

## PORK SAFETY

**Title:** Quantifying the Effect of Slow-Cooking Operations on the Thermal Resistance of *Salmonella* in Whole-Muscle Pork Products - **NPB #09-065**

**Investigator:** Bradley P. Marks, Ph.D., P.E.

**Institution:** Michigan State University

**Date Submitted:** 7 March 2011

### Industry Summary

The overall goal of this project was to improve the reliability of thermal process validation tools for *Salmonella* in pork products, by accounting for stress adaptation that can occur during slow cooking processes. The specific objectives were: (1) To modify, for ground and whole-muscle pork 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, and (2) To validate this model via pilot-scale challenge studies using ground and whole-muscle pork products inoculated with *Salmonella*. The project entailed thermal treatment (cooking) trials in four different systems, ranging from 1 g samples in a highly controlled laboratory system to ground pork patties and whole-muscle chops and roasts in two pilot-scale oven systems. The key results were: (1) The thermal resistance of *Salmonella* in pork products increases when subjected to sublethal injury during slow cooking processes, and an improved inactivation model accounts for that effect; (2) The thermal resistance of *Salmonella* is significantly greater (~50%) in whole-muscle pork than in ground pork; (3) Application of laboratory-based inactivation data for commercial process validations carries inherent uncertainty that is generally underreported and that increases with scale-up; and (4) Although slow cooking processes can allow *Salmonella* to adapt and become more thermally resistant, moist-air cooking to an endpoint  $\geq 160^{\circ}\text{F}$  generally results in sufficient cumulative lethality to overcome this effect. These findings mean that producers of ready-to-eat pork products need to ensure that process validations are based on inactivation data and models that are appropriate to their specific product and process (e.g., whole-muscle vs. ground) and that thermal processes are designed with a sufficient margin of safety to account for the inherent uncertainty associated with the application of inactivation models to thermal process validations.

Contact: Dr. Bradley Marks, Michigan State University, 210 Farrall Hall, East Lansing, MI 48824-1323; marksbp@msu.edu

**Keywords:** food safety, *Salmonella*, cooking, lethality, process validation

---

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.

---

For more information contact:

National Pork Board • PO Box 9114 • Des Moines, IA 50306 USA • 800-456-7675 • Fax: 515-223-2646 • pork.org

---

## Scientific Abstract

The overall goal of this project was to improve the reliability of thermal process validation tools for *Salmonella* in pork products, by accounting for stress adaptation that can occur during slow cooking processes. The specific objectives were: (1) To modify, for ground and whole-muscle pork 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, and (2) To validate this model via pilot-scale challenge studies using ground and whole-muscle pork products inoculated with *Salmonella*.

*Salmonella*-inoculated pork products were heated in four different heating/cooking systems at three different scales. Small samples (1 g) were heated in a thermocycler to generate a set of well-controlled temperature profiles and thereby generate inactivation data that were used to estimate parameters of a modified thermal inactivation model accounting for sublethal history and its effect on subsequent thermal resistance of *Salmonella*. Traditional inactivation models (D and z) and the modified model were applied to validation data generated by cooking inoculated pork patties and whole-muscle products in a custom, bench-scale, moist-air convection oven, a pilot-scale impingement oven, and a pilot-scale, moist-air convection oven (using cooking processes that mimicked commercial operations). Surviving *Salmonella* were enumerated, and experimental process lethality (log reductions) were compared to those calculated using the collected core temperature data and the inactivation models.

The laboratory-scale studies demonstrated that the traditional, log-linear inactivation models can over-predict *Salmonella* lethality ( $P < 0.001$ ) when applied to processes that subject the product to significant time in the critical sublethal temperature region (i.e., 40-50°C or 104-122°F). A modified inactivation model, in which the thermal inactivation rate is a function of current temperature and an integral of the prior sublethal history, eliminated this systematic over-prediction of lethality, reducing the root mean squared error (RMSE) by 82% and reducing the over-prediction bias from 2.6 log to an under-prediction (fail-safe) bias of 0.3 log.

Bench-scale oven treatments (20 g pork patties and mini-steaks, 1 cm thick) validated the above findings. For both the ground-and-formed and whole-muscle pork products, the traditional inactivation model (D and z values from prior isothermal laboratory studies) significantly ( $P < 0.001$ ) over-predicted lethality, with individual errors as large as 2.1 and 6.0 log, respectively. For the pork patties, the size of the computed lethality error increased ( $P < 0.01$ ) with increasing sublethal history (corresponding to cooking times up to 115 min).

At the pilot-scale, impingement cooking of pork patties and whole-muscle pork chops was used to test the utility and validity of D and z values previously determined in isothermal laboratory studies with both product types. For the whole-muscle product, the RMSE and bias were 1.5 and 0.8 log, respectively. For the patties, these values were larger (2.4 and 1.1), but 88% of the pilot-scale outcomes fell within the 95% prediction intervals for computed lethality. These outcomes are consistent with expectations, given that the fast cooking times in this system (< 9 min) did not cause any significant sublethal heating.

In pilot-scale, slow cooking trials (86-253 min) with inoculated (6.3 log CFU/g) whole-muscle pork roasts, processing to endpoint temperatures  $\geq 71.1^\circ\text{C}$  ( $\geq 160^\circ\text{F}$ ) resulted in no countable surviving *Salmonella*. Although sublethal injury causes increased thermal resistance, slow cooking to this endpoint yielded sufficient cumulative lethality to overcome this effect. Nevertheless, scale-up to the pilot-scale caused significant increases in the inherent uncertainty associated with the application of lethality models (0.08 to 1.5 log), and commercial processors should account for this factor when validating the safety of cooking operations.

## Introduction

This project was motivated by: (1) the dynamic nature of the challenges associated with microbial food safety, (2) prior research demonstrating that the thermal resistance of *Salmonella* is significantly affected by both product structure (whole vs. ground muscle) and the rate of heating, and (3) the insufficiency of commercially relevant data and tools to enable the industry to use this knowledge for improved product safety. Therefore, in order to best ensure consumer safety and protect market share at the center of the consumer's plate, the pork industry needs advanced knowledge and tools to produce the safest products possible.

Our prior research demonstrated that the thermal inactivation of *Salmonella* is significantly affected by meat product structure (i.e., whole-muscle vs. ground) and prior sub-lethal injury. Specifically, we demonstrated that thermal inactivation rates of *Salmonella* in whole-muscle beef, turkey, and pork were ~50% less than in ground product of equivalent composition (Orta-Ramirez and others 2005; Tuntivanich and others 2008; Velasquez and others 2010). Therefore, process validations based on ground meat data may not be valid for whole-muscle products.

Additionally, we previously published a study in which we: (1) demonstrated that slow heating rates (~1 to 2°F/min) can result in significant errors between observed and predicted lethality of *Salmonella* in ground turkey (up to 4 log<sub>10</sub> under-processed) when using traditional thermal inactivation models (i.e., D and z), (2) developed a modified, *path-dependent* model that predicts thermal inactivation as a function of product temperature *and* prior exposure to critical, non-lethal temperatures, and (3) showed that the modified model eliminated the prior systematic lethality error (Stasiewicz and others 2008). However, these results were generated based on 1 g samples of ground turkey in a highly controllable laboratory heating system.

We subsequently scaled-up these findings by conducting laboratory- and pilot-scale tests with *Salmonella*-inoculated beef roasts and turkey breasts in moist-air convection ovens in the MSU Biosafety Level-2 Pilot Plant. The same general trend held true in these tests; traditional lethality models (i.e., D and z) significantly under-predicted *Salmonella* inactivation as the degree of sub-lethal heating increased with longer cooking times. However, these recent results revealed two challenges: (1) The underlying uncertainty in the outcomes increases significantly with scale-up to pilot/commercial-scale operations, and (2) The apparent adaptation of *Salmonella* during sub-lethal heating in meat products appears to be different among different product species (beef vs. turkey). Therefore, there was a need to test these effects specifically in pork products, and to develop commercially-relevant, pork-specific lethality data that account for *Salmonella* adaptation to thermal injury during slow cooking processes.

In general, existing tools for computing *Salmonella* lethality are insufficient for robust process validation across diverse categories of meat products. USDA-FSIS regulations for whole-muscle, ready-to-eat (RTE) products state that validated models can be used to document whether a process achieves the target lethality (e.g., 6.5 log<sub>10</sub> reduction of *Salmonella*), but that those models "...will need to demonstrate the relationships between the lethality treatments and the specific characteristics of a product..." (USDA-FSIS 1999; USDA-FSIS 2001). Unfortunately, models are not yet available that account for all variables that impact lethality, such as product structure, specie, and heating rates. However, such tools are urgently needed, given increasing regulatory pressure and the economic importance of RTE products.

Microbial food safety failures are rare, but catastrophic, events in the meat industry. Given the dynamics of these challenges, and the known ability of *Salmonella* to adapt and become more thermally resistant during real processes relevant to pork products, improved models for process validation are critical tools for safeguarding the industry and its consumers.

## Objectives

The *overall goal of this project* was to improve the reliability of thermal process validation tools for *Salmonella* in pork products, by accounting for stress adaptation that can occur during slow cooking processes.

The *specific objectives* were:

1. To modify, for ground and whole-muscle pork 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 using ground and whole-muscle pork products inoculated with *Salmonella* and subjected to moist-air cooking in impingement and slow-roasting oven systems, respectively.

## Materials & Methods

### Overview

In this project, *Salmonella*-inoculated pork products were heated in four different heating/cooking systems, at three different scales (Fig. 1). First, a temperature-controlled programmable thermocycler (Fig. 1A) was used to heat very small inoculated samples (1 g) to generate data for estimating thermal inactivation model parameters. Subsequently, a computer-controlled, custom laboratory-scale, moist-air convection oven (Fig. 1B) was used to subject small samples (~20 g), both whole-muscle and patties, to a range of cooking schedules that yielded core temperature histories that mimicked commercial applications; the purpose of the small oven was to enable precise control of sample history, while retaining sample properties and oven conditions relevant to commercial applications. At the pilot-scale, a moist-air impingement oven (Fig. 1C) was used to cook *Salmonella*-inoculated pork patties and whole-muscle, boneless loin chops under conditions identical to those in commercial-scale impingement ovens. Lastly, a pilot-scale, moist-air convection oven (Fig. 1D) was used to subject *Salmonella*-inoculated, whole-muscle pork roasts to a range of typical commercial cooking schedules. All four oven/heating systems were computer controlled, with PC-based data acquisition systems for real-time monitoring of core temperatures and real-time calculations of predicted lethality. While the first system (the thermocycler) was used to generate data for estimating model parameters, the other three systems were used to generate model validation data and to test the effects of scale-up on the accuracy and repeatability of process validations relevant to commercial applications. The details associated with each system, and the specific sample treatments, are described below, following the description of the sample preparation and inoculation procedures.

### Meat

Fresh center cut pork loins (*Longissimus Dorsi* muscle) were acquired directly from a commercial supplier

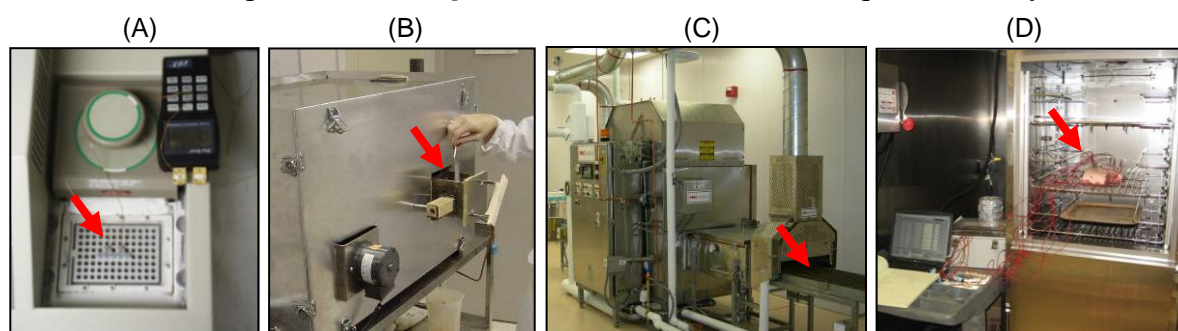


Fig. 1. Four oven/heating systems used in this project: (A) Thermocycler; (B) Lab-scale, moist-air convection oven; (C) Pilot-scale (JBT FoodTech), moist-air impingement oven; (D) Pilot-scale (Crescor), moist-air convection oven. The red arrows point to the sample location in each system.

from a federally inspected source and shipped to Michigan State University's meat processing facility. Product temperature at receipt was verified to be  $< 4.4^{\circ}\text{C}$ . Meat was sectioned into either ~680 g roasts or ~20 g (1.0 cm thick) mini-steaks, vacuum packaged in double plastic bags, and frozen ( $-20^{\circ}\text{C}$ ). Frozen roasts were then irradiated ( $>10$  kGy; FTSI, FDA registration 1054811, Tampa, FL) to eliminate indigenous microflora. Frozen irradiated samples remained frozen ( $-20^{\circ}\text{C}$ ) until use. Sterility of the samples was confirmed by randomly testing 3 roasts of each species. A 25 g core was removed aseptically from the roast using a sterile scalpel blade, placed in an 8 oz Whirl-Pak® bag, diluted 1:10 in sterile tryptic soy broth containing yeast extract (TSBYE), homogenized in a masticator (Neu-Tec Group Inc, Barcelona, Spain) for 180 s, and incubated for 24 h at  $37^{\circ}\text{C}$ . Following incubation, duplicate samples were plated on aerobic Pertrifilm™ plates (3M Microbiology Products, St. Paul, MN) and incubated at  $37^{\circ}\text{C}$  for 24 h. No bacteria were recovered. The moisture and fat content were

68.5 ± 0.9% and 10 ± 3.3%, respectively, based on the Association of Official Analytical Chemists (Chemists 1990) methods 950.46B and 991.36, respectively.

For tests utilizing ground product, roasts from the same lot, prior to irradiation, were ground twice (Hobart, model 4146, 4 mm plate). Samples were then vacuum packaged (in 100 or 500 g portions), frozen, and irradiated in the same manner as described for the whole-muscle roasts.

### ***Inoculum***

An 8-serovar *Salmonella* cocktail consisting of *S. Thompson* FSIS 120 (chicken isolate), *S. Typhimurium* DT 104 H3380 (human isolate), *S. Hadar* MF60404 (turkey isolate), *S. Copenhagen* 8457 (pork isolate), *S. Montevideo* FSIS 051 (beef isolate), and *S. Heidelberg* F5038BGI (human isolate), was previously obtained from V.K. Juneja (Agricultural Research Service, Eastern Regional Research Center, USDA-ARS, Wyndmoor, PA). Each serovar was maintained separately at -80°C in vials containing tryptic soy broth (Difco Laboratories, Sparks, MD) with yeast extract (TSBYE) and 20% glycerol. Cultures were grown separately in TSBYE at 37°C with a minimum of two consecutive 24 hour transfers prior to inoculation.

### ***Inoculation of Ground Pork***

Inoculum was prepared by combining 9 ml of each culture to yield a total of 72 ml. This cocktail was centrifuged (6000×g, 15 min) and the pellet was re-suspended in 9 ml of 1% sterile peptone water (Difco Laboratories, Sparks, MD). The entire 9 ml of inoculum was added to 25 g of the irradiated ground pork and hand mixed for 180 s. The 25 g sample was added to 1000 g irradiated ground pork and hand mixed for 180 s to obtain a uniform bacterial population of ~8.0 log CFU/g in the inoculated product. For tests in the laboratory-scale oven, the inoculated product was formed, in a small Petri dish, into 24 mm × 10 mm (Ø,H) patties (~20 g).

For tests utilizing pork patties in the pilot-scale impingement oven, the inoculated ground pork was formed into 108 mm × 12 mm (Ø,H) patties (~120 g) via a commercial patty maker (Hollymatic Corp., Countryside, IL). Patties were placed on sterile papers then on ice packs and placed in a freezer (-15°C) for 20 min, flipped, and then frozen another 20 min. These partially frozen patties were then sliced in half to form two thin patties 108 mm × 6 mm (Ø,H) using a commercial deli slicer (GFE Co., Dayton OH; FMA OMCAN, Niagra Falls, NY). A 36 gauge wire temperature probe (Omega Engineering, Stamford, CT) was wrapped around a pin and positioned in the center of each patty. Top and bottom halves were placed back together and allowed to thaw (4°C) for 20 min prior to use. This procedure was carried out to ensure very accurate placement of the thermocouple into the geometric center of the product.

### ***Inoculation of Pork Steaks/Chops***

Inoculated marinade was prepared by combining 72 ml of each culture to yield a total of 576 ml. This cocktail was centrifuged (6000×g, 15 min), and the pellet was re-suspended in 500 ml of a typical commercial marinade containing 11.5% (w/v) salt and 3.7% (w/v) phosphate to achieve a target *Salmonella* population of ~10<sup>9</sup> CFU/ml.

For tests in the laboratory-scale oven, the previously irradiated 20 g mini-steaks (1.0 cm thick) were each combined with 160 g of the inoculated marinade in a 24-oz sterile Whirl-Pak® bag (Nasco, Fort Atkinson, WI). After 20 min of exposure, resulting in an internal *Salmonella* population of ~7 log CFU/g, the sample was removed and chilled (4°C) until testing.

For the pilot-scale trials, the previously irradiated roasts were sliced into 12 mm thick chops (~120 g) using a commercial deli slicer. Chops were trimmed to ~88 mm (Ø) to maintain sample uniformity. Trimmed samples were placed into separate Whirl-Pak® bags, and 50 ml of inoculated marinade was added. Air was removed from bags and they were laid flat and allowed to marinate (4°C) for 45 min to obtain an initial bacterial population of ~7.4 log CFU/g within the whole-muscle product.

Temperature probes were placed via the use of a 16G x 12 cm hypodermic needle (Air-Tite Products Co. Inc., Virginia Beach, VA) and a needle guide designed to insert the needle at a height of 5.5 mm into the center of the sample. After penetration with the needle, the 36 gauge wire temperature probe was inserted into the

needle half way through the sample (~44 mm), and then the needle was withdrawn while the probe remained in place. This was done to ensure positioning of the probe at the geometric center (cold spot) of the sample.

***Inoculation of Whole-Muscle Roasts***

The *Salmonella* marinade, previously described, was combined with the whole-muscle roasts in a sterile tumbler (T-15 Vacuum Meat Tumbler, Kent Butcher Supply, Grandville, MI) that was modified with a stainless steel baffle insert, and a vacuum of ~84.6 kPa was pulled to facilitate vacuum tumbling, with a target marinade uptake of ~10% (w/w). *Salmonella* migration into intact, whole muscle during vacuum tumbling was shown previously to be an effective technique to introduce *Salmonella* cells into the interior of whole muscle without external intervention (Warsow and others 2008). Roast(s) were tumbled at 8 rpm for 20 min, rested 5 min, and then tumbled for an additional 20 min. The inoculation process was validated by excising a center core (16.4 cm<sup>3</sup>) from the inoculated roasts with an electrosurgical unit (Valleylab SurgiStat II, Boulder CO), and the mean core inoculation was 6.3 log CFU/g.

***Thermal Treatments – 1 g samples***

All treatments were carried out in a temperature-controlled programmable thermocycler (ENE Mate, Model: FPROGO2G, serial #85018-14, ISC Bioexpress, Kaysville, UT), with a stated manufacturer’s accuracy of ± 0.1°C. For each test, the 1 g sample was divided into 0.2 g portions and inserted into five 0.2 ml thin-walled PCR microtubes with attached caps (Dot Scientific Incorporated, Burton, MI). For triplicate testing, 15 microtubes were placed in the thermocycler and then thermally equilibrated to 25°C before being subjected to one of 53 different heating profiles (described below), in triplicate. Immediately after the heat treatment, all samples were cooled in ice and held at ~4°C for recovery and plating later the same day.

Each heating profile consisted of a randomly selected combination of a linear heating rate (1, 2, 3, 4, or 7 K/min), a variable-length sublethal holding period (40, 45, or 50°C), and a final lethal temperature (55, 58, 61, or 64°C). The sublethal holding period was determined depending on the selected sublethal history (τ) target (15, 25, 34, 50, 100, 200, 370, or 500 K·min), defined below in the “Model” section. The holding time at the lethal temperature was chosen to achieve a nominal target lethality of 3 or 5 log reductions. Total treatment times lasted between 8.2 and 252 min, and the full sample set consisted of 159 data points for each species.

***Thermal Treatments – 20 g (lab oven) samples***

The 20 g inoculated patties or whole-muscle mini-steaks were placed on a wire rack, and a temperature probe was inserted to the sample center, for continuous monitoring of core temperature. The sample on the rack was then inserted into the cooking chamber of the custom, laboratory-scale convection oven, which delivered conditioned air (~1.3 m/s) across the sample. Triplicate samples were cooked according to one of five cooking schedules (Table 1), designed to achieve core product temperature that approximated that achieved in a range of oven roasting conditions, with total cooking time ranging from 27 to 115 min.

*Table 1. Conditions during five different cooking schedules for inoculated, ground-and-formed patties and mini-steaks (20 g each) cooked in a laboratory-scale, moist-air convection oven.*

Cooking Stages	Dry Bulb Temperature (°C)	Wet Bulb Temperature (°C)	Cooking Schedule					
			Control	A	B	C	D	E
			Time in each stage (min)					
1	32.0	28.1	n/a	4.0	9.6	15.2	20.7	26.3
2	35.0	33.1	n/a	4.5	10.8	17.1	23.3	29.6
3	45.0	42.9	n/a	6.5	15.6	24.6	33.7	42.8



4	55.0	52.8	n/a	6.5	13.2	17.4	17.1	16.6
5	60.0	57.7	variable	variable	variable	variable	variable	variable

## **Thermal**

### **Treatments – pilot-scale impingement oven**

Each inoculated pork patty or whole-muscle chop was placed on the belt of the pilot-scale moist-air impingement oven (Fig. 2). Cooking conditions consisted of six combinations of air temperature (149 or 204°C; 300 or 400°F), humidity (20 or 50% moisture by volume), and target lethality (4 or 6 log reductions), with duplicate samples at each condition. The impingement fan was run at 80% full speed, which corresponded to an impingement jet velocity of ~2.7 m/s. The belt speed was set to mimic that which occurs in a commercial-scale oven with an ~30 ft cooking zone, so that the product in the pilot-scale oven oscillated under the impingement jets with the same relative velocities as would occur in the corresponding commercial-scale oven.



Fig. 2. Inoculated samples being prepared and processed for pilot-scale validation trials. (A) Pork patty being instrumented, (B) Pork chop entering into the impingement oven, (C) Pork roasts prepared for in-bag cooking in convection oven, and (D) Pork roasts in moist-air convection (roasting) oven.

The product was removed from the oven based on core temperature that was determined during preliminary trials to achieve the target lethalties. Following removal from the oven, the product was quenched in ~5 L of liquid nitrogen for ~15 s, in order to cease cooking at the desired endpoint lethality (without actually freezing the sample).

### **Thermal Treatments – pilot-scale, slow-roasting convection oven**

Before cooking, all roasts were inoculated as described above. Cooked-in-bag samples were inserted aseptically into boil-in-bags (Smurfit-MBI, Butcher and Pack Supply, Detroit, MI) and vacuum sealed (VacMaster, Kansas City, MO). Each bag had two septums (SSP 134, Spa, NY) glued to its surface 24 h prior to use. The septums maintained the vacuum seal of the bag after temperature probes were inserted through the septum into the roast (Fig 2C). Except for the bagging step, the in-bag and out-of-bag samples were prepared identically.

A commercial, moist-air convection oven (Fig 2D, CO151FWUA12B2083, Cres Cor®, Mentor, OH) was modified to control the cooking temperature profile and to log the time-temperature data, via a LabVIEW® data acquisition unit (CompactDAQ, National Instruments, Austin, TX). Thermocouple needle probes (type K, PA1454B, diameter 1.6 mm, barb end, accuracy  $\pm 1.1^\circ\text{C}$ , Datapaq, Inc., Wilmington, MA), were used to measure core temperature of the samples real-time during cooking. Oven humidity ranging from 20-100% rh

was nominally set with the oven's built-in humidity dial. Actual humidity was measured with a humidity sensor (Hydro Clip, serial number 36737 009, Rotronic, Huntington, NY) and the data logger.

Sterile corers were pre-strung onto sterile thermocouple wires before two temperature probes were inserted near the center of each roast. The temperature data from the probe at the coldest location were utilized to evaluate the product endpoint temperature/calculated lethality. During cooking, lethality was calculated real-time based on a log-linear model (described below) using parameters previously determined from isothermal inactivation studies with whole-muscle pork.

Roasts were processed using seven different industry-relevant cook protocols (Fig 3). The cooking schedules varied in time, temperature, and humidity (60 to 93.3°C; 0 to 100% rh; total cooking time 86-253 min). For each of the cooking schedules, two roasts were cooked to 71.1°C (160°F), and three roasts were cooked to a targeted lethality of 3.0 log reductions. Because the initial *Salmonella* concentration (6.3 log<sub>10</sub> CFU/g) was lower than the FSIS standard, it was not possible to cook the roasts to the USDA *Salmonella* lethality standard of 6.5 log<sub>10</sub> and still be able to recover surviving bacteria to test the inactivation models. In order to consistently quantify recoverable cells for every observation, to validate the model without biasing the lethality results, a targeted lethality below the initial *Salmonella* concentration was necessary. However, this procedure still allowed us to achieve the core objective, which was to validate whether the slow-cooking process affected the validity of using an inactivation model that was determined via prior laboratory-scale studies.

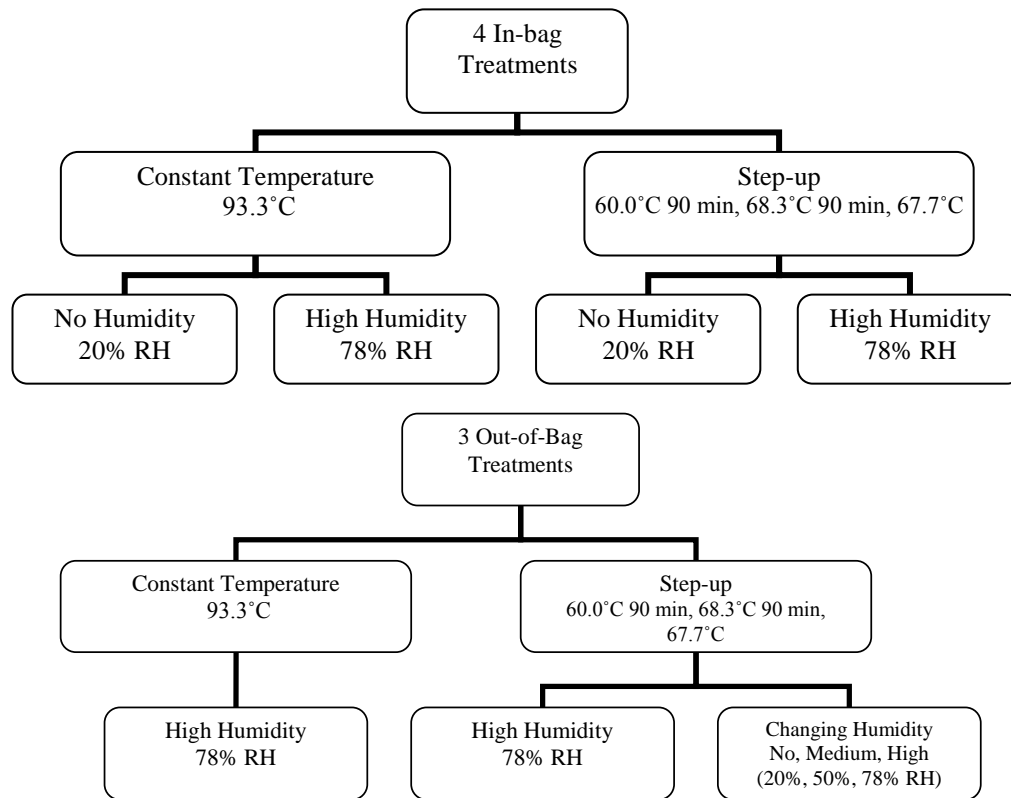


Fig. 3. Description of the seven different cooking schedules applied to the whole-muscle pork roasts in the pilot-scale, moist-air convection oven.

### Temperature Data during Cooking

In all of the cooking trials, sample core temperature was monitored and recorded real-time. In the pilot-scale tests, a Multipaq21 data logger (Datapaq Inc., Wilmington, MA) was used to record real-time sample core temperature and oven temperature as the data logger traveled with the samples through the oven and transmitted the data to a laptop computer outside the oven via a radio-frequency transmitter. These temperature data were



used to compute predicted *Salmonella* lethality real-time during cooking, based on the inactivation models described below.

### ***Post-Cooking Enumeration of Survivors***

For the laboratory-scale heating trials (samples of 1 or 20 g), the entire sample (treated sample or untreated control) was recovered, homogenized in a masticator (Model 0410, IUL Instruments USA, Cincinnati, OH), diluted (1:5), and serially diluted in 0.1% sterile peptone water (Difco™, Becton, Dickinson and Company, Sparks, MD) for duplicate plating on Petrifilm™ aerobic count plates (3M Microbiology Products, St. Paul, MN). Incubation was carried out at 37°C for ~48 h before enumeration. Inoculated but unheated samples served as positive controls against which the heated samples were compared to determine the process lethality (i.e., log reductions).

For the samples (ground and whole-muscle) cooked in the pilot-scale impingement oven and then quenched in the liquid nitrogen, each patty or steak was sliced into thirds (~4 mm thick) in order to obtain the middle slice of the product. The middle slice was cored (50 mm) and diluted 1:5 (w/w) in 1% peptone water. The core was homogenized in a masticator for 180 s and then serially diluted and plated on modified Tryptic Soy Agar (Difco Laboratories, Sparks, MD) plates, which were placed in an incubator for 48 h at 37°C. *Salmonella* colonies were counted, and the experimental log reductions were calculated and compared to the predicted values calculated using measured core temperature.

For the whole-muscle roasts cooked in the pilot-scale, moist-air convection oven, once the targeted end point was achieved, the roast was immediately removed from the oven. The cold spot was cored using the sterile corer that had been strung onto that thermocouple wire, the top and bottom 2.54 cm were aseptically removed, and the remaining core was immersed into 18 g of sterile, chilled 0.1% buffered peptone water (Difco Laboratories, Sparks, MD) to cool to <15°C in less than 10 s. The computed lethality used in the subsequent analysis included the entire temperature history of the core through cooking and cooling. After cooling, the ~8 g samples were diluted 1:5 with 0.1% buffered peptone water, and homogenized for 180 s in a masticator, serially diluted, plated using duplicate aerobic Petrifilm™ plates (3M Microbiology Products, St. Paul, MN), and enumerated after 48 h of incubation at 37°C. The limit of detection was 0.4 log<sub>10</sub> CFU/g.

### ***Inactivation Model – traditional***

Two forms of traditional, log-linear inactivation kinetics were applied. For the pilot-scale validation trials, the traditional D and z model was utilized, in which the D-value is the time required to achieve a 1.0 log reduction in bacterial population at a constant condition, and the z-value describes the temperature change required to cause a 10-fold change in the D-value, such that. Numerical integration (trapezoidal rule) was applied to the dynamic core temperature data to compute the cumulative predicted log reductions for each sample in those trials. The D and z values used in this study (Table 2) were based on prior work in our laboratory (Velasquez et al. 2010).

*Table 2. Log-linear inactivation parameters used for computing process lethality in the pilot-scale validation trials (based on methods from (Velasquez et al. 2010)).*

	<b>T<sub>ref</sub></b> <b>(°C)</b>	<b>D<sub>ref</sub></b> <b>(min)</b>	<b>z (C°)</b>
<b>ground pork</b>	<b>60</b>	<b>1.21</b>	<b>5.9</b>
<b>whole-muscle pork</b>	<b>60</b>	<b>1.73</b>	<b>5.2</b>

An alternative form of the traditional, state-dependent inactivation model is:

$$\log S = \log \frac{N}{N_0} = -bt \quad (1)$$

where S is the survival ratio, N is the number of microorganisms at time t,  $N_0$  is the initial microorganism population, and b is the rate of inactivation (equal to the inverse of the traditional D-value). This version of the model was used for the laboratory-scale trials, in order to enable the model modification described below. However, using either equation 1 or the D and z model yields the same predicted lethality, when using the same laboratory inactivation data, as both are merely different forms of log-linear inactivation kinetics.

### ***Inactivation Model – path-dependent***

According to our previous work (Stasiewicz et al. 2008), b (the inactivation rate) can be described with an Arrhenius-type dependency:

$$b(T) = b_{ref} \cdot \exp\left\{-\beta_1 \left[ \frac{1}{T(t)} - \frac{1}{T_{ref}} \right]\right\} \quad (2)$$

where  $\beta_1$  determines the effect of temperature on b. A new path-dependent inactivation model (Stasiewicz et al. 2008) takes the following modified form:

$$b(T) = b_{ref} \cdot \exp\left\{-\beta_1 \left[ \frac{1}{T(t)} - \frac{1}{T_{ref}} \right] - \beta_2 \tau\right\} \quad (3)$$

where the  $\beta_2 \tau$  term is added to account for the resistance bacteria can develop when exposed to sublethal thermal injury, as can occur during slow cooking processes.  $\beta_2$  scales the impact of this phenomenon, or sublethal history ( $\tau$ ) on b. The sublethal history is quantified as the integral of the temperature vs. time curve when the temperatures fall in the heat shock region, that is, where *Salmonella* appears to increase its thermal tolerance. Therefore:

$$\tau = \int_{T=HS_{lower}}^{T=HS_{upper}} (T(t) - HS_{lower}) dt \quad (4)$$

where T(t) is the dynamic produce temperature. Based on prior research (Stasiewicz et al. 2008), the heat shock region was considered to be between 38°C and 52°C ( $HS_{upper}$  and  $HS_{lower}$ ). The final path-dependent model is obtained by combining equations 1, 3, and 4, and the parameters for this model were estimated from the laboratory-scale (1 g) inactivation data. Equation 4 was also used independently, as a means to quantify the magnitude of the sublethal temperature history for the slow-cooked products, both in the laboratory- and pilot-scale trials.

## **Results**

As outlined at the beginning of the methods section, the four different types of heating trials in this study served four different purposes. The data from the 1-g samples (thermocycler) were used to estimate the parameters of the new model that accounts for the effect of sublethal injury on thermal resistance. The data from the 25-g samples (laboratory-scale oven) were used for initial testing of the validity of prior, product-specific (ground vs. whole-muscle) inactivation parameters and for testing the impact of slow cooking on model validity. Lastly, data from the two pilot-scale cooking trials (impingement vs. slow roasting) were used to further validate the model (in terms of the impact of slow roasting on process lethality validation) and to quantify the impact of scale-up on the underlying uncertainty associated with using lethality models for thermal process validations. Example product (core) temperature data for each of these four systems are shown in Figure 4.

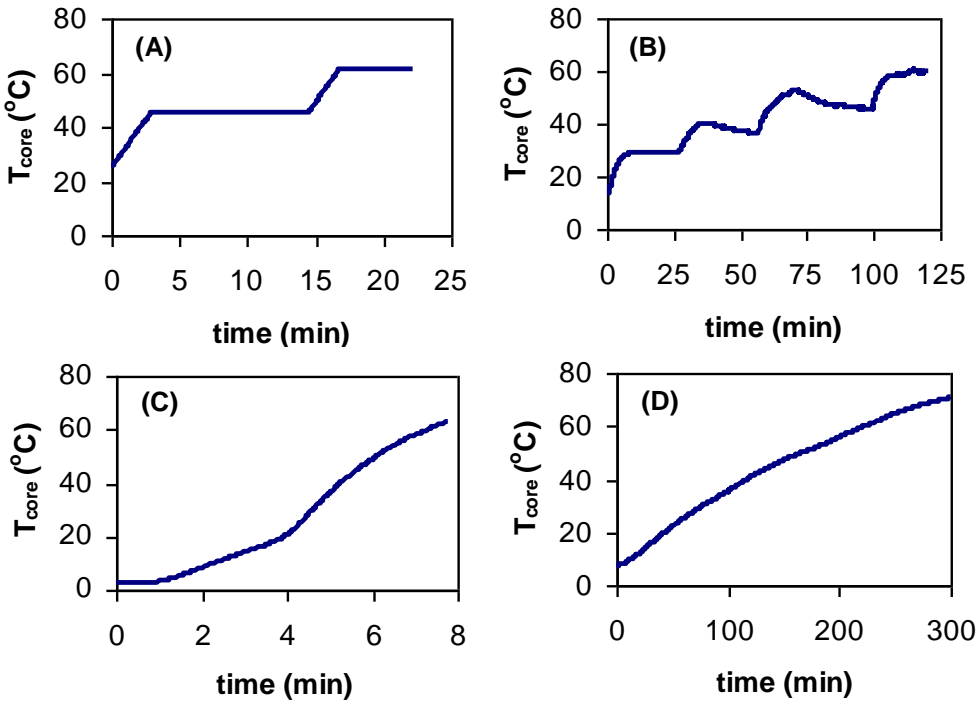


Fig. 4. Examples of sample core temperature profiles for pork products heated in: (A) Thermocycler (1 g); (B) Lab-scale, moist-air convection oven (~20 g); (C) Pilot-scale (JBT FoodTech), moist-air impingement oven (~120 g); (D) Pilot-scale (Crescor), moist-air convection oven (~1 kg). Note the wide range of cooking durations across the different cooking regimes.

### Model Development – 1 g samples

When the state-dependent model (i.e., the traditional log-linear model based on prior isothermal inactivation data) was applied to the non-isothermal validation sets, substantial over-prediction errors (as large as 14.7 log) were observed (Figure 5) at the largest tested sublethal history ( $\tau=500$  minK, corresponding to very slow heating). Analysis of variance (ANOVA) revealed a statistically significant relationship between the sublethal history ( $\tau$ ) and the traditional state-dependent model error ( $P<0.0001$ ), meaning that the traditional, state-dependent model error increased as  $\tau$  increased. This agrees with previous work (Stasiewicz et al. 2008), and reaffirms the importance of a model that can account for bacterial injury in a variety of heating profiles. In addition, this concurs with heat shock literature (Knabel and others 1990; Pagan and others 1997; Farber and Brown 1990) in that a combination of time and temperature in the sublethal region, effectively described by  $\tau$ , is what causes an increase in bacterial thermotolerance. Table 3 shows the statistical results of these validations. It can be seen that the root mean squared errors (RMSE) are the largest of all model fittings and that the biases are located in the fail-dangerous zone. These results show that the combined use of isothermal data and state-dependent models might carry risk when applied to slow cooking conditions where thermal injury can occur.

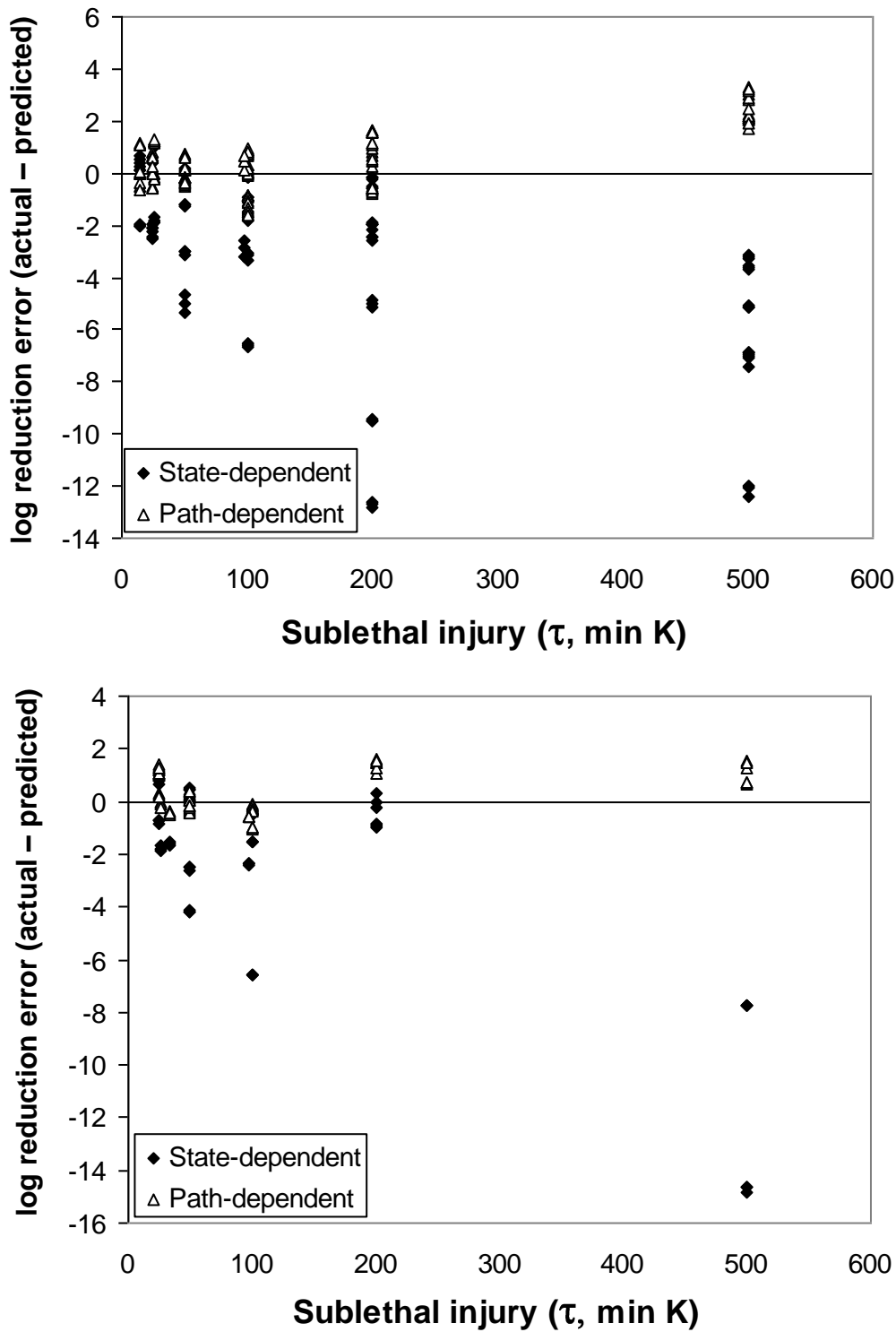


Fig. 5 Log reduction (lethality) errors for the traditional (state-dependent) and modified (path-dependent) inactivation models, for (A) the calibration data set ( $n=108$ ) and (B) the validation data set ( $n=51$ ) from the non-isothermal, laboratory-scale (1 g) inactivation tests. Larger negative error in these graphs reflects larger over-prediction (fail-dangerous) by the model.

The parameters and calibration/validation statistics for application of the modified, path-dependent inactivation model also are shown in Table 3. The use of the path-dependent model on the non-isothermal validation tests showed a substantial (82%) reduction in prediction error (RMSE) when compared to the state-dependent model (Table 3 and Figure 5). This shows that the path-dependent model provides greater accuracy

than the state-dependent model when applied to data where sublethal heating has occurred, and eliminates the systematic error that occurs when applying the traditional model to long heating times (i.e., those with large  $\tau$  values). Additionally, bias values for the path-dependent model are in the slight under-prediction range, which is a great improvement over the high over-prediction bias (-2.6 log) from the state-dependent model. The negative value reflects a fail-dangerous scenario, and suggests that a traditional state-dependent model cannot be relied upon to effectively predict microbial inactivation when significant sublethal heating occurs (e.g., during slow cooking processes).

Table 3. Thermal inactivation model parameters and statistics, as related to the traditional (state-dependent) and modified (path-dependent) models applied to *Salmonella* inactivation data from laboratory-scale (1 g) trials.

		Calibration					Validation against non-isothermal data (validation set, n=51)	
		Parameters			Statistics		Statistics	
Model	Data source	$b_{ref}$	$\beta_1$	$\beta_2$	RMSE	Bias	RMSE	Bias
		( $\text{min}^{-1}$ )	(K)	( $\text{K}^{-1} \text{min}^{-1}$ )				
State -dependent	Isothermal pork	0.90	42,590	NA	1.07	-0.06	4.55	-2.58
	Non-isothermal pork calibration set	0.45	52,382	NA	1.33	0.53	1.49	0.82
Path-dependent	Non-isothermal pork calibration set	0.82	53,952	0.0032	1.27	0.55	0.82	0.28

### Model Validation – 20 g samples

For the cooking trials with 20 g samples (pork patties and mini-steaks) in the laboratory-scale, moist-air convection oven, the actual *Salmonella* lethality was compared with the computed lethality, based on the previously reported D and z values for ground and whole-muscle pork. In both cases, the mean computed lethality was significantly ( $P < 0.001$ ) greater than the actual lethality, with individual errors as large as 2.1 and 6.0 log for the ground and whole-muscle samples, respectively. In the case of the patties, the size of the lethality error increased with increasing sublethal history ( $P < 0.01$ ), as quantified with equation 4 (Fig 6). Because sublethal history roughly corresponds with cooking time, this implies that the traditional inactivation model (D and z) became less reliable as the cooking time increased (up to 115 min in this test series).

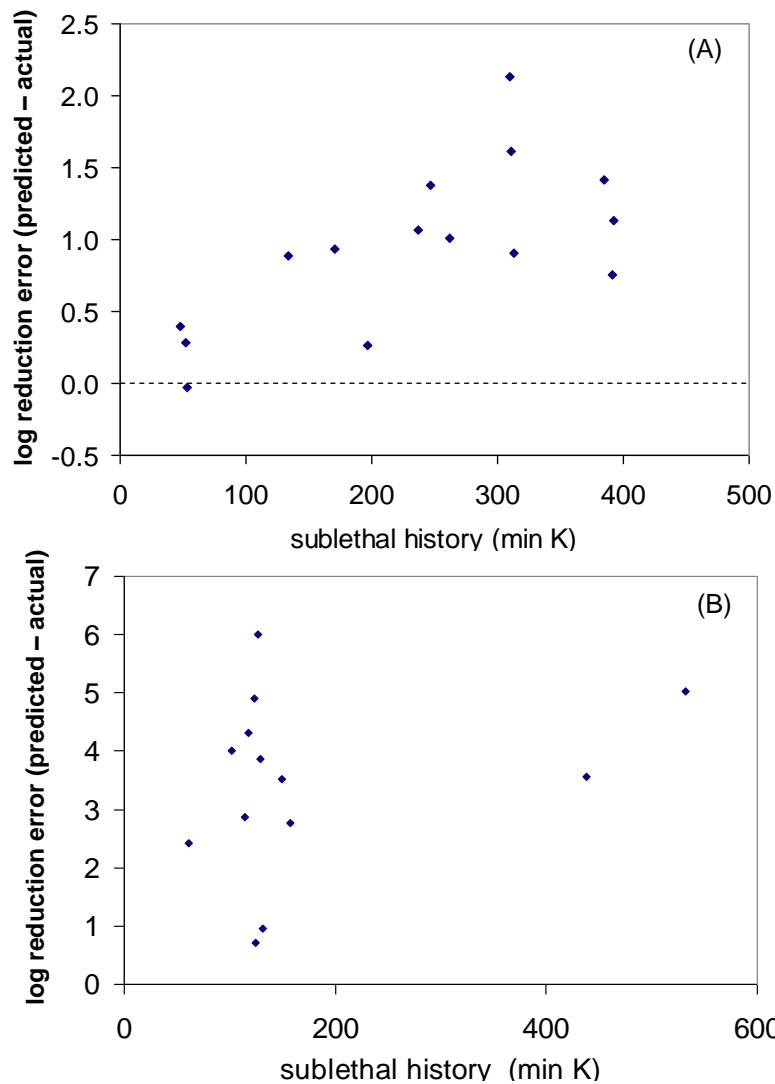


Fig. 6. Log reduction (lethality) errors when applying the traditional (state-dependent,  $D$  and  $z$ ) modified (inactivation model, for (A) ground pork patties and (B) whole-muscle mini-steaks (20 g each) in the laboratory-scale oven inactivation tests. Larger positive error in these graphs reflects larger over-prediction (fail-dangerous) by the model.

### Model Validation – pilot-scale impingement

For the impingement cooked products (patties and chops), the total cook time was much shorter than in the laboratory-scale or pilot-scale convection/roasting ovens (i.e., < 9 min). Therefore the cumulative sublethal history ( $\tau$ ) was less than 12 (min K), which was much less than occurred in the slow cook schedules, as reflected in Figure 6. Therefore, the hypothesis was that the traditional  $D$  and  $z$  model (not accounting for sublethal injury) should work reliably for this type of product and process, and should not be affected by cooking time. Based on analyses of variance, there was no significant effect ( $P > 0.05$ ) of the cooking treatment (temperature, humidity, duration) on the error between experimental and computed lethality for either of the product types.



In the case of the ground-and-formed pork patties and the traditional inactivation model (D and z from prior isothermal inactivation trials), the RMSE and bias across all 24 samples were 2.4 and 1.1 log(CFU/g), respectively. This represented a significant ( $P < 0.05$ ) mean over-prediction of the actual lethality. However, 87.5% of the pilot-scale outcomes fell within the 95% prediction intervals for the computed lethality based on the D and z values from the prior isothermal inactivation study (Fig 7), so that the model just slightly under-performed, in terms of the expected outcomes. If the D and z values were, however, determined directly from these pilot-scale cooking trials, then the RMSE and bias were reduced to 0.85 and 0.08 log(CFU/g), respectively. This indicates that the model form itself (i.e., D and z) could fit these data well; however, the main objective of the test was to validate inactivation parameters determined in isothermal laboratory studies against actual pilot-scale data, and the difference between the above validation statistics reflect the importance of this process.

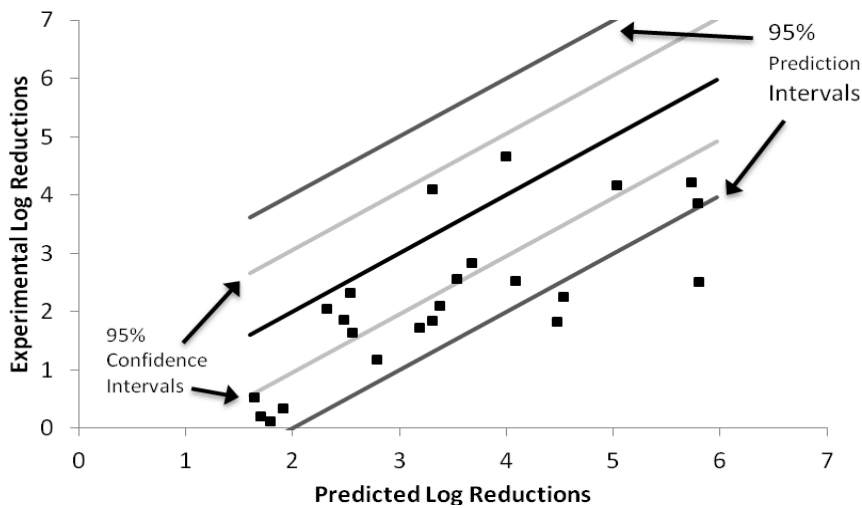


Fig 7. Experimental vs. predicted lethality (log reductions) for inoculated pork patties processed in a pilot-scale impingement oven under a variety of conditions. The confidence and prediction intervals are based on the inherent uncertainty in the D and z values from prior isothermal inactivation trials.

For the whole-muscle pork chops, the corresponding RMSE and bias were 1.5 and 0.18 log(CFU/g), respectively, indicating that the traditional D and z model in this case yielded no significant difference in the mean observed and predicted outcomes, but still resulted in a level of uncertainty (1.5 log) that needs to be considered when applying such models for commercial-scale validation.

**Model Validation – pilot-scale, slow roasting**

For the whole-muscle pork roasts processed to 71.1°C, there were no quantifiable *Salmonella* for the sample cores plated after the end of cooking. Complete elimination of all *Salmonella* was not confirmed, because enrichment was not performed on samples following cooking. However, given that the roasts were inoculated to contain an initial *Salmonella* population in the core of ~6.3 log<sub>10</sub> CFU/g, these results support the regulatory premise that an endpoint of 71.1°C is extremely likely to ensure a sufficient cook.

The mean predicted lethality of *Salmonella* in whole-muscle pork roasts (using the traditional D and z model) was not significantly different from the mean observed lethality ( $P=0.36$ ). Being that there was no significant difference between the predicted and observed lethality for the state-dependent model, the state-dependent model appeared to be a valid tool in this case, and the modified, path-dependent model actually under-predicted lethality (i.e., over-compensated for the effect of sublethal injury during slow cooking) with increasing sublethal history (Fig 8).

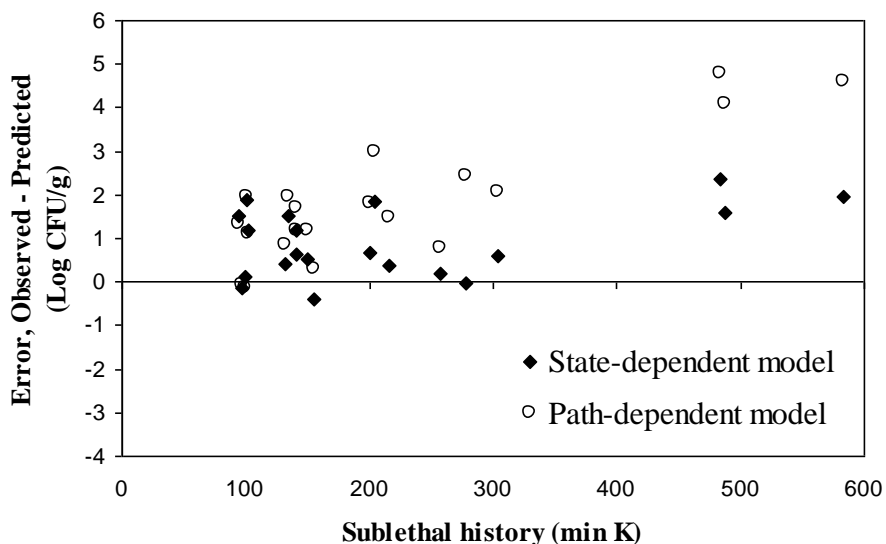


Fig 8. Log reduction (lethality) errors for the traditional (state-dependent) and modified (path-dependent) inactivation models applied to whole-muscle pork roasts cooked in a pilot-scale, moist-air convection oven. Larger positive error in these graphs reflects larger under-prediction (fail-safe) errors by the models.

### Uncertainty in Scale-Up of Process Validations

The replication error ( $\log_{10}$  CFU/g) increased with increase in sample size associated with scaling-up to the pilot-scale. Whereas the replication error (i.e., the standard deviation among replicates) was 0.08 log for the tests with 1 g samples, that error increased to 1.0-1.5 log when working with the actual patties and whole-muscle products. This increase reflects the inherent variability associated with tests involving larger samples and pilot-scale processes.

### Discussion

This project entailed thermal treatment (cooking) trials in four different systems, ranging from 1 g samples in a highly controlled thermocycler to ~1 kg whole-muscle roasts in a pilot-scale oven. Each served a different purpose in developing and testing thermal inactivation models and the importance of key factors (i.e., product structure, heating rate, scale-up) on the utility and validity of applying such models for validating the microbial safety of cooked pork products. The key findings and their relevance to industrial applications are listed below.

- *The thermal resistance of Salmonella in pork products increases when subjected to sublethal injury during slow cooking processes.* The laboratory-scale studies confirmed that cooking processes that subject pork products to critical, sublethal temperatures (i.e., ~40-50°C, or 104-122°F) for a sufficient period enable the bacteria to adapt, which is observed as significantly increased resistance to heat in the lethal temperature range (i.e., >55°C, or 131°F). Therefore, traditional inactivation models (D and z) significantly over-predicted lethality for processes with long exposure in the critical sublethal temperature range.
- *A modified thermal inactivation model can account for the effect of sublethal injury on thermal resistance.* The model that we previously developed was applied to *Salmonella* in pork, and the systematic over-prediction of lethality, noted above for slow cooking processes, was eliminated by the model.
- *The thermal resistance of Salmonella is significantly different in ground vs. whole-muscle pork products.* Although this was a result that we previously published, it is important to note that all of the lethality calculations in this study were done with inactivation parameters that were specific to either ground or whole-muscle pork. Given that the D value for *Salmonella* in whole-muscle pork is ~50% greater than in ground pork, it is critically important that processors use inactivation parameters that appropriately match the product they are producing; otherwise, significant under-predictions of process lethality could result.
- *Applying data and models developed from isothermal laboratory-scale inactivation studies to commercial-scale process validation carries inherent uncertainty that should be accounted for by commercial processors.* Very few published inactivation studies, based on laboratory-scale research, fully report the

uncertainty associated with D or z values. Application of such inactivation parameters to commercial-scale process validation carries the risk of under-representing the actual uncertainty associated with the resulting computed lethality. For example, if the target process lethality is 6.5 log reductions for *Salmonella*, and a processor uses published D and z values (and collected product temperature data) to compute that a given process results in 7.1 log reductions, then it might be tempting to conclude that 7.1 is greater than 6.5 and that the process is therefore sufficiently safe. However, our pilot-scale validation studies demonstrated two issues that impact this assumption. First, the true uncertainty associated with this type of lethality calculation is reflected in prediction intervals that illustrate the probability that a given observation will fall within those intervals surrounding the mean prediction (e.g., See Fig 7), and those intervals are inherently wider than the confidence intervals that are typically reported (and are wider than most users of such models assume). The key is that a processor of fully-cooked, ready-to-eat pork products needs to ensure that *every individual serving* of product produced is safe (reflected in the prediction intervals), not just that the *mean serving* of product is safe (reflected by the confidence interval around a predicted lethality). Secondly, this project demonstrated that the uncertainty associated with computed lethality increases significantly when scaling up from the laboratory- to pilot-scale. This is important to producers of ready-to-eat pork products, because there are almost no published data based on pilot- or commercial-scale testing, so that application of data from laboratory studies needs to be done cautiously. The practical impact is that processors need to be aware of the magnitude of this uncertainty and operate cooking processes with a sufficient margin of safety to ensure that each individual serving is processed sufficiently.

- *Processing slow-cooked, whole-muscle pork roasts to 71.1 °C (160 °F) appears to sufficiently reduce the risk of Salmonella survival, even for long (~4 h) cooking schedules.* This project confirmed that slow cooking can enhance *Salmonella* thermal resistance in pork products, and that there is a significant risk that traditional inactivation models over-predict lethality for slow-cooked products. However, when the roasts were cooked to a core  $\geq 71.1^{\circ}\text{C}$  ( $\geq 160^{\circ}\text{F}$ ) during the slow cooking processes in a moist-air convection oven, there were no countable *Salmonella* on post-cook plates (for an initial count of  $\sim 6.3$  log CFU/g). This suggests that the cumulative lethality at this endpoint, for these long cooks, was sufficient to overcome increased thermal resistance and therefore result in a safe product.

## **Publications and Presentations Resulting from this Study**

1. Breslin TJ, Marks BP, Booren AM, Ryser ET, Hall NO. 2009. Scaling-up *Salmonella* lethality calculations from laboratory to pilot-scale slow-cooking processes. RMC Abstract. American Meat Science Association. Reciprocal Meats Conference. June 2009.
2. Breslin TJ, Marks BP, Booren AM, Ryser ET, Hall NO. 2011. Validation of *Salmonella* thermal lethality in whole muscle meat products during pilot-scale slow roasting processes. *Journal of Food Science*. (to be submitted).
3. Breslin TJ. 2009. Validation of *Salmonella* thermal lethality in whole muscle meat products during pilot-scale slow roasting processes. M.S. Food Science. Michigan State University. Fall 2009.
4. Emery JM, Marks BP, Hall NO, James MK, Booren AM, Ryser ET. 2011. Pilot-scale validation of a *Salmonella* thermal inactivation model in ground meat patties. Abstract 037-40. To be presented at the IFT Annual Meeting. New Orleans, LA. 12 June 2011.
5. Hildebrandt IM, Marks BP, Hall NO, James MK, Booren AM, Ryser ET. 2011. Thermal inactivation model applied to whole-muscle meat and poultry products cooked in a moist-air impingement oven. Abstract accepted for presentation at the Annual Meeting of the International Association for Food Protection. Milwaukee, WI. 3 August 2011.
6. Tenorio-Bernal MI, Marks BP, Booren AM, Ryser ET. 2011. A universal thermal inactivation model for *Salmonella* in meat and poultry products. Abstract accepted for presentation at the Annual Meeting of the International Association for Food Protection. Milwaukee, WI. 3 August 2011.
7. Tenorio-Bernal MI, Marks BP. 2011. Evaluating the predictive ability of a path-dependent thermal inactivation model for *Salmonella* subjected to prior sublethal heating in ground turkey, beef, and pork. *Journal of Food Protection*. (to be submitted).

## Other references cited in this report

- AOAC. 1990. Official methods of analysis. Arlington, Va: Association of Official Analytical Chemists.
- Farber JM & Brown BE. 1990. Effect of prior heat-shock on heat-resistance of *Listeria monocytogenes* in meat. *Applied and Environmental Microbiology* 56(6):1584-1587.
- Knabel SJ, Walker HW, Hartman PA & Mendonca AF. 1990. Effects of growth temperature and strictly anaerobic recovery on the survival of *Listeria monocytogenes* during pasteurization. *Applied and Environmental Microbiology* 56(2):370-376.
- Orta-Ramirez A, Marks BP, Warsow CR, Booren AM & Ryser ET. 2005. Enhanced thermal resistance of *Salmonella* in whole muscle compared to ground beef. *Journal of Food Science* 70(7):M359-M362.
- Pagan R, Condon S & Sala FJ. 1997. Effects of several factors on the heat-shock-induced thermotolerance of *Listeria monocytogenes*. *Applied and Environmental Microbiology* 63(8):3225-3232.
- 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.
- 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.
- USDA-FSIS. 1999. Performance standards for the production of certain meat and poultry products. Federal Register. January 6, 1999:732-749.
- USDA-FSIS. 2001. Performance standards for the production of processed meat and poultry products; proposed rule. Federal Register February 27, 2001:12590-12636.
- Velasquez A, Breslin TJ, Marks BP, Orta-Ramirez A, Hall NO, Booren AM & Ryser ET. 2010. Enhanced thermal resistance of *Salmonella* in marinated whole muscle compared with ground pork. *Journal of Food Protection* 73(2):372-375.
- Warsow CR, Orta-Ramirez A, Marks BP, Ryser ET & Booren AM. 2008. Single directional migration of *Salmonella* into marinated whole muscle turkey breast. *Journal of Food Protection* 71(1):153-156.