FINAL PROJECT REPORT
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PROJECT TITLE: Determining the Likelihood that *Salmonella* Develops Heat Resistance during Thermal Processing of Commercial, Whole-Muscle, Ready-to-Eat Meat Products

PRINCIPAL INVESTIGATORS:

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PROJECT DURATION: April 2006 to September 2008

EXECUTIVE SUMMARY:

The purpose of this project was to directly address a targeted research need, identified by AMIF: "*Determine the likelihood that* Salmonella *will develop heat resistance in the relatively short times used to cook RTE foods, especially whole-muscle cuts.*" To do so, the project entailed three objectives, aimed at: (1) modifying a new thermal inactivation model that accounts for the effect of sub-lethal injury on subsequent heat resistance of *Salmonella*, (2) validating the model via laboratory- and pilot-scale challenge studies, and (3) evaluating whether the resulting increase in *Salmonella* thermal resistance has practical impact on the compliance of typical commercial cooking operations with USDA-FSIS lethality performance standards for ready-to-eat (RTE) products. To achieve these objectives, turkey and beef products were inoculated with an eight-serovar *Salmonella* cocktail and subjected to thermal process trials at three scales: (1) non-isothermal heating of 1 g samples (ground) in a thermocycler, (2) cooking of 25 g samples (whole-muscle and ground) in a laboratory-scale, moist-air convection oven, and (3) cooking of ~1 kg samples (whole-muscle) in a pilot-scale, moist-air convection oven according to a variety of typical cook schedules (1.4-5.5 h total cooking time). Results from all three test series showed that traditional inactivation parameters (D and z, previously determined via isothermal laboratory studies) over-predicted actual mean *Salmonella* lethality ($P<0.05$), with over-prediction errors as high ~8, 4, and 5 $\log_{10}$ for the three cooking systems, respectively. Additionally, the error increased with increasing sub-lethal heating (as occurs with increasing total cook time). Therefore, the data from the 1 g samples were used to estimate parameters for the new inactivation model, which accounts from the effect of sub-lethal history (which occurs during the early phase of cooking) on subsequent lethality. When applied to these data, the improved model reduced the mean over-prediction errors from 4.6 and 3.5 $\log_{10}$ (for turkey and beef, respectively) to -0.03 and -0.02 $\log_{10}$, which were insignificant mean errors ($\alpha=0.05$). The pilot-scale data also revealed two significant results. First, variability and uncertainty in *Salmonella* lethality increases significantly when scaling-up inactivation results from laboratory to pilot-scale, which needs to be considered when computing process lethalities. Secondly, for inoculated whole-muscle roasts cooked to a core temperature of 71.1°C (160°F) in the pilot-scale oven, no salmonellae were recovered via standard plate counts, indicating that there was no significant under-processing of those products. However, for slow-cooked roasts cooked to a target computed lethality (e.g., 6.5 $\log_{10}$ reductions), the results indicate that there is significant risk of not meeting the lethality performance standards. Therefore, particular caution (and/or improved modeling methods) should be exercised for marginally-processed products.

GOALS AND OBJECTIVES

This project was part of an overall research program with the following long-term mission: *To develop improved methods for the design and operation of thermal processing systems for meat and poultry products, based on the criteria of microbial safety, processing yield, and product quality.* The specific objectives were:

1. To adapt, for whole-muscle products, a model recently developed at MSU to predict the rate of *Salmonella* thermal inactivation as a function of both product temperature *and* prior (sub-lethal) thermal history.
2. To validate this model via pilot-scale challenge studies with whole-muscle products inoculated with *Salmonella* and subjected to moist-air cooking that emulates commercial processes.
3. To evaluate whether any resulting increase in *Salmonella* thermal resistance would have practical impact on the compliance of typical commercial cooking operations with the USDA-FSIS lethality performance standards for RTE products.

MATERIALS AND METHODS

The overall logistics for the various product types and cooking systems in this project are illustrated in Figure 1. The project included turkey and beef products, ground and whole-muscle products, and three different cooking systems, which are all described in the following sections.

Meat

Whole-muscle turkey breasts and beef top rounds were obtained fresh, directly from local processers. The products were fabricated to the sizes needed for the different phases of this project (e.g., ~25 g for the laboratory-scale tests and ~1 kg for the pilot-scale tests), and random portions of each lot were also ground to yield ground and whole-muscle product of equivalent composition. All samples were then vacuum packaged and frozen at -12°C. The samples were then shipped frozen to FTSI (Mulberry, FL) and irradiated (>10 kGy) to eliminate background microflora. Sterility after irradiation was tested by diluting random samples from each box 1:5 in peptone water (Difco, Detroit, MI), followed by plating on Petrifilm™ Aerobic Count Plates (3M, St. Paul, MN). Frozen samples were thawed (~4 h @ 4°C) prior to inoculation.

Inoculation

The inoculum consisted of a *Salmonella* cocktail, comprised of the following 8 serovars, previously obtained from Dr. V.K. Juneja at the USDA-ARS, Eastern Regional Research Center: *S.* Thompson FSIS 120*, S.* Enteriditis H3527 and H3502, *S.* Typhimurium H3380 (human isolate), *S.* Hadar MF60404, *S.* Copenhagen 8457, *S.* Montevideo FSIS 051, and *S.* Heidelberg F5038BG1. Each serovars was cultured separately in TSB containing 0.6% yeast extract (37°C; 2 consecutive 24 h transfers) to obtain ~10^9 CFU/ml. On the day of inoculation, equal volumes of the 8 cultures were combined, centrifuged, and re-suspended in sterile marinade (11.5% salt, 3.7% phosphate in distilled water) to ~10^9 CFU/ml, confirmed by plating on trypticase soy agar containing 0.6% yeast extract.

The whole-muscle samples were vacuum tumbled (~25 in Hg; 8 rpm) in the inoculated marinade for 40 min at 4°C in a laboratory-scale tumbler, which achieved ~$10^{6.9}$ and $10^{6.7}$ CFU/g

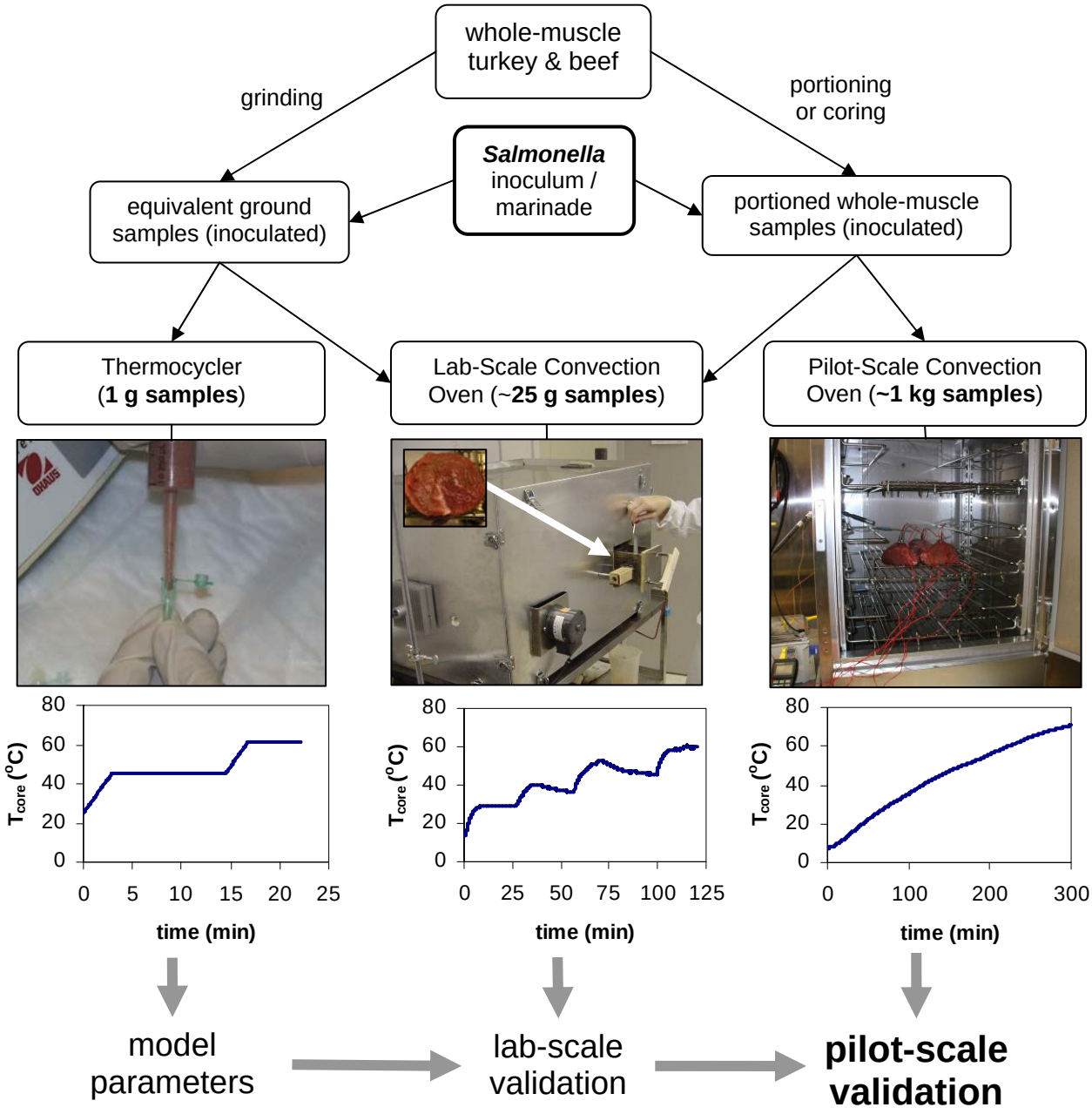

Figure 1. *Conceptual flowchart illustrating: (a) sample preparation logistics, (b) the three thermal processing methods (and example core temperature profiles from each), and (c) the outcomes from each of the project phases.*

at the center of whole-muscle turkey and beef roasts, respectively. The ground samples for the laboratory studies were inoculated by adding the same inoculated marinade dropwise (at the same proportion as the uptake in the whole-muscle samples) and mixing to ensure uniform distribution.

Cooking

The project entailed three different scales of cooking trials, as illustrated in Figure 1. The first (1 g samples in a thermocycler) was designed to achieve very precise control of sample temperature and to thereby estimate the parameters of a new thermal inactivation model (described below). The second (25 g samples in a laboratory-scale, moist-air convection oven) was designed to test the new inactivation model and the effect of slow-cooking on *Salmonella* lethality. The third (whole-muscle roasts in a pilot-scale, moist-air convection oven) was designed to quantify the impact of slow-cooking on the accuracy and variability of lethality predictions for commercial-type processes.

1 g samples (non-isothermal in thermocycler)

The inoculated 1 g samples of ground product were divided into five 0.2 g sub-samples, which were inserted into samples tubes (Figure 1), inserted in a thermocycler, and equilibrated to 25°C. The thermocycler was then programmed for a specific linear heating rate (1, 2, 3, 4, or 7 K/min), sub-lethal holding temperature (40, 45, or 50°C), sub-lethal holding period (0-86 min), final holding temperature (55, 58, 61, 64), and final holding period (set to achieve a nominal lethality of 3 or 5 log reductions). Out of the total possible combinations of these parameters, 46 were randomly selected (with total heating times ranging from 10.5 to 113 min) to generate data to estimate the model parameters. After thermal treatment, the five sub-samples were immediately placed on ice, and subsequently recombined prior to enumeration of *Salmonella* survivors on aerobic Petrifilm™.

25 g samples (laboratory-scale, moist-air convection oven)

The inoculated 25 g samples (ground and whole-muscle; ~1 cm thick and ~2.6 cm diam; triplicate) were cooked in a custom-built, computer-controlled, moist-air convection oven (Figure 1). Oven temperature was controlled (±1°C) by electrical heaters. Oven humidity was monitored by a polymer sensor (Vaisala DRYCAP®, Vaisala, Helsinki, Finland) and controlled (±1%) by a steam generator. Air velocity through the sample chamber (10 cm x 10 cm x 10 cm) was ~1.3 m/s. Oven temperature and core temperature of the samples were monitored using a T-type thermocouple.

The experimental design consisted of three base cooking schedules (Table 1), designed to ramp-up the product temperature to approximate the change in core temperature that occurs during cooking of full-sized commercial products. During cooking, *Salmonella* lethality was computed real-time, based on the transient core temperature and D and z values previously determined for the same *Salmonella* cocktail in ground and whole-muscle beef and turkey[1,2].

[1] Orta-Ramirez A, Marks BP, Warsow CR, Booren AM, Ryser ET. 2005. Enhanced thermal resistance of *Salmonella* in whole muscle vs. ground beef. *Journal of Food Science*. 70(7):359-362.

[2] Tuntivanich V, Orta-Ramirez A, Marks BP, Ryser ET, Booren AM. 2008. Thermal inactivation of *Salmonella* in whole muscle and ground turkey breast. *Journal of Food Protection*. 71(12):2548-2551.

When the computed lethality reached 7.0 or 6.5 $\log_{10}$ reductions for the turkey and beef products, respectively, the samples were removed from the oven, immediately cooled in sterile, chilled peptone water, and subsequently serially diluted and plated (on aerobic count Petrifilm™) for enumeration of *Salmonella* survivors.

Table 1. The temperatures, relative humidities, and durations of the sequential cooking stages (1-5) for the three base cooking schedules in the laboratory-scale, moist-air convection oven. (n/a = not applicable, because target lethality was achieved prior to reaching stage 5).

Cooking stages	Dry bulb temp (°C)	Wet bulb temp (°C)	% RH	Cooking Schedule		
				A	C	E
				Time (min)		
1	32.0	28.1	80.0	4.0	15.2	26.3
2	35.0	33.1	90.0	4.5	17.1	29.6
3	45.0	42.9	90.0	6.5	24.6	42.8
4	55.0	52.8	90.0	6.5	variable	variable
5	60.0	57.7	90.0	variable	n/a	n/a

1-2 kg samples (pilot-scale validation trials)

Inoculated, whole-muscle roasts (~1 kg, in duplicate or triplicate) were cooked (Figure 1) in a pilot-scale, moist-air convection oven (Aqua-Temp, CresCor, Mentor, OH). The seven different combinations of cooking conditions are illustrated in Figure 2, and were selected to represent a range of typical commercial cooking strategies and schedules. Oven conditions and roast temperatures were monitored and recorded using a PC-based data acquisitions system (LabView, National Instruments). For the high humidity condition, the oven was capable of operating at >98% relative humidity; however, because the oven door was opened 2-3 times during a cooking cycle (to remove samples), the average relative humidity during a high humidity cooking cycle was actually ~78%. In order to best ensure that the measured product temperature represented the cold-spot, two thermocouple probes were inserted to the product center, and both temperatures were monitored real-time. The colder of the two probes was presumed to best represent the core (cold-spot) temperature, and *Salmonella* lethality was calculated real-time from those data, as described for the laboratory-scale trials. Roasts were removed when either: (a) the core temperature reached 71.1°C (160°F), or (b) the calculated *Salmonella* lethality reached 7.0 or 5.5 $\log_{10}$ reduction, for turkey and beef, respectively. A core sample (~20 g) was immediately removed from the center of the roast, cooled, and plated to enumerate *Salmonella* survivors.

Model

Isothermal inactivation parameters

As indicated above, the laboratory-scale and pilot-scale oven cooking tests were designed to achieve target lethalities (computed real-time), based on a Bigelow-type (log-linear) inactivation models. The T_{ref}, D_{ref}, and z values used for these calculations (determined in previous studies) are given in Table 2.

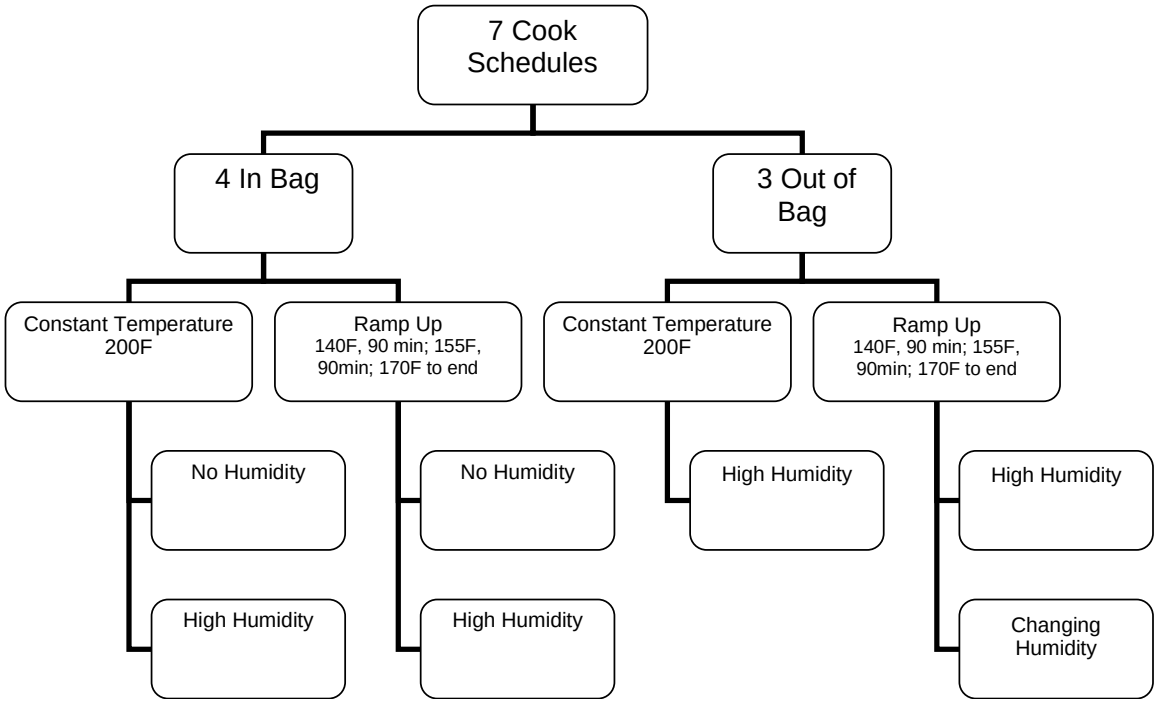

Figure 2. Experimental design for pilot-scale validation trials in moist-air convection oven.

Table 2. Thermal inactivation parameters, from prior isothermal laboratory studies, used to compute process lethalities in the laboratory-scale and pilot-scale oven cooking trials in this study.

	T_{ref} (°C)	D_{ref} (min)	z (C°)
turkey breast			
whole-muscle	60	1.91	5.4
ground	60	1.03	5.2
beef round			
whole-muscle	60	1.86	5.5
ground	60	1.07	5.6

New, path-dependent model

Our recently developed, *path-dependent* model for thermal inactivation quantifies the degree of sub-lethal injury (τ) in terms of the cumulative time-temperature integral in the temperature range known to elicit an injury response in *Salmonella*, such that:

$$\tau = \int_{t_{T=HS_{lower}}}^{t_{T=HS_{upper}}} [T(t) - HS_{lower}]dt$$

Inserting this expression into a modified Arrhenius-type model for thermal inactivation rate, and inserting that model into standard first-order kinetics, yields the complete inactivation model:

$$ln\frac{N}{N_0} = -k_{ref}\int_0^t e^{\underbrace{-\beta_1\left(\frac{1}{T(t)}-\frac{1}{T_{ref}}\right)}_{state}\underbrace{-\beta_2\int_{t_{T=HS_{lower}}}^{t_{T=HS_{upper}}}(T(t)-HS_{lower})dt}_{sub\text{-}lethal\ history}} dt$$

which describes cumulative inactivation of *Salmonella* [$\ln(N/N_0)$] as a function of both product temperature (state) and the integrated thermal history for the time the product spent in the sub-lethal temperature zone (e.g., HS_{lower} to HS_{upper} = 38 to 52°C). The labeled portions of the model above are the modified-Arrhenius-type model, accounting for the combined effect of current temperature and prior thermal history. The parameters for this model (k_{ref}, β_1, β_2) were previously determined for *Salmonella* in ground turkey thigh[3]. The data from the 1 g (thermocycler) samples in this study (ground turkey breast and ground beef round) were similarly used to estimate these parameters for these products, by minimizing the sum of squared errors between the computed and observed lethality (i.e., log reductions of *Salmonella*).

Lethality error vs. sub-lethal history

In order to quantify the validity of traditional modeling methods (D and z) in predicting *Salmonella* lethality in oven-cooked turkey and beef products, the expression for sub-lethal history described above (τ) was applied to all of the time-temperature profiles from both the laboratory-scale and pilot-scale cooking trials. Subsequently, the relationship between lethality error (i.e., the difference between computed and actual *Salmonella* inactivation) and τ was tested to determine whether significant lethality errors occur for slow cooked products, and to determine whether any such error increases with the degree of sub-lethal history.

Results

The results of the three project phases are presented here in reverse order, in order to illustrate first the final result (effect of slow cooking on lethality errors) and then the capabilities of the new, path-dependent inactivation model to mitigate systematic errors.

Cooking to 71.1°C (160°F)

For the seven different pilot-scale cooking conditions (Figure 2), no surviving salmonellae were recovered (plate counts) from any of the roasts (turkey and beef) cooked to a core temperature of 71.1°C (160°F). Although the initial core inoculation level was slightly below the lethality target for the turkey (6.9 instead of 7.0 $\log_{10}$), we can still conclude that there was no significant risk of survivors for any of these cases. In applying the traditional, log-linear, inactivation kinetics, the computed lethalities for these processes were all >>25 $\log_{10}$. Therefore, even if there was a meaningful error between computed and actual lethality (say, for example, a 5-$\log_{10}$ error), then such an error could not be detected experimentally and also would not have any bearing on the microbial safety of a product that is processed so significantly beyond the lethality performance standards.

Lethality error vs. sub-lethal history

However, for the samples processed to a target lethality, the traditional inactivation model (D and z) over-predicted the mean *Salmonella* inactivation ($P<0.05$), with errors as high as ~8, 4, and 5 $\log_{10}$ for the 1 g, 25 g, and ~1 kg (pilot-scale) studies, respectively (Figures 3 and 4).

[3] Stasiewicz MJ, Marks BP, Orta-Ramirez A, Smith DM. 2008. Modeling the effect of prior sublethal thermal history on the thermal inactivation rate of *Salmonella* in ground turkey. *Journal of Food Protection*. 71(2):279-285.

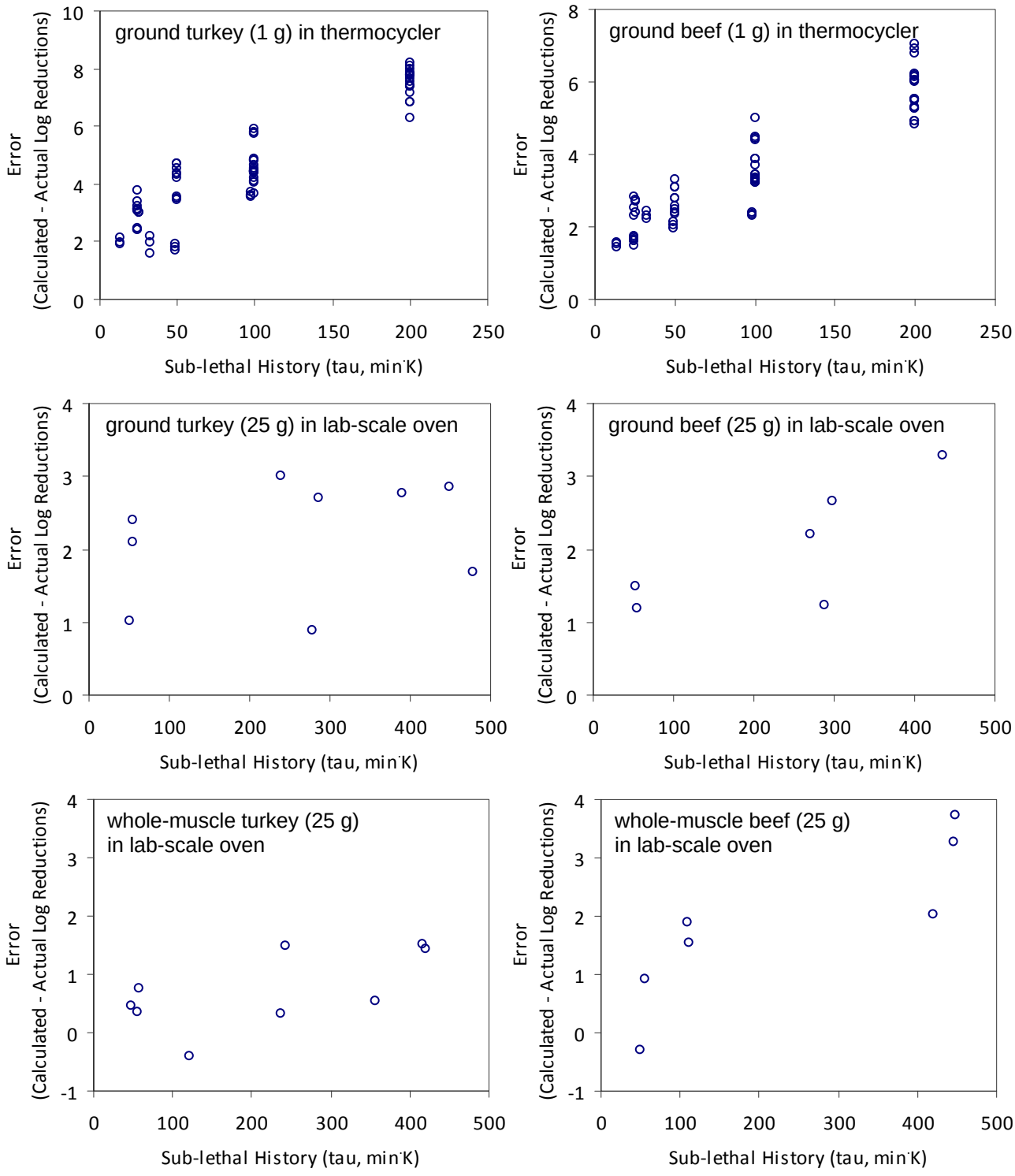

Figure 3. Plots of Salmonella *lethality error (i.e., calculated minus actual log reductions) vs. the extent of sub-lethal history, when calculations were based on D and z values determined in isothermal laboratory studies. The top two graphs are for ground product (1 g) heated in a thermocycler. The bottom four graphs are for ground and whole-muscle product (25 g) cooked in a laboratory-scale, moist-air convection oven. Larger sub-lethal history generally corresponds to longer cooking times, particularly with slow come-up through the critical temperature range affecting sub-lethal injury (38-52 ℃).*

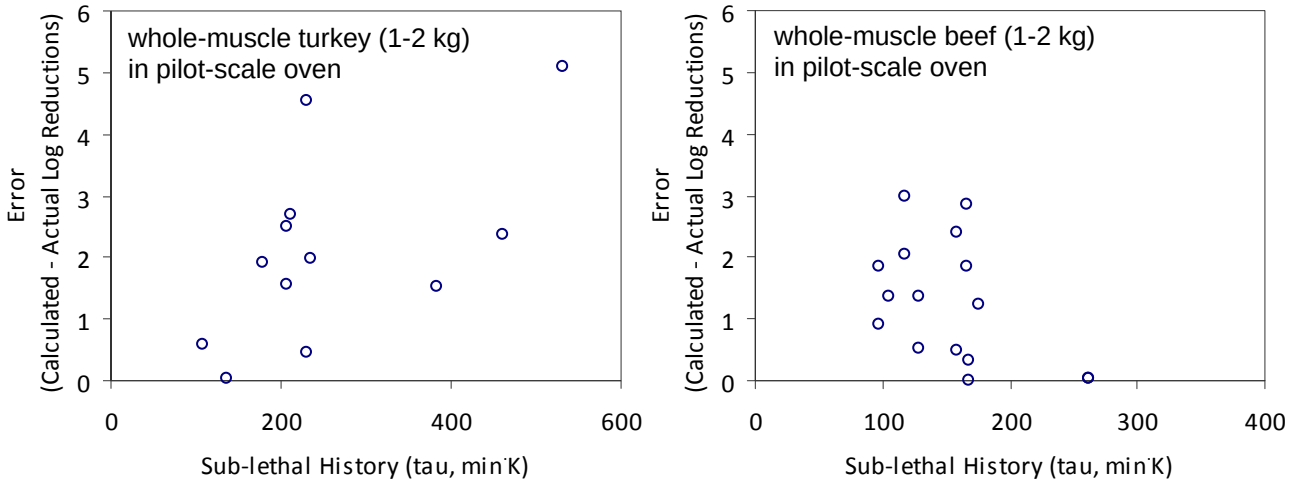

Figure 4. Plots of Salmonella *lethality error (i.e., calculated minus actual log reductions) vs. the extent of sub-lethal history, when calculations were based on D and z values determined in isothermal laboratory studies. These data are for whole-muscle roasts (1-2 kg) cooked in a pilot-scale (CresCor), moist-air convection oven. Larger sub-lethal history generally corresponds to longer cooking times, particularly with slow come-up through the critical temperature range affecting sub-lethal injury (38-52°C).*

Additionally, for all of above cases, except the pilot-scale trials with the whole-muscle beef roasts, the lethality error increased ($P<0.05$) with increasing sub-lethal history (τ). This suggests that a greater degree of sub-lethal history corresponds to greater sub-lethal injury, which corresponds to an adaptive response in *Salmonella*, which in term corresponds to an increased thermal resistance (showing up as an over-prediction of the observed lethality). It is unknown why the pilot-scale trials with the whole-muscle beef roasts so clearly deviated from this trend, and we are continuing to investigate this phenomenon with further analysis and experiments.

The other observation to make from Figures 3 and 4 is the significant increase in variability that occurred as the lethality trials were scaled-up from 1 g to 25 g to ~1 kg. Most published inactivation parameters are determined via very small (e.g., ~1 g) samples in laboratory tests (often isothermal water bath studies); therefore, it is mission-critical to recognize that there is significant uncertainty underlying any application of these methodologies and models to commercial-scale applications, and process validations should account for this fact.

New, path-dependent model

The parameters of the new, path-dependent inactivation model were successfully estimated based on temperature and inactivation results from the 1 g samples. When applying traditional, log-linear inactivation kinetics to these data, the mean over-prediction errors were 4.6 and 3.5 $\log_{10}$ for turkey and beef, respectively. When applying the new model, the systematic over-prediction error was completely eliminated ($\alpha=0.05$) (Figure 5). This confirms that the new model successfully accounts for the effects of sub-lethal heating (τ) on the increased thermal resistance of *Salmonella* in slow-cooked turkey and beef.

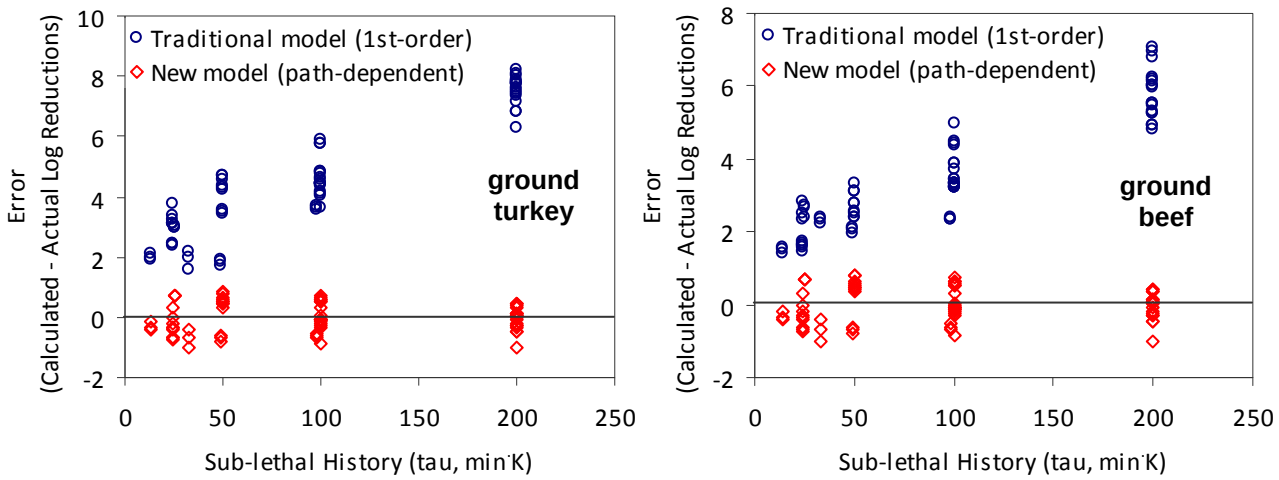

Figure 5. Plots of Salmonella *lethality error (i.e., calculated minus actual log reductions) vs. the extent of sub-lethal history, when calculations were based on: (the blue circles) D and z values determined in isothermal laboratory studies and (the red diamonds) the new, path-dependent model developed and tested in this study. These data are for the 1-g, ground samples processed in the thermocycler. Larger sub-lethal history generally corresponds to longer cooking times, particularly with slow come-up through the critical temperature range affecting sub-lethal injury (38-52 °C).*

Application of model to laboratory-scale data

The new, path-dependent model was then applied to lethality data from the laboratory- and pilot-scale trials. In most of the cases, the new model over-compensated for sub-lethal effect, given that the lethality errors in the 1 g samples were much larger than in the oven tests, for equivalent τ values. Additionally, the tests with 1 g samples could be conducted with only ground (not whole-muscle) product; given prior studies, we know that the thermal resistance of *Salmonella* in whole-muscle product can be ~2× greater than in equivalent ground product, and additional data are needed from the laboratory-scale oven to improve the estimates of model parameters for whole-muscle products. However, Figure 6 illustrates one example, in which the path-dependent model successfully eliminated the systematic error between the predicted and actual *Salmonella* lethality for whole-muscle beef samples cooked in the laboratory-scale convection oven.

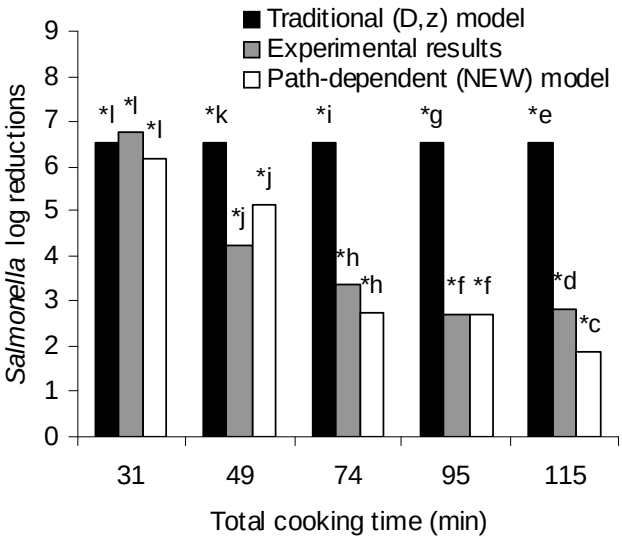

Figure 6. Experimental log reductions of Salmonella *in whole-muscle beef cooked in a laboratory-scale, moist-air convection oven, compared to predictions using traditional and new (path-dependent) inactivation models. Results labeled with identical letters (e.g., *k) are not significantly different (α=0.05).*

Conclusions

The results of this study have confirmed the following:

1. *Salmonella* can develop significantly increased thermal resistance due to sub-lethal injury that can occur during slow cooking of whole-muscle meat and poultry products.
2. Traditional inactivation models (D and z) based on isothermal inactivation studies can significantly over-predict the actual lethality of *Salmonella* in slow-cooked meat and poultry products, with the degree of over-prediction increasing with the extent of sub-lethal heating.
3. The uncertainty underlying thermal process validations increases significantly when scaling predictions from laboratory- to pilot-scale (and presumably commercial-scale) applications.
4. Whole-muscle turkey and beef products cooked in a moist-air convection oven to a core temperature of 71.1°C (160°C) all exceeded the lethality performance standards.
5. There was a significant risk of *not* achieving the lethality performance standards for whole-muscle turkey and beef products cooked just to the target lethality (i.e., 7.0 or 6.5 $\log_{10}$ reductions, respectively), computed via traditional methods (D and z from laboratory studies).

Next Steps

The tests that were completed during this study currently are being extended as part of a three-year project funded by the USDA National Integrated Food Safety Initiative. Inoculated challenge studies are being conducted with ~12 different classes of meat and poultry products cooked in both moist-air convection and impingement ovens in the MSU Biosafety Level-2 Pilot Processing Facility. The aim of this large project is to translate results from this (AMIF) and the USDA project into a web-based process lethality tool that will account for the effects of product species, structure, composition, and heating profile (including sub-lethal injury) in computing process lethality and reliable estimates of uncertainty.

Presentations and Publications

Several manuscripts are in preparation for submission to the *Journal of Food Science* and *Journal of Food Protection* within the next few months. These manuscripts are based on the following abstracts that have been (or will be) presented, based on the results of this project.

1. Mogollon MA, Marks BP, Jeong S, Stasiewicz MJ, Booren AM. 2007. Effect of cooking profiles and sub-lethal history on *Salmonella* thermal inactivation in whole-muscle beef. IFT Abstract 098-09. Presented at the Institute of Food Technologists Annual Meeting. Chicago, IL . July 2007.
2. Jones SL, Marks BP, Booren AM, Ryser ET, Hall NO. 2009. Effect of sub-lethal heating on *Salmonella* lethality during slow-cooking of turkey and beef products. Abstract, submitted for presentation at the 2009 IFT Annual Meeting. Anaheim, CA.
3. Breslin TJ, Marks BP, Booren AM, Ryser ET, Hall NO. 2009. Pilot-scale validation of *Salmonella* thermal inactivation in whole-muscle turkey breast. Abstract, submitted for presentation at the 2009 IFT Annual Meeting. Anaheim, CA.
4. Marks BP, Tenorio-Bernal MI, Breslin TJ, Ryser ET, Booren AM. 2009. Validating a *Salmonella* thermal lethality model that accounts for prior sublethal injury during commercial meat and poultry cooking processes. Abstract, submitted for presentation at the 2009 ASABE International Meeting. Reno, NV.