Evaluation of *Salmonella* Thermal Inactivation Model Validity for Slow Cooking of Whole-Muscle Meat Roasts in a Pilot-Scale Oven

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ABSTRACT

Sublethal heating can increase subsequent thermal resistance of bacteria, which may compromise the validity of thermal process validations for slow-roasted meats. Therefore, this research evaluated the accuracy of a traditional log-linear inactivation model, developed via prior laboratory-scale isothermal tests, and a novel path-dependent model accounting for sublethal injury, applied to pilot-scale slow cooking of whole-muscle roasts. Irradiated turkey breasts, beef rounds, and pork loins were inoculated with an eight-serovar *Salmonella* cocktail via vacuum tumble marination in a salt-phosphate marinade. The resulting initial *Salmonella* population in the geometric center (core) was 7.0, 6.3, and 6.3 log CFU/g for turkey, beef, and pork, respectively. Seven different cooking schedules representing industry practices were evaluated in a pilot-scale, moist-air convection oven. Core temperatures recorded during cooking were used to calculate lethality real-time via the log-linear model. The path-dependent model reduced the bias (mean residual) and root mean square error by 4.24 and 4.60 log CFU/g respectively, in turkey; however, the new model did not reduce the prediction error in beef or pork. Overall, results demonstrated that slow-cooked roasts, processed to a computed lethality at or near that required by the regulatory performance standards, as calculated with a state-dependent model, may be underprocessed.

*Salmonella* has remained the leading cause of bacterial foodborne illness in the United States and annually is responsible for an estimated 1.0 million cases of foodborne illness, including 19,336 hospitalizations and 378 fatalities (12). To decrease the incidence of salmonellosis associated with meat and poultry products, the U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) has set lethality performance standards of 7 log (poultry) and 6.5 log (beef) for certain ready-to-eat products (18).

Predictive microbiology is used to estimate process lethality during cooking. The most common models utilized by industry today are first-order kinetic models, which presume log-linear inactivation of bacteria under isothermal conditions. Typically developed using controlled, laboratory-scale studies, these models have generally not been validated in full-scale processing environments. However, actual process outcomes can be affected by a variety of factors rarely considered in laboratory studies, such as bacterial uptake during marination (21) and the effect of product structure on bacterial resistance (10, 17, 20). Bacterial inactivation of bacteria in commercial settings is also far more complicated than a simple laboratory experiment, with the different conditions, sample sizes, and other uncontrolled variables all affecting the process outcome.

Slow cooking processes, in particular, challenge the modeling approach because traditional models fail to account for sublethal injury of bacteria. This phenomenon occurs when microorganisms are held at nonlethal temperatures that are above the limit for growth. With the production of heat-shock proteins, cells are able to adapt and, subsequently, better survive lethal temperatures (5, 7). For example, Stephens et al. (15) demonstrated that slow heating (<5°C/min) increased the thermotolerance of *Listeria monocytogenes*, and Wesche et al. (22) reported enhanced *Salmonella* survival at 60°C in ground turkey previously heat shocked at 43°C for 5, 30, and 60 min. Wiegand et al. (23) reported that inactivation parameters based on isothermal laboratory data generally overpredicted end-point lethality outcomes for *Escherichia coli* O157:H7 in whole-muscle beef roasts slow-cooked in a pilot-scale oven. Because sublethal injury of microorganisms might result in an overestimation of lethality, predictive models need to be evaluated and potentially modified to account for this phenomenon (8).

Corradini and Peleg (3) and Valdramidis et al. (19) proposed lethality models intended to account for the effect of slow heating on bacteria during thermal processing. Both of these studies modeled the inactivation parameters as functions of temperature and heating rate ($\bar{C}T/\bar{h}$). However, prior research (11, 14) has shown that sublethal injury or the
associated physiological response of heat shock is a function of the time-temperature history rather than heating rate. Valdramidis et al. (19) heated tubes containing suspended bacterial cells in a circulating water bath, whereas Corradini and Peleg (3) estimated their model parameters using previous thermal inactivation data generated from other laboratories. Most importantly, neither model was validated using a food product, which is essential if it is to be used in any food application.

Alternatively, the thermal inactivation model proposed by Stasiewicz et al. (14) accounts for sublethal injury by quantifying the cells’ thermal history in the heat shock temperature range during thermal processing. In their laboratory-scale study, Salmonella lethality was significantly overestimated in ground turkey thigh when a state-dependent model was used for slowly heated samples. Therefore, they proposed a novel path-dependent inactivation model that accounted for sublethal history and demonstrated that the new model eliminated the systematic errors that occurred with the traditional, state-dependent model. However, the model was validated for 1-g samples in a well-controlled laboratory-scale heating system, so its utility was not tested in products or processes appropriate to commercial conditions. Therefore, the objective of this study was to quantitatively compare the performance of a traditional state-dependent model and a path-dependent model, when applied to industry-relevant, slow-cooking processes for whole-muscle turkey, beef, and pork roasts in a pilot-scale moist air convection oven.

MATERIALS AND METHODS

Overall, this study entailed the inoculation of whole-muscle roasts with a Salmonella cocktail, cooking those roasts via several slow-roasting protocols, and comparing the lethality outcomes to those predicted by state-dependent and path-dependent models.

Meat. Fresh, skin-off turkey breast (pectoralis major and pectoralis minor muscles), top beef round (semimembranosus, adductor, and pectineus muscles), and center-cut pork loin (longissimus dorsi muscle) were acquired from federally inspected commercial producers and shipped to Michigan State University’s meat processing facility (refrigerated at <4.4°C). Meat was sectioned into ~680-g roasts, vacuum packaged in double plastic bags, frozen (~20°C), and irradiated (>10 kGy; Food Technology Services, Inc., Mulberry, FL; shipped frozen, overnight both directions) to eliminate indigenous microflora. Irradiated samples remained frozen (~20°C) until use. Irradiation effectiveness was confirmed by randomly testing three roasts of each species; a 25-g core was removed aseptically from each roast using a sterile scalpels placed in a Whirl-Pak bag, diluted 1:10 in sterile tryptic soy broth containing 0.6% (wt/vol) yeast extract (TSBYE; Difco Laboratories, Sparks, MD), and homogenized in a masticator (Neu-Tec Group Inc., Barcelona, Spain) for 3 min. Following 24 h of incubation at 37°C, duplicate samples were plated on aerobic Petrifilm plates (3M Microbiology Products, St. Paul, MN), incubated at 37°C for 24 h, and then enumerated. The moisture and fat composition of all meat species was determined using AOAC International methods 950.46B and 991.36, respectively (1).

Bacterial cultures. Eight Salmonella serovars with previously documented thermal resistance were used: Thompson FSIS 120 (chicken isolate), Enteritidis H3527 and H3502 (clinical isolates, phage types 13A and 4, respectively), Typhimurium DT 104 H3380 (human isolate), Hadar MF60404 (turkey isolate), Copenhagen 8457 (pork isolate), Montevideo FSIS 051 (beef isolate), and Heidelberg F5038BGI (human isolate), all previously obtained from Dr. V. K. Juneja (Eastern Regional Research Center, USDA, Agricultural Research Service, Wyndmoor, PA). Each serovar was separately maintained at ~80°C in vials containing tryptic soy broth (TSB; Difco Laboratories) supplemented with 20% (vol/vol) glycerol. Cultures were started by transferring one loop of frozen culture into 9 ml of TSBYE, and incubating at 37°C for 18 to 24 h. All serovars were separately maintained using consecutive daily transfers for up to 1 week, with a minimum of two consecutive transfers prior to use (10, 13). One day prior to each test, the serovars were transferred separately into either 250-ml (for turkey) or 500-ml (for beef and pork) bottles of sterile TSBYE, which were incubated at 37°C for 18 to 24 h before use.

Marinade. A typical commercial marinade containing 11.5% (wt/wt) salt and 3.7% (wt/wt) phosphate was prepared by completely dissolving (via continuous stirring on a stir plate [model PC-420, Corning Inc., Corning, NY]) 169 g of liquid phosphate (50% food grade liquid potassium phosphates, Butcher and Packer Supply Company, Detroit, MI) and then 253 g of NaCl in 1,775 g of deionized distilled water. Thereafter, 520-ml aliquots were added into multiple twist-cap autoclavable bottles containing a stir bar and were autoclaved at 121°C for 20 min.

Inoculum. On the day of experimentation, 16 ml of each Salmonella serovar culture was pipetted into each of four 250-ml centrifuge bottles (for turkey), or 20 ml into each of 18 (for beef) or 22 (for pork) 250-ml centrifuge bottles. After pelleting the eight-serovar Salmonella cocktail by centrifugation (6,000 × g for 15 min at 4°C), the supernatant was removed by pipette, and the remaining pellets were transferred into a 500-ml bottle of sterile salt-phosphate marinade and were mechanically stirred with the stir bar for 10 min.

Inoculation. The Salmonella marinade (Salmonella population ≥10⁹ CFU/ml) and roasts were combined in a sterile tumbler (T-15 vacuum meat tumbler, Kent Butcher Supply, Grandville, MI) modified with a stainless steel baffle insert; then they were tumbled under vacuum (~84.65 kPa) at 8 rpm for 20 min, rested 5 min, and then tumbled for an additional 20 min to obtain marinade uptake of ~15% (wt/wt) for turkey or ~10% (wt/wt) for beef and pork. After vacuum tumbling, a center core sample (~16.4 cm³) was aseptically removed from representative roasts (n = 6 for each species) using an electrosurgical unit (Valleylab SurgiStat II, Boulder, CO), for subsequent Salmonella enumeration.

Preparation for cooking. After inoculation, roasts were prepared for either in-bag or out-of-bag cooking treatments. The in-bag samples were vacuum sealed (VacMaster, Kansas City, MO) in boil-in-bags (Smurfit-MBI, Butcher and Pack Supply, Detroit, MI). For each roast, two sterile, rigid thermocouple probes (diameter 1.6 mm, barb end, accuracy ± 1.1°C; type K, PA1454SB, Datapaq Inc., Wilmington, MA) were threaded through sterile metal corers (2.54-cm diameter) and were inserted, from orthogonal directions, to the geometric center of each roast. For the in-bag roasts, the probes were inserted through two rubber septa (SSP 134, Specialty Silicone Products, Ballston Spa, NY) that were previously affixed, using silicone, to the surface of each bag to maintain the vacuum. Except for the bagging step, the in-bag and out-of-bag samples were prepared identically.
Oven control and data acquisition. A commercial, moist-air convection oven (CO151FWUA12B2083, Cres Cor, Mentor, OH), housed in the Michigan State University Biosafety Level-2 Pilot Processing Facility, was modified to control cooking temperature profiles and to log time-temperature data, via a LabVIEW data acquisition unit (CompactDAQ, National Instruments, Austin, TX), composed of thermocouple signal conditioning modules (NI 9211), a digital output module (NI 9401), and a universal relay module (URM-800, Omega Engineering, Inc., Stamford, CT). Oven relative humidity (20 to 100%) was nominally set with the oven’s built-in humidity dial. Actual humidity was measured with a humidity sensor (Hygroclip, Rotronic, Huntington, NY) and a data logger (Multipaq21, Datapaq, Inc., Wilmington, MA).

Cooking. Roasts were processed using seven different predetermined, industry-relevant cooking protocols, which varied in time, temperature, and humidity (60 to 93.3°C; 20 to 78% RH; total cooking time 86 to 253 min; Fig. 1). For each cooking schedule, process end points were based on the lower of the two real-time core temperatures (Fig. 2) measured for a given roast. Using those data, two roasts for each schedule were cooked to an end-point center temperature of 71.1°C, and three roasts were cooked to a target end-point center lethality of 7.0-, 5.5-, or 3.0-log reductions for turkey, beef, and pork, respectively, based on real-time calculations of lethality (described below).

Turkey roasts were cooked to an end-point lethality of 7.0 log to be consistent with FSIS Salmonella lethality standards (18). Because initial Salmonella populations in the center of vacuum-tumbled beef and pork roasts were <6.5 log CFU/g (see “Results”), both products were cooked to an end point less than the USDA Salmonella lethality standard of 6.5 log, in order to be able to quantify some Salmonella survivors and, therefore, process lethality (target 5.5 log for beef in this study). Additionally, because of the variability associated with the initial concentration in pork (see “Results”), an even lower targeted lethality (3.0 log) was selected, in order to ensure reliable, quantitative results. Although the targets were lower than the USDA lethality performance standards, the goal was to validate model performance, not process compliance; therefore, this design still enables achievement of this overall goal.

Salmonella enumeration. After achieving the targeted end point for a given schedule or treatment, each roast was immediately removed from the oven and rapidly cored by pushing the sterile corer through the coldest spot of the roast, as measured by the colder of the two thermocouples. The top and bottom 2.54 cm of the core sample were then aseptically removed, and the remainder (~8 g) was cooled to <15°C in less

FIGURE 1. Industry-relevant cooking schedules used to process Salmonella-inoculated whole-muscle roasts in a pilot-scale, moist-air convection oven. All temperatures are oven air temperatures.

FIGURE 2. Sample oven temperature profiles for whole-muscle roasts in a pilot-scale, moist-air convection oven at (a) constant temperature (91.1°C) and (b) step-up temperature (60°C for 90 min, 68.3°C for 90 min, and 76.7°C).
than 10 s by immersion in 18 g of sterile, chilled 0.1% peptone water (Difco Laboratories). The computed lethality used in subsequent analysis included the entire temperature history of the core through cooling to <15°C. Additional 0.1% sterile peptone water was added to achieve a 1:5 dilution, and the samples were homogenized for 3 min in a masticator, serially diluted, and plated using duplicate aerobic Petrifilm plates (3M Microbiology Products, St. Paul, MN), with *Salmonella* survivors enumerated after 48 h of incubation at 37°C. The limit of detection was 0.4 log CFU/g.

**Model-computed lethality.** Previously developed state-dependent and path-dependent models were used to estimate the lethality (i.e., log reductions) of *Salmonella* in the whole-muscle roasts during cooking, based on the core temperature data. Predictions were then compared to the experimental results (aggregated across all of the cooking treatments) to assess the models’ usefulness in scaled-up applications.

For the state-dependent model, the lethality calculations were performed using a first-order, modified-Arhrenius model previously reported by Stasiewicz et al. (14):

$$
\log S = \log N_0 - b(T(t)) \times t
$$

(1)

where $S$ is the survivor ratio, $N_0$ is the number of microorganisms at time $t$, and $N_0$ is the initial *Salmonella* population. Parameter $b$ is the temperature-dependent rate of inactivation (i.e., the inverse of the $D$-value). To be consistent with Stasiewicz et al. (14), the effect of temperature on $b$ was modeled as a modified Arrhenius dependence:

$$
b(T) = b_{ref} \cdot \exp \left\{ -\beta_1 \left[ \frac{1}{T(T)} - \frac{1}{T_{ref}} \right] \right\}
$$

(2)

where $\beta_1$ determines the effect of temperature on $b$, and $b_{ref}$ is the inactivation rate at a reference temperature $T_{ref}$. The state-dependent model parameters ($b_{ref}$ and $\beta_1$) were obtained by minimizing the sum of squared errors (Solver, Microsoft Excel 2003, Redmond, WA) on isothermal inactivation data from previous studies with the same *Salmonella* cocktail as in this study, in whole-muscle turkey, beef, and pork (Table 1).

For the path-dependent model, the rate of inactivation ($b$) was modified to account for the effect of sublethal injury (14):

$$
b(T) = b_{ref} \cdot \exp \left\{ -\beta_1 \left[ \frac{1}{T(T)} - \frac{1}{T_{ref}} \right] - \beta_2 \tau \right\}
$$

(3)

where $\beta_2 \tau$ accounts for the sublethal thermal injury of *Salmonella*. Sublethal history was quantified as an integral of the temperature versus time curve within the heat shock (HS) region (38 and 52°C) (14):

$$
\tau = \int_{T(HS)}^{T(T)} \left( T(t) - T_{HS} \right) dt
$$

(4)

The path-dependent model parameters ($b_{ref}$, $\beta_1$, and $\beta_2$; Table 2) were obtained from a previous study that used the same *Salmonella* cocktail in ground beef, pork, and turkey (16).

**RESULTS AND DISCUSSION**

**Product composition and sterility.** The moisture content of the turkey, beef, and pork was 74.0% ± 0.8%, 73.8% ± 0.3%, and 68.5% ± 0.9%, respectively. The fat content of the turkey, beef, and pork was 1.05% ± 0.2%, 2.32% ± 0.65%, and 9.99% ± 3.3%, respectively. Although the fat contents were not identical, all three products had relatively low fat content, so that any differential impact on model performance would be expected to be minimal. No bacteria were recovered from uninoculated samples that were irradiated.

**Initial *Salmonella* population.** Roast inoculation via tumble marination resulted in initial core *Salmonella* populations of 6.96 ± 0.53, 6.26 ± 0.89, and 6.27 ± 1.06 log CFU/g for turkey, beef, and pork, respectively.

**End-point temperature lethality.** When processed to 71.1°C, near-complete elimination of *Salmonella* was observed for all inoculated roasts. For turkey and beef, 11 of the 14 roasts cooked to 71.1°C yielded no detectable *Salmonella* (plating 1 ml from the 1:5 dilution), and the remaining 3 roasts yielded *Salmonella* levels at or near the limit of detection (i.e., one or two colonies), which corresponded to *Salmonella* reductions of 6.3 and 5.6 log, respectively. The pork roasts yielded no quantifiable *Salmonella* when cooked to 71.1°C; however, complete elimination of *Salmonella* was not confirmed, because enrichment was not performed on the samples following cooking. Because these roasts were inoculated to contain an

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**TABLE 1. State-dependent model parameters for whole-muscle turkey, beef, and pork**

<table>
<thead>
<tr>
<th>Product</th>
<th>$b_{ref}$ (min$^{-1}$)</th>
<th>$\beta_1$ (K)</th>
<th>Data source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-muscle turkey</td>
<td>0.774</td>
<td>37,889</td>
<td>17</td>
</tr>
<tr>
<td>Whole-muscle beef</td>
<td>0.553</td>
<td>45,311</td>
<td>2</td>
</tr>
<tr>
<td>Whole-muscle pork</td>
<td>0.593</td>
<td>46,724</td>
<td>20</td>
</tr>
</tbody>
</table>

**TABLE 2. Path-dependent model parameters and fitting statistics from nonisothermal calibration data sets for ground turkey, beef, and pork$^a$**

<table>
<thead>
<tr>
<th>Product</th>
<th>$b_{ref}$ (min$^{-1}$)</th>
<th>$\beta_1$ (K)</th>
<th>$\beta_2$ (K-min$^{-1}$)</th>
<th>RMSE (log CFU/g)</th>
<th>Bias (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground turkey</td>
<td>0.91</td>
<td>50,787</td>
<td>0.0017</td>
<td>0.66</td>
<td>0.07</td>
</tr>
<tr>
<td>Ground beef</td>
<td>0.94</td>
<td>44,710</td>
<td>0.0018</td>
<td>0.93</td>
<td>0.12</td>
</tr>
<tr>
<td>Ground pork</td>
<td>0.70</td>
<td>54,713</td>
<td>0.0016</td>
<td>0.87</td>
<td>0.18</td>
</tr>
</tbody>
</table>

$^a$ See reference 16.
Salmonella

(ii) 25-g ground- and 11 beef, u (16),

Bias, actual minus predicted lethality (log reductions).
tb

O157:H7 lethality 2 ~

Replication error for measured process lethality at three experimental scales

<table>
<thead>
<tr>
<th>Species</th>
<th>1 g of ground</th>
<th>25 g of ground</th>
<th>25 g of whole</th>
<th>500–1,000 g of whole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turkey</td>
<td>0.11</td>
<td>0.81</td>
<td>0.80</td>
<td>1.37</td>
</tr>
<tr>
<td>Beef</td>
<td>0.13</td>
<td>0.95</td>
<td>0.65</td>
<td>0.93</td>
</tr>
<tr>
<td>Pork</td>
<td>0.12</td>
<td>0.41</td>
<td>1.13</td>
<td>1.02</td>
</tr>
</tbody>
</table>

*Values expressed as log CFU per gram. Experimental scales: 1 g (16); 25 g (4); 500 to 1,000 g (this study).

initial Salmonella core population of 6.3 to 7.0 log CFU/g, these results generally support the regulatory premise that an end-point temperature of 71.1°C is extremely likely to ensure a sufficient cook. However, the very limited number of survivors makes these data unsuitable for quantifying model accuracy, which is described in subsequent sections.

Replication error. When comparing the replicate lethality results between (i) 1-g ground-meat samples cooked in a thermocycler (16), (ii) 25-g ground- and whole-muscle samples cooked in a bench-top convection oven (4), and (iii) 500- to 1,000-g whole-muscle samples cooked in a pilot-scale moist air convection oven (this study), the replication error (log CFU per gram) for turkey, beef, and pork increased with sample size (Table 3). These findings suggest that substantial variability should be expected and accounted for when applying lethality models to validate actual cooking processes. Wiegand et al. (23) reported very similar results for E. coli O157:H7 lethality when cooking inoculated beef products; the variability in their results increased markedly when scaling up from 25-g ground beef samples in laboratory trials to full-sized, whole-muscle roasts (2.2 to 8.9 kg) in a commercial smokehouse. The associated variability in cooking times for the 71.1°C end-point trials and the lethality end-point trials in this study (in terms of the mean standard deviation among replicates within treatments) for turkey, beef, and pork was 6.7, 8.0, and 1.0 min, respectively (for 71.1°C end points) and 2.1, 7.8, and 4.9 min, respectively (for lethality end points), and the mean total cook times for all treatments were 184, 145, and 126 min, for turkey, beef, and pork, respectively.

Comparison of the state-dependent and path-dependent models. When using model parameters from controlled laboratory experiments, it would be ideal to apply parameters generated from ground-muscle samples to ground-muscle products, and those generated from whole-muscle samples to whole-muscle products. However, the path-dependent model parameters were obtained using ground-muscle samples (16), given the impracticality of creating whole-muscle samples of less than 1 g that retain their muscle structure. Nevertheless, to understand the usefulness of the path-dependent model in pilot-scale, slow-cooking processes, these parameters were applied to the whole-muscle roast data that had quantifiable Salmonella survivors (n = 9 turkey, n = 11 beef, n = 20 pork). The whole-muscle, state-dependent model also was applied to the data to compare both models’ predictive ability. All of the model performance measures were computed by aggregating the predicted and experimental lethality results from all of the cooking treatments for a given product species.

The path-dependent model performed better than the state-dependent model (i.e., considerably improved RMSE and bias) for validating turkey samples, but produced fail-dangerous results for beef (i.e., overprediction of lethality), even more so than the state-dependent model (Table 4). For the pork roasts, the path-dependent model produced more conservative results (i.e., underpredictions of lethality) than the state-dependent (Table 4). The deviation between species, in terms of the statistical performance of the models, could be attributed to differences in the targeted final log reduction. Pork and beef had lower targeted lethalities (3.0 and 5.5 log CFU/g, respectively) as compared with turkey (7.0 log CFU/g), which allowed for more positive (fail-safe, underpredicted) lethality errors to be quantified.

To verify whether the prediction errors from both models were significantly different, and specifically to determine whether the state-dependent model error was more fail-dangerous than that resulting from the path-dependent model predictions, t tests were conducted on the prediction bias for all three species. In the case of turkey and pork, the test results showed that, as expected, the state-dependent model’s errors were more fail-dangerous (P < 0.05); but, for the beef roasts, the t test revealed that the path-dependent model prediction errors actually were more fail-dangerous. This divergence from the anticipated results could be due to these samples having more than 60% of the data points with a sublethal history (τ) of <200 K–min, and only one at ~500 K–min, which meant that the effect of β2 in the path-dependent model was much less for the beef data.

To assess whether prediction error directly correlated to sublethal injury (τ) when using the state-dependent model,
ANOVAs were performed on the data sets from the three species. Error and sublethal injury correlated for beef ($P < 0.05$) but not for turkey and pork ($P > 0.10$) (Fig. 3). In the beef samples, error tended to decrease with sublethal injury, which is opposite to what Tenorio-Bernal et al. (16) and Stasiewicz et al. (14) reported. However, this project analyzed a much smaller data set than previous studies, which entailed laboratory-based thermal treatments.

**Challenges of whole-muscle pilot-scale research.** During this study, many challenges centered on developing a reliable experimental method that also would be relevant to industry practices, because very little prior thermal inactivation research has used inoculated whole-muscle products, particularly at the commercial scale.

One major challenge was introducing and removing the pathogen from the product cores, while still replicating industry practices as closely as possible. Several studies have documented the migration of pathogens into intact whole muscle (6, 21); however, a reliable inoculation method for achieving high numbers of the target pathogen in the product core has not been previously reported. Warsow et al. (21) did show penetration of Salmonella into whole-muscle turkey breast, but at a core concentration of $<3.0$ log CFU/g. In this study, an inoculated marinade and vacuum tumble marination method was used to achieve a Salmonella core population of $>7.0$ log CFU/g in a 500- to 1,000-g sample. However, the targeted population was still difficult to consistently achieve, as evidenced by the relatively large standard deviations for the initial population, particularly for pork ($1.06$ log CFU/g).

Accurate recovery of Salmonella from large roasts after thermal treatment also was a challenge. Luchansky et al. (6) quantified E. coli O157:H7 reductions in blade-tenderized steaks, after commercial-scale open-flame grilling. Similar to this study, quantifying the postcooking pathogen reduction was a challenge, as evident from their large range of standard deviations (0.16 to 2.13 log CFU/g).

Very few pathogen inactivation studies have been performed with intact whole-muscle products (23), likely due to the challenges associated with inoculation and postprocessing recovery. Intact, whole-muscle products are typically used in studies where the sample sizes are relatively small and, therefore, are not directly applicable to industry relevant processing. For example, in a prior bench-top study, small (5-g) samples of whole-muscle beef were immersed in a Salmonella-inoculated marinade ($10^8$ CFU/ml); the reported initial Salmonella population was $\sim 7.8$ log CFU/g (9). By comparison, the inoculated marinade in the present study had a concentration of $9.54 \pm 0.56$ log CFU/g, but the average Salmonella population at the center of the roast following vacuum tumble marination was only 6.27 log CFU/g.

Overall, the state-dependent model evaluated in this study has the potential to overestimate Salmonella lethality during slow cooking of whole-muscle roasts, likely due to the effects of sublethal heating. The path-dependent model, which accounts for those effects, was more accurate in predicting inactivation of Salmonella in turkey roasts, resulting in a safer end product as compared with the state-dependent model. Additionally, the practical conclusions of these results are the following: (i) although slow cooking can impart enhanced heat resistance to Salmonella, cooking to a 71.1°C end point still is reasonably likely to achieve sufficient lethality and (ii) if a traditional ($D, z$), state-dependent model is used to compute lethality and validate a slow-cooking process to achieve a marginally sufficient outcome (i.e., at or near that required by the regulatory performance standards), there is a real possibility that the actual outcome could be an underprocessed product.

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