Research Note

Enhanced Thermal Resistance of *Salmonella* in Marinated Whole Muscle Compared with Ground Pork

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**ABSTRACT**

The internal muscle environment may enhance thermal resistance of bacterial pathogens. Based on the migration of pathogens into whole muscle products during marination, the validity of current thermal inactivation models for whole muscle versus ground products has been questioned. Consequently, the objective of this work was to compare thermal resistance of *Salmonella* in whole muscle versus ground pork. Irradiated samples of whole and ground pork loin (5.5 to 7.5 g) were exposed to a *Salmonella*-inoculated (10⁶ CFU/ml) marinade (eight serovar cocktail) for 20 min, placed in sterile brass tubes (12.7 mm diameter), sealed, and heated isothermally at 55, 58, 60, 62, or 63 °C, and surviving salmonellae were enumerated on Petrifilm aerobic count plates. The thermal lag times and initial bacterial counts were similar for both whole muscle and ground samples (P > 0.05), with all samples having equivalent compositions, inocula, and thermal histories. Heating temperature and physical state of the meat (whole versus ground muscle) affected *Salmonella* inactivation, with greater thermal resistance observed in whole than in ground muscle (P < 0.05). Assuming log-linear inactivation kinetics, *Salmonella* was 0.64 to 2.96 times more heat resistant in whole muscle than in ground pork. Therefore, thermal process validations for pork products should also account for the physical state of the product to ensure microbial safety.

*Salmonella* is the leading cause of bacterial foodborne illness in the United States (3). Additionally, *Salmonella* is the target pathogen for the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) performance standards that require a 6.5-log reduction during cooking of ready-to-eat meat products (12). Cooking processes utilized by the industry are generally effective at destroying vegetative foodborne bacterial pathogens. However, salmonellae might survive these processes if changes in product formulation or other characteristics are not accounted for in process validations or if there are defects in the process itself.

Tenderization of whole muscle products may result in increased risk of bacterial presence and survival. Mechanical tenderization can transfer surface bacteria to the interior of the muscle (8), where they may proliferate (given adequate extrinsic and intrinsic conditions) and shorten the shelf life of the product (9). Recently, several researchers have shown that *Salmonella* can migrate into and survive inside intact whole muscle products (11, 14, 16). Warsow et al. (16) found that exposure to a vacuum significantly enhanced pathogen migration into the product core during marination of turkey breasts.

Several other researchers have reported enhanced thermal resistance of *Salmonella* in whole muscle beef and turkey products compared with ground products of equivalent composition (6, 11). However, these results also confirmed that thermal resistance is different across different meat species (e.g., ground turkey versus ground beef). Therefore, there is a need for product-specific thermal resistance data and model parameters to ensure the highest degree of reliability in process validations. The objective of this study was to quantify the effect of product structure (whole versus ground muscle) on thermal resistance of *Salmonella* in pork products.

**MATERIALS AND METHODS**

**Inoculum preparation.** Eight serovars of *Salmonella* previously shown to have moderate to high thermal resistance (5) were obtained from Dr. V. K. Juneja (USDA, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA); *Salmonella* Thompson FSIS 120 (chicken isolate), *Salmonella* Enteritidis H3527 and H3502 (chicken isolates, phages 13A and 4, respectively), *Salmonella* Typhimurium DT104 H3380 (human isolate), *Salmonella* Hadar MF60404 (turkey isolate), *Salmonella* Copenhagen 8457 (pork isolate), *Salmonella* Monte-video FSIS 051 (beef isolate), and *Salmonella* Heidelberg F5038BG1 (human isolate). All isolates were maintained at −80 °C in tryptic soy broth (TSB; Becton Dickinson, Sparks, MD) containing 20% glycerol. After the frozen stock cultures were subjected to at least two consecutive transfers at 18 to 24 h and

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37°C, 9-ml volumes of each culture were combined and centrifuged at 6,000 x g for 20 min at 4°C. The resulting cell pellet was then resuspended in 500 ml of sterile marinade to obtain ~1.21 x 10^5 CFU/ml, which was confirmed by duplicate plating of appropriate dilutions on Petrifilm aerobic count plates (3M, St. Paul, MN).

Marinade preparation. A typical aqueous marinade solution for whole muscle foods contains 96.0% deionized water, 3.2% (wt/vol) NaCl, and 0.8% (vol/vol) potassium phosphate solution (Butcher and Packer Supply Co. Detroit, MI). This marinade was prepared according to the method of Pearson and Dutson (7) by adding NaCl to the phosphate solution to ensure complete solubility. Marinade was autoclaved (15 min at 121°C) in 520-ml aliquots to ensure sterility and stored no longer than 30 days at ~22°C before use.

Meat preparation. Nine nonenhanced pork loins (longissimus dorsi muscle) were obtained locally, segmented into roasts (~0.75 kg each), packaged, vacuum sealed, stored at ~20°C, transported on dry ice to Food Technology Service (Mulberry, FL) for irradiation (~10 kGy), returned frozen, and stored at ~20°C. After irradiation, sterility was confirmed by examination of three randomly selected roasts. Roast samples (25 g) were diluted 1:10 in TSB containing 0.6% yeast extract, homogenized in a masticator (model 0410, IUL Instruments USA, Inc., Cincinnati, OH), incubated at 37°C for 24 h, and plated in duplicate on Petrifilm aerobic count plates, which were incubated for 24 h at 37°C. No growth was observed on any of these plates.

Surface fat was trimmed from the roasts using a sterile knife, and samples were manually removed using a coring device (1.27-cm diameter; G. R. Electrical Mfg. Co., Manhattan, KS) to produce whole muscle plugs (5.5 to 7.0 g, 6.0 to 8.0 cm long), with the muscle fibers running parallel to the length. The remaining sterile muscle was ground through a 4-mm-diameter plate using a Kitchen Aid grinder (model k5-A, Hobart, Troy, OH).

Proximate analyses. Moisture and fat content were determined using AOAC methods 950.46B and 991.36, respectively (2).

Meat inoculation. Whole muscle cores previously thawed for 48 h at 4°C were immersed in the inoculated marinade for 20 min at 4°C. To determine marinade uptake, each core was weighed before and after marination. The average marinade uptake was ~14% in the whole muscle cores. The same amount of marinade was added dropwise to the ground product, which was then hand mixed with a sterile spatula in a sterile plastic container to ensure uniform distribution of Salmonella. Unheated inoculated samples of whole muscle and ground pork were randomly selected and tested to confirm uniformity of mixing. All whole and ground muscle samples were aseptically packed into sterile brass tubes (1.27-cm diameter, 10 cm long), which were sealed at both ends with sterile rubber stoppers and Teflon tape and then heated within 2 h of storage at 4°C.

Thermal inactivation. All tubes of meat were placed in a plastic rack and immersed in an agitated temperature-controlled water bath (NESLAB Instruments Inc., Newington, NH) for isothermal heating at 55, 58, 60, 62, or 63°C, with the temperature of the water bath set 0.5°C above the target temperature. A thermocouple (1.0 mm; Type T, Omega Engineering, Stamford, CT) was inserted into the center of one sample in each replicate and attached to a data logger (DuoLogK Thermocouple Thermometer, model 91100-50, Cole Parmer Instrument Company, Vernon Hills, IL) to monitor internal temperature. After the thermal lag time (defined as the time when the sample core temperature was within 0.5°C of the set point), the tubes of meat were removed from the water bath at predetermined intervals and immediately placed in an ice-water bath. All treatments were tested in triplicate.

Salmonella recovery. After being heated and cooled, the samples were diluted 1:5 in 0.1% peptone water and homogenized for 90 s in the masticator. Surviving salmonellae were enumerated by plating serial dilutions in duplicate on Petrifilm aerobic count plates and incubating the plates at 37°C for 48 h.

Statistical analyses. An analysis of variance (ANOVA) was conducted for log survivors versus time, temperature, and structure (whole versus ground). First-order (log-linear) inactivation kinetics was assumed, and linear regression of ln N versus time was used to estimate the inactivation rate constant at each temperature. The corresponding D-values were computed as the inverse of the slope from linear regression of log N versus time.

<table>
<thead>
<tr>
<th>Variable</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grinding</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Temp</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Temp x time</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Grinding x time</td>
<td>0.0062</td>
</tr>
<tr>
<td>Grinding x temp</td>
<td>0.3102</td>
</tr>
</tbody>
</table>

FIGURE 1. Thermal inactivation of Salmonella in whole versus ground pork muscle at 55, 58, 60, 62, and 63°C.
TABLE 2. First-order inactivation rate constant, k, for thermal inactivation calculated by linear regression from the Salmonella survivor data for whole and ground pork at 55, 58, 60, 62 and 63°C

<table>
<thead>
<tr>
<th>Product</th>
<th>55°C</th>
<th>58°C</th>
<th>60°C</th>
<th>62°C</th>
<th>63°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole muscle</td>
<td>0.10 ± 0.01</td>
<td>0.67 ± 0.04</td>
<td>1.33 ± 0.13</td>
<td>3.00 ± 0.46</td>
<td>3.13 ± 0.35</td>
</tr>
<tr>
<td>Ground muscle</td>
<td>0.26 ± 0.01</td>
<td>1.53 ± 0.11</td>
<td>3.80 ± 0.41</td>
<td>4.93 ± 1.01</td>
<td>8.37 ± 0.96</td>
</tr>
</tbody>
</table>

Salmonella survivor data were calculated as ln CFU per gram versus time.

RESULTS

Proximate analyses. The raw pork contained 2.5% ± 0.93% fat and 73.6% ± 2.7% moisture.

Salmonella populations in marinated whole muscle and ground pork. After inoculation, there were no significant differences (α = 0.05) in numbers of salmonellae between the whole muscle (7.27 ± 0.2 log CFU/g) and ground samples (7.29 ± 0.2 log CFU/g). Based on previous work by Warsow (15), in which Salmonella migrated to depths >2 cm, Salmonella distribution within the small whole muscle cores was assumed to be uniform.

Thermal inactivation of Salmonella in marinated samples. Thermal lag times ranged from 2.3 to 3.7 min for whole muscle and 2.1 to 3.5 min for ground samples. The Salmonella counts after the thermal lag time (time 0 of inactivation curves) were not significantly different (α = 0.05) between the whole and ground muscle samples.

Replication error (i.e., the standard deviation among replicates) for microbial survivors was not affected by heating time or grinding (α = 0.05). However, replication error tended to increase with heating temperature, with values of 0.37, 0.41, 0.80, 0.91, and 0.57 log CFU/g at 55, 58, 60, 62, and 63°C, respectively.

At all five temperatures, Salmonella was more heat resistant (P < 0.001) in whole muscle than in ground pork (Fig. 1). The ANOVA revealed that grinding, temperature, heating time, the interaction between time and temperature, and the interaction between time and grinding significantly affected thermal inactivation of Salmonella in pork (Table 1). Overall, Salmonella was 0.64 to 2.96 times more resistant in the whole muscle than in the ground pork (Table 2). The corresponding D-values at 55, 58, 60, 62, and 63°C were 23.4, 3.43, 1.74, 0.77, and 0.74 min, respectively, for whole muscle and 8.75, 1.50, 0.61, 0.47, and 0.28 min, respectively, for ground pork.

DISCUSSION

Whole and ground muscle samples in this study were similar in composition, bacterial load, and thermal histories, suggesting that differences in Salmonella thermal inactivation were due only to differences in the physical structure of the meat. The fundamental reasons for this effect were not tested in this study and are not definitively known. However, destruction of the original muscle structure during grinding results in a change in water status and a more uniform distribution of the fat and protein within the product. Water also is more likely to be bound within the muscle fibers of whole muscle samples, which may result in increased heat resistance.

The protective effect of fat against thermal inactivation of bacteria (1, 13) also may be partially lost after the fat has been uniformly distributed as a result of grinding. Bacteria attached to intramuscular fat in whole muscle tissue may be able to utilize this protective effect in a way that is not possible in more homogenous ground products. During cooking, meat proteins denature and contract, thereby also changing the meat microstructure (10).

The literature contains limited information on thermal inactivation of Salmonella in ground pork, and any direct comparisons with our results are complicated by differences in bacterial serovars, meat species, muscle type, formulations, and other environmental factors. Juneja et al. (5) studied the heat resistance of 35 Salmonella strains at 58 and 60°C in different substrates and found that the D-values in meat were higher than those in broth. Salmonellae are generally more heat resistant when attached to meat surfaces (or to surfaces such as stainless steel or glass) than when unattached and dispersed throughout a food or broth (4).

In the present work, Salmonella was more heat resistant in whole muscle than in ground pork. Consequently, thermal process validations for meat products should consider the physical state (whole muscle versus ground) of the product. Further work is needed to better understand the mechanisms of thermal resistance in Salmonella in whole muscle compared with ground products during cooking.

ACKNOWLEDGMENT

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REFERENCES

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