Generalized Linear Mixed Model Analysis of Risk Factors for Contamination of Moisture-Enhanced Pork with Campylobacter jejuni and Salmonella enterica Typhimurium

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Generalized Linear Mixed Model Analysis of Risk Factors for Contamination of Moisture-Enhanced Pork with *Campylobacter jejuni* and *Salmonella enterica* Typhimurium

Xuesong Wen, Jing Li, and James S. Dickson

**Abstract**

Translocation of foodborne pathogens into the interior tissues of pork through moisture enhancement may be of concern if the meat is undercooked. In the present study, a five-strain mixture of *Campylobacter jejuni* or *Salmonella enterica* Typhimurium was evenly spread on the surface of fresh pork loins. Pork loins were injected, sliced, vacuum packaged, and stored. After storage, sliced pork was cooked by traditional grilling. Survival of *Salmonella* Typhimurium and *C. jejuni* in the interior tissues of the samples were analyzed by enumeration. The populations of these pathogens dropped below the detection limit (10 colony-forming units/g) in most samples that were cooked to 71.1°C or above. The general linear mixed model procedure was used to model the association between risk factors and the presence/absence of these pathogens after cooking. Estimated regression coefficients associated with the fixed effects indicated that the recovery probability of *Salmonella* Typhimurium was negatively associated with increasing level of enhancement. The effects of moisture enhancement and cooking on the recovery probability of *C. jejuni* were moderated by storage temperature. Our findings will assist food processors and regulatory agencies with science-based evaluation of the current processing, storage condition, and cooking guideline for moisture-enhanced pork.

**Introduction**

Moisture enhancement through multineedle injection has been well developed in the pork industry. Brine containing various ingredients such as salt, phosphates, sodium lactate, and lemon juice have been reported to improve pork juiciness, tenderness, and flavor (Brewer et al., 1999). The concentration of ingredients used in brine solution is dependent on the level of moisture to be added to pork. Since the brine is diluted once injected into the pork, the ingredients of the brine are at higher concentrations than the target concentration in the finished product (Feiner, 2006). For example, if the target concentration of sodium chloride in pork is to be 0.2% wt/wt and the product is to be enhanced by 10% of its initial weight, then the concentration of sodium chloride in the brine should be 2.2% wt/wt (that is, 0.2% [final concentration of ingredient] × 110% [final weight of moisture-enhanced product]/10% [enhancement weight of brine]). After injection, pork loins are usually sliced to a desired weight or thickness for consumer preference and then delivered to retail stores sealed in vacuum packages.

To meet consumer demands, a pork processor must consistently produce a high-quality product that is microbiologically safe. In industry, brines are usually recirculated within the equipment used for injecting pork. A significant increase in the numbers of *Listeria monocytogenes* has been observed in brine with time during this process (Greer et al., 2004). Moisture enhancement also has the potential to introduce bacteria from the surface into the interior muscle (Bohaychuk et al., 2003). For these reasons, injection with brine contaminated with bacteria may increase the incidence and severity of contamination in the interior tissues of pork. Concerns about the microbiological safety of moisture-enhanced pork have been raised because consumers preparing dishes with such products may regard them as an intact product, and may thoroughly cook the surface by grilling without raising all deep tissues to temperatures that are sufficient to destroy all pathogenic microorganisms. *Salmonella enterica* and *Campylobacter jejuni* were isolated in moisture-enhanced pork samples from retail stores in the United States (Duffy et al., 2001). Up to now, there has no...
available information to determine whether commonly used cooking temperatures are lethal for internalized *S. enterica* and *C. jejuni* in moisture-enhanced pork. During the processing and storage, microbial contaminants may be exposed to food-related stresses, such as salt and cold, which may influence their thermal tolerance during cooking. Using information regarding the effects of processing and storage conditions on the survival of *S. enterica* and *C. jejuni* in moisture-enhanced pork after cooking, food processors and regulatory agencies can develop decision-support systems for monitoring, controlling, or optimizing processes for this meat product. Unfortunately, such information is scarce. Thus, the objective of the present study was to examine the survival of *Salmonella enterica* serovar Typhimurium and *C. jejuni* in moisture-enhanced pork after cooking. The effects of processing and storage conditions on the survival were also assessed.

**Materials and Methods**

**Bacterial strains**

Five strains of *Salmonella Typhimurium* phage type DT104 and five strains of *C. jejuni* were studied. The five strains of *Salmonella Typhimurium* were human clinical isolates. Strains G29, G30, G32, and G33 were obtained from the Food Microbiology Research Laboratory in Iowa State University, whereas strain G34 was obtained from the Centers for Disease Control and Prevention. The five strains of *C. jejuni* were obtained from Dr. Zhang (Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA). Of these strains, three strains (CT 1:1, CT 2:2, CT 3:7) were isolated from turkeys and two strains (Clev 9100, F 12469) were isolated from humans.

The individual strains of *Salmonella Typhimurium* were maintained on tryptic soy agar (TSA; Difco, BD, Sparks, MD) slants at 4°C. A loopful of culture from a TSA slant was individually transferred into 10 mL of trypticase soy broth (Difco) and incubated for 24 h at 35°C. The individual strains of *C. jejuni* were stored in glycerol broth at −70°C and re-suscitated by streaking on Columbia Blood Free agar (Oxoid, United Kingdom) with CCDA Selective Supplement (Oxoid). Plates were incubated for 24 h at 37°C in a CO2 incubator (model 3130, Thermo Forma, Marietta, OH). The atmosphere in the incubator was programmed and automatically adjusted to be 5% O2, 10% CO2, and 85% N2. A loopful of culture from the plate was transferred to 20-mL Bolton selective enrichment Broth (Oxoid) with 5% lysed horse blood and incubated for 24 h at 37°C in the CO2.

To prepare inoculums of each pathogen, a 1-mL aliquot of culture was removed from each individual strain, and the aliquots were combined to give a 5-mL mixed culture; 2 mL of this mixed culture was transferred to 98 mL of peptone water, with a final population of approximately 10^6 colony-forming units (CFU)/mL.

**Inoculation and injection**

For each experiment, three boneless pork loins were inoculated with *Salmonella Typhimurium* and three boneless pork loins were inoculated with *C. jejuni*. The inoculum of *Salmonella Typhimurium* or *C. jejuni* was evenly spread on the surface of each whole boneless pork loin by using a 2.5-cm-wide foam paintbrush. The bacteria were allowed to adhere for 5 min. A brine solution (18.925 L) was prepared in a 19-L (5-gallon) bucket with sterile distilled water. The brine was formulated to produce a final concentration of 0.2% sodium chloride and 0.3% sodium tripolyphosphate in the enhanced loins. Inoculated pork loins were enhanced by 10% and 20% (wt/wt) of their initial weight using a needle injector (P-10 Pokomat Injector; Quality Food Equipment, El Monte, CA). The pork loins were weighed before and after injection to determine enhancement level. All the equipment was cleaned and sanitized with Vanquish disinfectants (Total Solutions, Milwaukee, WI) and hot water.

**Slicing**

After injection, each pork loin was aseptically sliced into ten 1-cm thick slices with a sterile scalpel. An inoculated pork loin without injection (enhancement level was 0%) was also sliced in the same way. The experiment was independently replicated on 3 different days. The total number of slices in the present study was 180.

**Storage and cooking**

Each slice was placed individually in a vacuum bag (Cryovac Packaging, Duncan, SC). All packages bags were sealed under vacuum (model A300 vacuum packager, Multivac Inc., Kansas City, MO) and stored at 4°C for 21 days or at 10°C for 14 days. After storage, individual packages were aseptically opened with a sterile knife and each slice was cooked in a George Foreman clamshell grilling machine (Lake Forest, IL). All experimental units (pork loin and sliced pork) were randomly allocated to treatments including bacterial inoculation, injection, vacuum-packaged storage, and cooking.

**Sampling procedure**

The target endpoint temperatures for cooking were 0°C (control), 68.3°C, 71.1°C, 73.9°C, and 76.7°C. A type J thermocouple (Oakton Instruments, Vernon Hills, IL) was sterilized in alcohol and inserted into the geometric center of each slice to monitor internal temperature. When slices reached their target endpoint temperatures, they were immediately removed from the grill, and then a 20-g portion was aseptically excised from the interior tissues of a meat slice with a sterile scalpel and forceps. Samples were homogenized by stomaching in a laboratory blender (Stomacher 400; Seward Medical, London, UK) for 60 s with 0.1% peptone water in a sterile Whirl-Pak stomacher bag (Nasco, Ft. Atkinson, WI).

**Microbial analysis**

Serial dilutions of sample homogenate were surface plated in duplicate onto xylose lysine deoxycholate (BD, Franklin Lakes, NJ) medium for enumerating *Salmonella Typhimurium*. Plates were incubated at 37°C for 24 h and colony-forming units were manually counted. Serial dilutions of sample homogenate were surface plated in duplicate onto Columbia Blood Free agar (Oxoid) with CCDA Selective Supplement (Oxoid) for enumerating *C. jejuni*. Plates were incubated at 42°C for 48 h in the CO2 incubator, and colony-forming units were manually counted. The populations of
Salmonella Typhimurium and C. jejuni obtained from enumeration were converted to log\(_{10}\) CFU/g. When counts of Salmonella Typhimurium and C. jejuni were under the detection limit for surface plating (10 CFU/g), presence or absence of viable cells in these samples was investigated. For Salmonella, homogenates were incubated for 48 h at 37°C, then 100 µL of an enriched homogenate was transferred onto 9.9 mL of Rappaport-Vassiliadis (Oxoid) broth. After incubation at 42°C for 48 h, 10 µL of the enrichment culture was streaked onto BBL CHROMagar Salmonella (BD, Sparks, MI), and incubated at 37°C for 48 h. For C. jejuni, 10 mL of a homogenate sample was transferred to 90-mL Bolton Broth (Oxoid). The enrichment cultures were incubated at 42°C in the CO\(_2\) incubator. After 48 h, 10 µL of the enrichment culture was streaked onto Columbia Blood Free agar (Oxoid) with CCDA Selective Supplement (Oxoid). Presumptive Salmonella colonies were confirmed by using the Reveal Salmonella test kits (Neogen Corp., Lansing, MI) and BAX® PCR System Salmonella test kit (Du Pont Qualicon, Germany). Presumptive C. jejuni colonies were confirmed by using the DrySpot Campylobacter test kit (Oxoid) and BAX® PCR System Campylobacter test kit (Du Pont Qualicon).

Model fitting

Separate statistical analyses for Salmonella Typhimurium or C. jejuni were performed by using the statistical software package R (version 2.11.1, R Development Core Team, 2010). The experimental design for each pathogen was a split-plot design. Pork loin was the whole-plot unit with moisture enhancement (0%, 10%, 20%) as the whole-plot factor. Sliced pork was the split-plot unit with storage temperature (4°C and 10°C) and cooking temperature (68.5°C, 71.1°C, 73.9°C, 76.7°C) as the split-plot factors (Fig. 1). After cooking, enumeration results of Salmonella Typhimurium or C. jejuni were presence or absence. A logistic General Linear Mixed Model with random effect was used to model the association between potential risk factors and the presence/absence outcome. Sliced samples within a pork loin were not independent; therefore, pork loin was included in the model as a random effect. All the fixed-effect terms \(M_i\), \(S_j\), \(C_j\) and the random-effect term \(g_i\) in the model are described in Table 1.

A Likelihood Ratio Test (Self and Liang, 1987) was conducted to test whether the variance of the random effect differs significantly from zero. A stepwise backward deletion of nonsignificant terms was performed. The coefficients

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**Table 1. Description of the Variables that Were Used in the Logistic Regression Models (Equations 1 and 2)**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>(M_i)</td>
<td>Level of moisture enhancement applied on the (i^{th}) pork loin</td>
</tr>
<tr>
<td>(S_j)</td>
<td>Storage temperature applied to the (j^{th}) sliced pork on any pork loin</td>
</tr>
<tr>
<td>(C_j)</td>
<td>Target internal cooking temperature applied to the (j^{th}) sliced pork on any pork loin</td>
</tr>
<tr>
<td>(g_i)</td>
<td>The random effect of the (i^{th}) pork loin in equation 1, independently and identically normally distributed as (N(0, \sigma_g^2))</td>
</tr>
<tr>
<td>(g_i')</td>
<td>The random effect of the (i^{th}) pork loin in equation 2, independently and identically normally distributed as (N(0, \sigma_g'^2))</td>
</tr>
</tbody>
</table>

---

**FIG. 1.** Flow diagram of the experimental set-up in the split-plot design. Pork loin (□) was the whole-plot unit and sliced pork (□) was the split-plot unit.
retained in the reduced model were those significant at a \( p \) value of <0.05. Analysis of deviance, based on a likelihood-ratio test (LRT), was used to assess the statistical significance of the exclusion of each variable. When the coefficient for the logistic regression varied as a function of a retained term in the reduced model that moderated the impact of an independent variable on the dependent variable, the 95% confidence interval of the coefficient was calculated with the asymptotic covariance matrix (Preacher et al., 2006). Contour plots with 50% recovery probability of *Salmonella Typhimurium* and *C. jejuni* were created to assess the interaction effects that were in the reduced models.

**Results**

*Survival of Salmonella Typhimurium and C. jejuni after cooking*

Survival of *Salmonella Typhimurium* and *C. jejuni* in samples after cooking is shown in Figures 2 and 3. The populations of *Salmonella Typhimurium* and *C. jejuni* in the interior tissues of samples without cooking (control) ranged from 4.08 to 5.94 \( \log_{10} \) CFU/g and from 4.12 to 5.94 \( \log_{10} \) CFU/g, respectively. There were 18 samples inoculated with *Salmonella Typhimurium* or *C. jejuni* and cooked to each endpoint temperature. *Salmonella Typhimurium* was recovered from 10 samples that were cooked to 68.3°C, 3 samples that were cooked to 71.1°C, and 3 samples that were cooked to 73.9°C. When the internal temperature reached 76.7°C, the populations of *Salmonella Typhimurium* dropped below the detectable level. The surviving populations of *Salmonella Typhimurium* ranged from 1.3 to 2.94 \( \log_{10} \) CFU/g. *C. jejuni* were recovered from the interior tissues for every endpoint temperature. *C. jejuni* was recovered from 6 samples that were cooked to 68.3°C, 4 samples that were cooked to 71.1°C, 4 samples that were cooked to 71.1°C, and 1 sample that was cooked to 76.7°C. The surviving populations of *C. jejuni* ranged from 1.3 to 3.15 \( \log_{10} \) CFU/g.

**Model development**

There was substantial and significant variation (\( p < 0.05 \)) between the loins that were inoculated with *Salmonella Typhimurium*, and the variance of the random effect (\( \sigma_r^2 \)) was 1.85. The variation between the slices (\( \sigma_t^2 \)) was 0.35. After

![FIG. 2](image-url). Survival of *Salmonella Typhimurium* in moisture-enhanced pork after cooking. Before cooking, all inoculated samples were stored at 4°C for 21 days (A) or stored at 10°C for 14 days (B). Control samples were analyzed for *Salmonella Typhimurium* after storage. Other samples were cooked to target endpoint temperatures (68.3°C, 71.1°C, 73.9°C, and 76.7°C). After cooking, these samples were analyzed for *Salmonella Typhimurium*. The bars represent the mean ± standard error. CFU, colony-forming units.
variable selection, a reduced model used to describe the 
\[ \text{logit}(p_{ij}) = \beta_0 + \gamma_i + \beta_1 C_j + \beta_2 S_j + \beta_3 M_i \times S_j \]  

(1)

where \( p_{ij} \) is the probability of *Salmonella* Typhimurium presence in the \( i \)th loin, \( j \)th slice.

There was also substantial and significant variation \( (p < 0.05) \) between the loins that were inoculated with *C. jejuni* and the variance of the random effect \( (\sigma^2_r) \) was 105.37. The variation between the slice \( (\sigma^2_s) \) was 0.11. After variable selection, a reduced model that used to describe the logit\( (p_{ij}) \) of *C. jejuni* was obtained as follows:

\[ \text{logit}(p_{ij}) = \beta_0' + \gamma_i' + \beta_1' C_j + \beta_2' S_j + \beta_3' M_i + \beta_4' M_i \times S_j + \beta_5' C_j \times S_j \]  

(2)

where \( i = 10, 11, \ldots, 18 \)th loin.

All the coefficients retained in the reduced model (1) and (2) were significantly nonzero. Comparing the full model and the reduced model suggested that the difference between these models was not statistically significant \( (p > 0.05) \), based upon the LRT.

**Risk factors associated with *Salmonella* Typhimurium and *C. jejuni* contamination**

Effects of moisture enhancement, storage, and cooking temperature on the recovery probability of *Salmonella* Typhimurium and *C. jejuni* are presented in Tables 2 and 3, respectively. The upper bounds of the confident intervals for the estimated coefficients associated with \( M_i \) and \( C_j \) in equation 1 were negative. The estimated coefficients associated with \( M_i \) and \( C_j \) in equation 2 were dependent on storage temperature. At 4°C, the confident intervals for these coefficients included zero. At 10°C, the upper bounds of the confident intervals for these coefficients were negative. The lower bounds of the confident intervals for the estimated coefficients associated with \( S_j \) in equation 1 were positive when enhancement levels were 0% and 10%, but the confident interval included zero when enhancement level was 20%. The estimated coefficients associated with \( S_j \) in equation 2 were dependent on enhancement level and cooking temperature.

**FIG. 3.** Survival of *C. jejuni* in moisture-enhanced pork after cooking. Before cooking, all inoculated samples were stored at 4°C for 21 days (A) or stored at 10°C for 14 days (B). Control samples were analyzed for *C. jejuni* after storage. Other samples were cooked to target endpoint temperatures (68.3°C, 71.1°C, 73.9°C, and 76.7°C). After cooking, these samples were analyzed for *C. jejuni*. The bars represent the mean ± standard error. CFU, colony-forming units.
Discussion

To assure the microbiological safety of meat products, 71.1°C is recommended as the safe minimum internal cooking temperature for pork (USDA-FSIS, 2009). In the present study, after samples were cooked to 71.1°C or above without holding time, the abundance and frequency of recovery of *C. jejuni* and *Salmonella* Typhimurium in samples of enhanced pork were both lower than those in samples of intact pork (without injection). This result may imply that *C. jejuni* and *Salmonella* Typhimurium are less heat resistant in the interior of moisture-enhanced pork than in intact pork. It is generally agreed that the intact pork is safe. Thus, cooking to a temperature above 71.1°C should be adequate for assuring the microbiological safety of moisture-enhanced pork. *C. jejuni* and *Salmonella* Typhimurium were still recovered from some samples that were cooked above 71.1°C. During cooking, the heating of the slices maybe uneven and consequently bacteria survived at cold spots within slices (Luchansky et al., 2012). The levels of inoculum used in the present study were relatively high compared to levels of natural contamination and this may also affect the results.

Examination of the coefficient showed that moisture enhancement had a negative effect on the survival of *Salmonella Typhimurium*. Although moisture enhancement has the potential to carry *Salmonella Typhimurium* from the surface to the interior of pork, the vacuum storage and final cooking practices that were followed did not result in a higher heat resistance of *Salmonella Typhimurium* in moisture-enhanced pork than in intact pork. Moisture enhancement also had a negative effect on the survival of *C. jejuni* when pork samples were stored at 10°C. However, the effect of moisture enhancement was uncertain on the survival of *C. jejuni* when pork samples were stored at 4°C.

We further observed that the effect of cooking on the survival of *C. jejuni* was moderated by storage temperature. After pork samples were stored at 10°C, cooking had the inactivation effect on the survival of *C. jejuni*. However, cooking may not have an inactivation effect after pork samples were stored at 4°C. The low-temperature response of *C. jejuni* is very different from that of *Salmonella* (Park, 2005; Rychlik and Barrow, 2005). For example, *C. jejuni* cells are unable to alter their fatty-acid composition during rapid chilling, and the unaltered fatty-acid composition is more suited to survive when cells are exposed to subsequent heat stresses (Hughes et al., 2009). In the meat industry, vacuum packaging and storage at strictly controlled temperatures of 4°C are widely used to store and export raw meat. It is therefore recommended to reconsider the effect of chilling by including...

### Table 2. General Linear Mixed Model (Equation 1) of Risk Factors That Influence the Recovery Probability of *Salmonella Typhimurium*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>Description</th>
<th>Estimate</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_i$</td>
<td>$b_3S_i$</td>
<td>Storage temperature was 4°C</td>
<td>−0.11</td>
<td>−0.17, −0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Storage temperature was 10°C</td>
<td>−0.28</td>
<td>−0.44, −0.12</td>
</tr>
<tr>
<td>$S_j$</td>
<td>$b_3M_i + b_2$</td>
<td>Enhancement level was 0%</td>
<td>0.51</td>
<td>0.25, 0.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhancement level was 10%</td>
<td>0.23</td>
<td>0.03, 0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhancement level was 20%</td>
<td>−0.05</td>
<td>−0.30, 0.19</td>
</tr>
<tr>
<td>$C_j$</td>
<td>$b_1$</td>
<td>All levels of cooking temperature</td>
<td>−1.00</td>
<td>−1.36, −0.65</td>
</tr>
</tbody>
</table>

CI, confidence interval.

### Table 3. General Linear Mixed Model (Equation 2) of Risk Factors That Influence the Recovery Probability of *Campylobacter jejuni*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>Description</th>
<th>Estimate</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_i$</td>
<td>$b_3 + b_4S_i$</td>
<td>Storage temperature was 4°C</td>
<td>0.34</td>
<td>0.58, 1.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Storage temperature was 10°C</td>
<td>−1.52</td>
<td>−2.57, −0.47</td>
</tr>
<tr>
<td>$S_j$</td>
<td>$b_2 + b_4M_i + b_5C_j$</td>
<td>Enhancement level was 0%, cooking temperature was 68.3°C</td>
<td>4.10</td>
<td>2.46, 5.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhancement level was 0%, cooking temperature was 71.1°C</td>
<td>2.13</td>
<td>1.31, 2.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhancement level was 0%, cooking temperature was 73.8°C</td>
<td>0.15</td>
<td>0.13, 0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhancement level was 0%, cooking temperature was 76.6°C</td>
<td>−1.83</td>
<td>−2.68, −0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhancement level was 10%, cooking temperature was 68.3°C</td>
<td>1.00</td>
<td>0.33, 1.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhancement level was 10%, cooking temperature was 71.1°C</td>
<td>−0.98</td>
<td>−1.48, −0.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhancement level was 10%, cooking temperature was 73.8°C</td>
<td>−2.95</td>
<td>−4.15, −1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhancement level was 10%, cooking temperature was 76.6°C</td>
<td>−4.93</td>
<td>−6.92, −2.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhancement level was 20%, cooking temperature was 68.3°C</td>
<td>−2.10</td>
<td>−3.15, −1.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhancement level was 20%, cooking temperature was 71.1°C</td>
<td>−4.08</td>
<td>−5.73, −2.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhancement level was 20%, cooking temperature was 73.8°C</td>
<td>−6.06</td>
<td>−8.45, −3.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhancement level was 20%, cooking temperature was 76.6°C</td>
<td>−8.03</td>
<td>−11.21, −4.85</td>
</tr>
<tr>
<td>$C_j$</td>
<td>$b_1 + b_5S_j$</td>
<td>Storage temperature was 4°C</td>
<td>−0.62</td>
<td>−2.88, 1.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Storage temperature was 10°C</td>
<td>−4.89</td>
<td>−7.15, −2.64</td>
</tr>
</tbody>
</table>

CI, confidence interval.
our results to avoid underestimation of potential risks and to enhance food safety for moisture-enhanced pork.

The effect of storage temperature on the survival of *Salmonella* Typhimurium decreased with increasing of enhancement level. When the enhancement level was 0% or 10% and other variables were fixed, increasing the storage temperature would increase the survival of *Salmonella* Typhimurium. However, increasing the storage temperature would decrease the survival of *Salmonella* Typhimurium when the enhancement level was 20%. It is generally assumed that microorganisms grown at higher temperatures have greater resistance to heat (Ng et al., 1969; Dega et al., 1972; Pagán et al., 1999). The results in the present study may be attributable to the increasingly negative effect of moisture enhancement on the survival of *Salmonella* Typhimurium.

Considerable variations in the chemical compositions of meat (fat content and dry-matter content) were observed among pork loins (Corino et al., 2009). Fat content and dry-matter content are the nuisance factors in the present study that may influence the heat resistance of foodborne pathogens in pork (Ghazala et al., 1995; Veeramuthu et al., 1998). Thus, pork loins are created as blocks in which these nuisance factors are held constant. Within blocks, it is possible to assess the effect of different levels of the risk factors without worrying about variations due to changes of the nuisance factors. The contour plot with the 50% recovery probability shows that the random effect of pork loin contributed the largest amount of variance on the survival than other fixed effects (not shown). Compared with *Salmonella* Typhimurium, the inactivation of *C. jejuni* by cooking was much more affected by the variation among pork loins.

Research previously conducted to investigate cooking inactivation of foodborne pathogens in nonintact meat products has been focused on evaluating the inactivation efficacy of common cooking practices on *Escherichia coli* O157:H7 internalized in nonintact beef. This study provides quantitative data to evaluate the effects of moisture enhancement and storage condition on the cooking inactivation of *Salmonella* Typhimurium and *C. jejuni* in moisture-enhanced pork. The findings will assist food processors and regulatory agencies in evaluating the current processing, storage condition, and cooking guidelines for moisture-enhanced pork.

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Disclosure Statement

No competing financial interests exist.

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