observed values by >0.3 log CFU (defined as fail-safe), 20 were within 0.3 log CFU of the observed values (accurate), and 10 were more than 0.3 log CFU lower than the observed values (fail-dangerous).

Data from earlier pork inoculation studies in other laboratories were also compared with THERM predictions. In one such study, ground pork with 0 to 5% added sodium chloride was inoculated with *Salmonella* and stored at 10°C for up to 14 days. Growth occurred by 2, 4, and 7 days, respectively, when the pork contained 0, 2, and 3.5% NaCl. No growth was reported for ground pork containing 5% NaCl *(1)*. The THERM tool for ground pork predicted an LPD at 10°C of about 2.25 days compared with ca. 0.7 log CFU of growth observed at 2 days in the ground pork with 0% NaCl. In this product stored for 4 days, observed *Salmonella* growth was 2.5 log CFU, and THERM predicted 2.6 log CFU of growth. Use of the THERM tool for sausage (containing salt) to predict *Salmonella* growth in the salt-added ground pork was problematic because the lowest experimental temperature used in developing this THERM tool was 21.1°C. Using this temperature as a default temperature resulted in a predicted growth of 4.9 log CFU after 2 days; however, no growth was observed in the salt-added products. Similarly, an early study of *S. aureus* growth on raw whole-muscle pork reported a population increase of approximately 7 log CFU during 72 h at 30°C. The *S. aureus* population at the end of this storage period (1.9×10^8 CFU/g) was clearly near the level expected for stationary phase on meat *(5)*. The THERM pork tool predicted an increase of 30.4 log CFU, obviously not realistic, but also clearly indicating that stationary phase would be reached. As seen in this example, the THERM tools differ from some predictive models by not accounting for a maximum population density. Therefore, THERM is likely to predict unrealistically high levels of growth during long periods of temperature abuse. However, this shortcoming is inconsequential when THERM is used as

intended. As an example, in the situation above regardless of whether the predicted level of *S. aureus* growth is 7 log CFU or higher, it is clearly enough to create an unsafe product.

Another situation for which the THERM tools may give unrealistically high quantitative predictions of pathogen growth is the intentional holding of sausage batter at growth-promoting temperatures to achieve pH reduction via the growth of added or indigenous lactic acid bacteria, and concurrent drying. Because the inoculated sausage mix used in developing the THERM sausage tool did not contain a fermentable carbohydrate, had relatively low levels of indigenous bacteria, and was not stored long enough for significant pH reduction, the predictions this tool produces do not account for pathogen growth being hindered by lower pH. For example, Ananou et al. *(2)* found that levels of *S. aureus* increased by only about 1.0 log CFU in sausage batter during 9 days of storage at 20°C. During this period, the sausage pH fell from about 6.0 to about 5.5. The THERM sausage tool predicted an unrealistically high level of *S. aureus* growth for this length of time, as did the THERM pork tool. However, if only qualitative predictions were considered, both of the THERM tools were accurate, i.e., they predicted growth.

In earlier work done in our laboratory using the same *Salmonella* and *E. coli* O157:H7 strains as in the present study, we observed no growth of either pathogen in the following situations: pork chop surface held 4 h at 10°C, pork loin surface held 8 h at 10°C or 2 h at 22°C, and bratwurst interior and surface held 8 h at 10°C or 2 h at 22°C *(10)*. Neither the THERM sausage tool nor the THERM pork tool predicted growth of either pathogen under these time and temperature conditions, even when use of default temperatures was necessary.

In experiments G through L, growth of each pathogen was on average highest during a second period of temperature abuse following freezing and thawing, e.g., G-3 and H-3, and lowest in the initial period of temperature abuse, e.g., G-1 and H-1. Differences in pathogen growth between temperature abuse periods were not significant ($P > 0.05$). However, of the 15 individual trials for which the THERM sausage tool made a fail-dangerous quantitative prediction, none involved the initial temperature abuse period, eight involved temperature abuse after refrigeration, and seven involved temperature abuse after freezing and thawing. Similarly, of four trials for which the THERM pork tool made a fail-dangerous quantitative prediction, none involved the first temperature abuse period, one involved temperature abuse after refrigeration, and three involved temperature abuse after freezing and thawing. The only fail-dangerous qualitative predictions were made by the THERM pork tool and occurred in six trials, three each involving temperature abuse after refrigeration and after freezing and thawing, respectively. Overall, the THERM tools appeared to be adequate for evaluating growth of *S. aureus, Salmonella,* and *E. coli* O157:H7 during repeated instances of temperature abuse, but the degree to which the tools were conservative decreased with repeated temperature abuse. The only fail-dangerous quantitative predictions made by the THERM sausage tool involved *S. aureus* (nine trials) and *E. coli* O157:H7 (six trials). All fail-dangerous qualitative predictions made by the THERM pork tool involved *S. aureus*. Further research to refine and test the THERM tools over a larger range of temperature abuse situations, particularly involving *S. aureus,* is anticipated as the tools undergo continuous improvement.

The THERM tools as currently constructed are far more likely to lead to a fail-safe prediction and thus potential loss of safe product than to a fail-dangerous prediction and the potential consumption of dangerous product. The USDA will not accept predictions from computer-based predictive models as the sole supporting information for planning a corrective action in response to deviations *(19)*. Processors must therefore obtain additional information, such as indicator bacteria test results from samples during the actual process or a process simulation, or pathogen testing on product, to fully support corrective action decisions. This additional information may lead to a determination of safety for a product whose time and temperature history resulted in THERM predictions of pathogen growth. Heeding this additional information would reduce the possibility of incorrectly concluding that the product is unsafe. Testing for levels of aerobic mesophilic bacteria also might be used as an additional tool in making decisions about corrective actions.

Ideally, predictive tools such as THERM should be part of an overall assessment of the risk of pathogen growth in raw meats that should include estimating the probability of occurrence of the targeted hazard, i.e., pathogen growth, and assessing the severity of the hazard when it does occur. These determinations then inform strategies for risk management and risk communication. The risk of pathogen growth in raw meat during processing is dependent on the likelihood of the pathogen being present and the product's time and temperature history. As exemplified 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 *(18)* and 2007 *(20)*, the USDA assumes that pathogenic bacteria are present in raw meats and poultry. Tools such as THERM evaluate time and temperature history and provide either a binary (growth versus no growth) prediction or a quantitative (Δ log CFU) prediction of pathogen growth in the product. To obtain a more correct hazard probability estimate, tools should estimate the probability, e.g., 90%, of pathogen growth resulting from a particular time and temperature history. Future research should aim toward development of such tools.

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 *(17)*. 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 provided that 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. Some unknown proportion of purchasers probably will mishandle or undercook raw meat or poultry, so accurately predicting the severity of the hazard resulting from pathogen growth in raw meat or poultry is difficult. In spite of these difficulties, THERM is an important research-based tool that could be useful when conducting risk assessments for raw meat and poultry processing.

In conclusion, THERM tools generally provide accurate or fail-safe growth and no-growth predictions of pathogen growth in raw pork products subjected to a range of temperature abuse conditions. Processors can use the THERM sausage tool for predicting pathogen growth in pork products containing salt and the THERM pork tool for making predictions about additive-free pork products. These tools will be useful to processors for supporting corrective actions taken after a deviation.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the USDA National Food Safety Initiative for funding the 2004 through 2006 portion of this work (project no. 2004-51110-02165), the U.S. Army Natick Soldier Research, Development & Engineering Center, Combat Feeding Directorate for funding the 2008 portion of this work, and Amy Haen, Ryan Algino, Erica Schoeller, and Melissa Talbot for laboratory assistance.

REFERENCES

1. Alford, J. A., and S. A. Palumbo. 1969. Interaction of salt, pH, and temperature on the growth and survival of salmonellae in ground pork. *Appl. Microbiol.* 17:528–532.
2. Ananou, S., M. Maqueda, M. Martínez-Bueno, A. Gálvez, and E. Valdivia. 2005. Control of *Staphylococcus aureus* in sausages by enterocin AS-48. *Meat Sci.* 71:549–556.
3. Baranyi, J., and T. A. Roberts. 1994. A dynamic approach to predicting bacterial growth in food. *Int. J. Food Microbiol.* 23:277–294.
4. Baranyi, J., T. P. Robinson, A. Kaloti, and B. M. Mackey. 1995. Predicting growth of *Brochothrix thermosphacta* at changing temperature. *Int. J. Food Microbiol.* 27:61–75.
5. Casman, E. P., D. W. McCoy, and P. J. Brandly. 1963. Staphylococcal growth and enterotoxin production in meat. *Appl. Microbiol.* 11:498–500.
6. Fanslau, M. A. 2006. Development of a tool for predicting pathogen growth during the short-term temperature abuse of raw pork and beef products. M.S. thesis. University of Wisconsin–Madison.
7. Fujikawa, H., A. Kai, and S. Morozumi. 2004. A new logistic model for *Escherichia coli* growth at constant and dynamic temperatures. *Food Microbiol.* 21:501–509.
8. Ingham, S. C., M. A. Fanslau, G. M. Burnham, B. H. Ingham, J. P. Norback, and D. W. Schaffner. 2007. Predicting pathogen growth during short-term temperature abuse of raw pork, beef, and poultry products: use of an isothermal-based predictive tool. *J. Food Prot.* 70:1445–1456.
9. Ingham, S. C., B. H. Ingham, D. Borneman, E. Jaussaud, E. L. Schoeller, N. Hoftiezer, L. Schwartzburg, G. M. Burnham, and J. P. Norback. 2009. Predicting pathogen growth during short-term temperature abuse of raw sausage. *J. Food Prot.* 72:75–84.
10. Ingham, S. C., J. A. Losinski, and K. L. Becker. 2004. Growth of *Escherichia coli* O157:H7 and *Salmonella* serovars on raw beef, pork, chicken, bratwurst and cured corned beef: implications for HACCP plan critical limits. *J. Food Saf.* 24:246–256.
11. Ingham, S. C., J. A. Losinski, B. K. Dropp, L. L. Vivio, and D. R. Buege. 2004. Evaluation of *Staphylococcus aureus* growth potential in ham during a slow-cooking process: use of predictions derived from the U.S. Department of Agriculture Pathogen Modeling Program 6.1 predictive model and an inoculation study. *J. Food Prot.* 67:1512–1516.
12. Koutsoumanis, K. 2001. Predictive modeling of the shelf life of fish under nonisothermal conditions. *Appl. Environ. Microbiol.* 67:1821–1829.
13. Koutsoumanis, K., A. Stamatiou, P. Skandamis, and G.-J. E. Nychas. 2006. Development of a microbial model for the combined effect of temperature and pH on spoilage of ground meat, and validation of the model under dynamic temperature conditions. *Appl. Environ. Microbiol.* 72:124–134.
14. Sutherland, J. P., A. J. Bayliss, and D. S. Braxton. 1995. Predictive modeling of growth of *Escherichia coli* O157:H7: the effects of temperature, pH and sodium chloride. *Int. J. Food Microbiol.* 25:20–49.
15. Tamplin, M. L. 2002. Growth of *Escherichia coli* O157:H7 in raw ground beef stored at 10°C and the influence of competitive bacterial flora, strain variation, and fat level. *J. Food Prot.* 65:1535–1540.
16. U.S. Department of Agriculture, Food Safety and Inspection Service. 1996. Pathogen reduction; hazard analysis and critical control point (HACCP) systems: final rule. *Fed. Regist.* 61:38805–38989.
17. U.S. Department of Agriculture, Food Safety and Inspection Service. 1998. Lethality and stabilization performance standards for certain meat and poultry products: technical paper. Available at: http://www.fsis.usda.gov/OPPDE/rdad/FRPubs?Docs_97-013P.htm. Accessed 28 January 2009.
18. U.S. Department of Agriculture, Food Safety and Inspection Service. 2002. *E. coli* O157:H7 contamination of beef products. *Fed. Regist.* 67:62325–62334.
19. U.S. Department of Agriculture, Food Safety and Inspection Service. 2005. Use of microbial pathogen computer modeling in HACCP plans, FSIS notice 25-05. Available at: http://www.fsis.usda.gov/regulations/notice_25-05/default.asp. Accessed 28 January 2009.
20. U.S. Department of Agriculture, Food Safety and Inspection Service. 2007. Notice 65-07, notice of reassessment for *Escherichia coli* O157:H7 control and completion of a checklist for all beef operations. Available at: http://www.fsis.usda.gov/OPPDE/rdad/FSISNotices/65-07.pdf. Accessed 28 January 2009.
21. Vold, L., A. Holck, Y. Wasteson, and H. Nissen. 2000. High levels of background microflora inhibit growth of *Escherichia coli* O157:H7 in raw ground beef. *Int. J. Food Microbiol.* 56:219–225.
22. Zwietering, M. H., J. C. De Wit, H. G. A. M. Cuppers, and K. Van'T Riet. 1994. Modeling of bacterial growth with shifts in temperature. *Appl. Environ. Microbiol.* 60:204–213.