Enhanced Thermal Resistance of *Salmonella* in Whole Muscle Compared to Ground Beef

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ABSTRACT: Irradiated beef (whole muscle and ground product with identical fat, protein, and moisture composition) was exposed to a *Salmonella*-inoculated marinade and heated in brass tubes in a water bath at 55 °C, 60 °C, and 62.5 °C. The bacterial load and thermal lag time were similar ($\alpha = 0.05$) for both whole and ground muscle; therefore, all samples had equivalent composition, inoculation levels, and thermal histories. Assuming 1st-order kinetics, the inactivation rate constants (k values) in whole muscle were 50% lower than those in ground product at each temperature ($P = 0.0001$), and Arrhenius-type models described the temperature dependency of k ($R^2 > 0.95$). Because thermal processing regulations are generally based on ground product studies, thermal process validations for meat and poultry products may need to consider the physical state (whole muscle versus ground) of the product being manufactured.

Keywords: thermal resistance, *Salmonella*, whole muscle, ground meat

Introduction

In contrast to comminuted and ground products, the interior of intact, undamaged, whole muscle is often assumed to be sterile (Elmossalami and Wasef 1971). Limited research, however, has shown that bacteria can indeed migrate into and survive inside whole muscle products. Elmossalami and Wassef (1971) found that *Salmonella* Enteritidis migrated into beef muscle 5, 10, and 15 cm after 12, 24, and 36 h, respectively, at 30 °C; the rate was slower but still observable at 7 °C and –10 °C. Gill and Penney (1977) showed that *S.* Typhimurium penetrated 3, 7, and 10 cm/h into beef muscle at 20 °C, 30 °C, and 37 °C, respectively.

The potential for *Salmonella* migration into intact, whole muscle turkey breasts during marination was specifically evaluated by Warsow and others (2003). Irradiated whole muscle turkey breasts were exposed to *Salmonella*-inoculated marinade. The meat was then either held at 4 °C or exposed to vacuum (101.3 kPa) at 4 °C for various durations. Significant numbers of *Salmonella* penetrated to depths greater than 1 cm ($P < 0.001$). Counts decreased with depth below the inoculated surface, increased ($P < 0.001$) with application of the vacuum, and perhaps increased ($P = 0.12$) with marination time. Although these tests did not exactly mimic a commercial process, they did demonstrate that significant migration can occur into whole muscle product during marination, even without any mechanical action.

If bacteria can gain access to the inner portions of the muscle, then a wide variety of microbial, product, and environmental factors can affect their thermal resistance. In terms of product attributes, meat species, muscle type, pH, carbohydrates, fat content, and salts can affect the heat resistance of pathogens. For example, thermal resistance of pathogens tends to increase with increasing fat content in the substrate (Ahmed and others 1995; Juneja and others 1997; Veeramuthu and others 1998), although some have not seen this effect (Fain and others 1991; Young and others 1991; Kotrola and Conner 1997). Also, additives such as salts, lactates, and phosphates may enhance the thermal resistance of pathogens (Kotrola and Conner 1997; Maurer and others 2001). Although a large body of literature has shown that intrinsic chemical factors affect thermal resistance, almost no previous literature has reported the potential effect of intrinsic physical factors, such as differences in the microstructure of whole muscle versus ground meat products.

Thermal resistance of bacteria is generally higher in meat products than in buffer solutions, peptone, agar, or other model systems (Ghazala and others 1995; Murphy and others 1999; Juneja and others 2001). Not only do food components appear to enhance heat resistance, but cell location (surface attachment versus interior dispersion) may also affect the resistance of *Salmonella* (Doyle and Mazzotta 2000). Therefore, applying thermal-inactivation data developed in liquid media (Ghazala and others 1995; Smith 1995), meat slurries (Juneja and others 2001), or surface-inoculated meat (Humphrey and others 1997) to whole muscle products may not be valid. Additionally, although the physical arrangement of various components within a food matrix (for example, homogeneous fat distribution in ground product as opposed to tissue segregation in whole muscle product) may cause differences in bacterial thermal resistance, no known literature has specifically tested this factor or has explored the mechanisms involved in this phenomenon. Thus, the objectives of this study were (1) to compare the thermal resistance of *Salmonella* in whole muscle and ground beef product and (2) to describe the differences via a secondary inactivation model.

Materials and Methods

Bacterial cultures

The inoculum was a *Salmonella* cocktail comprising the following 8 serovars, which were previously obtained from Dr. V. K. Juneja (USDA-ARS, Eastern Regional Research Center, Wyndmoor, Pa.) and shown to have moderate to high thermal resistance (Juneja and others 2001): *S.*Thompson FSIS 120 (chicken isolate), *S.* Enteriditis H3527 and H3502 (chicken isolates phage type 13A and 4, respectively), *S.* Typhimurium DT104 H3380 (human isolate), *S.* Hadar MF60404 (turkey isolate), *S.* Copenhagen 8457 (pork isolate), *S.*

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Montevideo FSIS 051 (beef isolate), and *S.* Heidelberg F5038BG1 (human isolate). Cultures were maintained at –80 °C in tryptic soy broth (TSB) (Difco, Detroit, Mich., U.S.A.) containing 20% glycerol. Before use, each serovar was cultured separately in TSB at 37 °C using a minimum of 2 consecutive 24 h transfers to obtain cells in late log phase (Smith and others 2002).

Marinade

A typical marinade solution for ready-to-eat whole muscle foods was prepared according to Pearson and Dutson (1987) as follows: 80.7% water, 12.6% NaCl, and 6.65% phosphate solution (Butcher and Packer Supply Co., Detroit, Mich., U.S.A.). After formulation, the marinade was autoclaved (121 °C, 20 min) and stored at room temperature (about 22 °C).

Meat preparation

Beef shoulder clods, specifically NAMP 114E, the *Triceps brachii* muscle, which included the long, medial, and lateral heads (NAMP 1997) were obtained from a local supplier and randomly divided into second lots. One lot was ground twice through a plate with 4-mm--dia holes in a Kitchen Aid grinder (Model k5-A, Hobart, Troy, Ohio, U.S.A.), vacuum packaged in 100-g portions, and frozen at –24.4 °C. In the s lot, muscle samples were manually removed using a coring device (1.27-cm dia, G.R. Electrical Mfg. Co., Manhattan, Kans., U.S.A.) to produce whole muscle cylinders (5.5 to 7.0 g) 1.27 cm in diameter and 6.0 to 8.0 cm in length, with the muscle fibers running parallel to the long dimension. The cores were vacuum packaged and frozen at –12 °C. Both ground beef and whole muscle cylinders were transported on dry ice to Iowa State Univ. and irradiated (about 10 kGy). Sterility of the meat after irradiation was tested by diluting the beef 1:5 in peptone water (Difco) followed by plating on Petrifilm™ aerobic count plates (3M, St. Paul, Minn., U.S.A.).

Moisture, fat, and protein contents were determined using AOAC methods 950.46B, 991.36, and 981.1, respectively (AOAC 1996). To measure the pH, ground beef (10 g) was homogenized for 30 s in distilled water (90 mL) using a Polytron homogenizer (Model PT 10/35, Brinkmann Instruments, Westbury, N.J., U.S.A.) at speed setting 3 (Smith and others 2001). The pH of the mixture was measured using a combination electrode (Model 145, Corning, Medfield, Mass., U.S.A.). All analyses were done in triplicate on portions randomly selected from the original lots.

Inoculation

On the day of the experiment and immediately before meat inoculation, equal volumes of each culture were combined, centrifuged (6000 · *g*, 20 min at 4 °C), and re-suspended in 525 mL of sterile marinade to a concentration of approximately 10^8 colony forming units (CFU)/mL. All marinades were used within 10 min of inoculation, with no loss in *Salmonella* viability observed (Warsow 2003).

The thawed whole muscle cores were immersed in the inoculated marinade for 20 min at 4 °C. Each core was weighed before and after marination to determine the resulting average uptake (about 0.15 g marinade/g meat). For the ground samples, the inoculated marinade was added dropwise, under aseptic conditions, in the same proportions as the uptake in the whole muscle samples. The inoculated ground meat was then hand-mixed in a sterile basin using sterile gloves to ensure uniform distribution of *Salmonella*. Uniformity of inoculation was tested by random sampling of inoculated, unheated whole muscle and ground beef.

All samples (whole and ground muscle, 5.5 to 7.0 g) were aseptically packed into sterile brass tubes (1.27cm dia, 10cm length). The ends of the tubes were sealed with sterile rubber stoppers and wrapped with Teflon tape. Samples were stored at 4 °C and heated within 2 h.

Thermal inactivation

After preparation, inoculation, and insertion into the brass tubes, all samples were subjected to isothermal heat treatment at 55 °C, 60 °C, or 62.5 °C. Samples in tubes were placed in a wire rack and submerged in an agitated, temperature-controlled water bath (NESLAB Instruments Inc., Newington, N.H., U.S.A.) set 0.5 °C above the target temperature. Internal temperatures were monitored using a thermocouple (Type T, 1.0-mm, Omega Engineering, Stamford, Conn., U.S.A.) inserted into the sample center and attached to a data logger (DuaLogR Thermocouple Thermometer, Model 91100-50, Cole Parmer Instrument Co., Vernon Hills, Ill., U.S.A.). The thermal lag time was defined as the time required for the meat temperature to increase to within 0.5 °C of the target temperature. At and beyond the thermal lag time ("time zero"), tubes were removed from the water bath at predetermined intervals and immediately placed in an ice-water bath. Whole muscle and ground beef samples were tested in duplicate and triplicate, respectively.

Salmonella enumeration

After heating and cooling, the samples were diluted 1:10 in 0.1% peptone water and homogenized for 90 s using a masticator (Model 0410, IUL Instruments USA, Inc., Cincinnati, Ohio, U.S.A.). Surviving salmonellae were enumerated after plating serial dilutions in duplicate on Petrifilm™ aerobic count plates followed by incubation at 37 °C for 48 h.

Data analysis

Salmonella survivor curves were determined for whole muscle and ground beef by plotting the logarithm of the survival ratio versus the holding time at each temperature. Analysis of variance (ANOVA) was used to assess the effect of time, temperature, and state of meat (whole versus ground) on *Salmonella* survival. Mean values were compared using Tukey-Kramer's test ($\alpha = 0.05$).

Assuming a 1st-order (that is, log-linear) inactivation response, thermal inactivation rate constants (k/min^{-1}) were obtained by linear regression of the natural logarithm of survivors over time, $Ln(N/N_o) = -kt$, for each replication. ANOVA was then used to evaluate the effects of temperature and grinding on k. Subsequently, an Arrhenius-type equation, $k = \beta_o e^{-\beta_1/T}$, was parameterized for both whole muscle and ground beef via linear regression of Ln (k) versus (1/T).

Results and Discussion

Raw meat

The raw beef had a pH of 5.7 and contained 5.6 ± 0.6% fat, 70.4 ± 0.7% moisture, and 20.3 ± 2.8% protein. No background microorganisms were found in uninoculated whole muscle or ground product after irradiation.

Inoculation of marinade and meat

Salmonella counts in the marinade were determined before sample inoculation. The target level of 10^8 CFU/mL (8.2 ± 0.1 Log CFU/mL) was consistently achieved for all treatments.

After marinade exposure, no differences ($\alpha = 0.05$) in initial *Salmonella* counts were observed between whole muscle and ground samples, which contained 7.5 ± 0.1 and 7.3 ± 0.1 Log(CFU/g), respectively. It was previously shown (Warsow 2003) that the whole muscle marination treatment used in this project yields *Sal-*

Table 1—*P* values from analysis of variance of *Salmonella* thermal inactivation data for whole and ground beef muscle.

Test factors	*P* values
Grinding	<0.0001
Temperature	0.1953
Time	<0.0001
Grinding · Temperature	<0.0001
Grinding · Time	<0.0001
Temperature · Time	<0.0001

Table 2—First-order inactivation rate constants, k (mean ± S) calculated by linear regression of *Salmonella* survivor data (Ln CFU/g versus time) for whole and ground beef muscle

	Heating temperature (°C)		
h	55	60	62.5
Whole muscle	0.13 ± 0.00	1.53 ± 0.54	2.06 ± 0.10
Ground muscle	0.32 ± 0.06	3.31 ± 0.24	5.28 ± 0.72

monella counts of about 10^6 CFU/g in the volume 0 to 1 cm below the surface exposed to inoculated marinade and about 10^4 CFU/g in the volume 1 to 2 cm below the exposed surface (for samples with a half thickness >2.5 cm). Given that the initial counts for the whole cores in this study were about $10^{7.5}$ CFU/g and the critical dimension (radius) was 0.635 cm, it can be assumed (in the context of the previous research) that the distribution of *Salmonella* cells in the marinated, whole muscle cores was relatively uniform across the sample volume.

Thermal history

The heating lag time, defined as the time necessary to reach within 0.5 °C of the target temperature, ranged from 2.3 to 3.0 min for whole muscle and 2.6 to 3.1 min for ground samples. The differences in heating lag time between whole and ground muscle were not statistically significant ($\alpha = 0.05$). Additionally, no differences ($\alpha = 0.05$) were seen between whole and ground muscle in terms of *Salmonella* counts before and after the thermal lag time (time zero of inactivation curves).

First-order kinetics

As expected, *Salmonella* survival in both whole and ground muscle decreased as the heating temperature increased (Figure 1). The semilogarithmic curves were generally linear, and influenced by the product state (whole muscle versus ground). ANOVA (Table 1) revealed that all 2-term interactions among time, temperature, and grinding affected *Salmonella* survival ($P < 0.0001$).

The 1st-order kinetic rate constants, k (Table 2), had R^2 values ranging from 0.84 to 0.96. The rate constants were approximately 50% lower in whole muscle than in ground product at each temperature ($P < 0.001$). Linear regression of Ln (k) versus the reciprocal absolute temperature (1/K) for whole muscle and ground product yielded R^2 values of 0.95 and 0.97, respectively. Thus, the reaction rate constants could be expressed in an Arrhenius-type format:

$$k_{ground} = 2.33 \cdot 10^{56}\, e^{-42918/T}$$

$$k_{whole} = 1.24 \cdot 10^{56}\, e^{-43002/T}$$

When the above secondary models were inserted into the 1st-order kinetic model, the root mean square errors for prediction of the $Ln(N/N_0)$ were 1.6 and 1.1 for the ground ($n_{obs} = 80$) and whole muscle ($n_{obs} = 39$) cases, respectively. Translating to prediction of $Log(N/N_0)$, these errors correspond to 0.69 and 0.46, indicating that the model fit the experimental survivor data relatively well. However, these statistics describe the quality of these models only within the temperature range (55 °C to 62.5 °C) and the specific conditions (beef muscle, 5.6 ± 0.6% fat, 70.4 ± 0.7% moisture, and 20.3 ± 2.8% protein) used in this study, and no extrapolations should be made outside this domain.

Murphy and others (2000) determined the kinetic parameters of *Salmonella* in both chicken breast meat and peptone-agar solution at 55 °C to 70 °C. Their results yielded kinetic rate constants of 0.08, 0.39, and 0.92min^{-1} for meat, and 0.10, 0.56, and 1.35 min^{-1} for peptone-agar at 55 °C, 60 °C, and 62.5 °C, respectively. Compared with our findings, *Salmonella* was more heat-resistant in chicken meat and broth than in beef muscle at the 3 temperatures tested. Although Murphy and others (2000) used a cocktail of 6 *Salmonella* serovars, 3 of which (*S.* Typhimurium, *S.* Heidelberg, and *S.* Montevideo) were also included in our study, they also included *S.* Senftenberg, a serovar reportedly 10 to 20 times more resistant than the

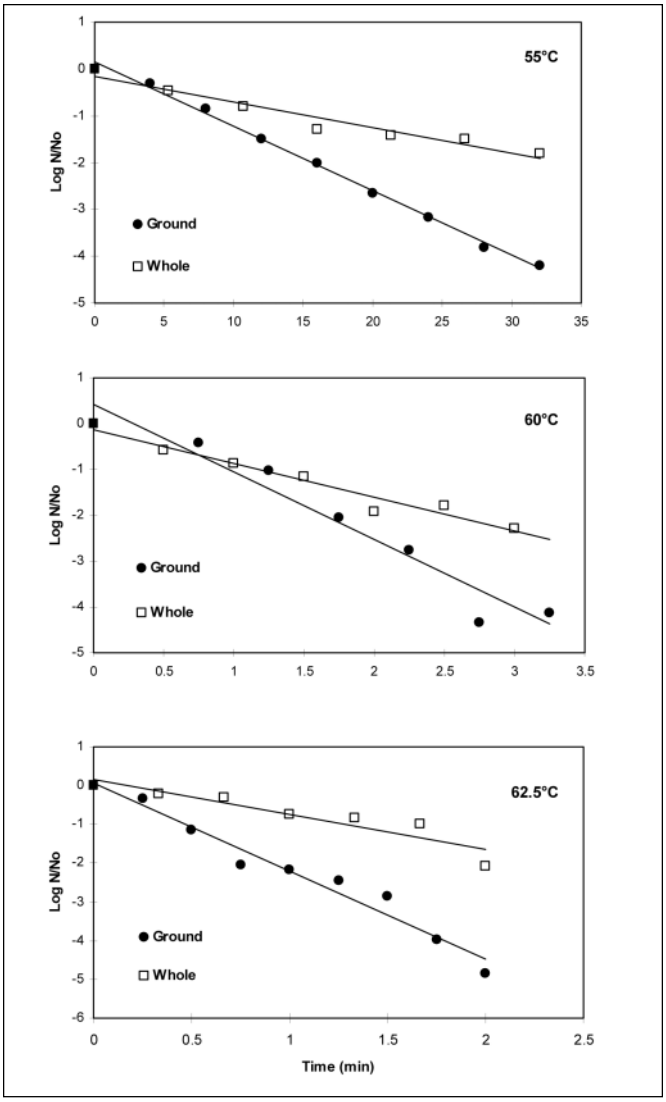

Figure 1—*Salmonella* survival ratios (N/N_0) in whole and ground beef muscle held at 55 °C, 60 °C, and 62.5 °C

average *Salmonella* spp (Doyle and Cliver 1990), which was not present in our cocktail.

Heat resistance of any *Salmonella* cocktail is based on the sum of the individual serovar resistances (Murphy and others 1999). Thus, including a microorganism of unusually high or low heat resistance will affect overall lethality. The USDA-FSIS (2001) recommends using a combination of *Salmonella* serovars to verify compliance with the thermal processing regulations for meat and poultry. These results illustrate the importance of the *Salmonella* cocktail make-up when conducting these types of studies.

In this study, whole muscle and ground samples were equivalent in composition, bacterial load, and thermal history; hence, differences in *Salmonella* thermal resistance, were due to differences in the physical state of meat components in the sample. Perhaps the bacteria have greater opportunities for surface attachment within the tissue structure of the whole muscle samples, resulting in differential resistance. Also, because fat has a protective effect against thermal inactivation (Shipp and others 1992;, Ahmed and others 1995; Juneja and others 1997; Veeramuthu and others 1998; Smith and others 2001), homogeneous distribution of the fat in a ground sample, as opposed to tissue segregation in whole muscle products, may "dilute" the protection factor. For example, bacteria attached to or by intramuscular fat in whole muscle samples may benefit from this protective effect in a way that is not possible in the more homogenous ground product. Clearly, the data from this study cannot explain the fundamental mechanism for this observed difference in thermal resistance, and there is a need to further elucidate the mechanisms by which muscle structure/arrangement protects pathogens against thermal inactivation during cooking.

Conclusions

A substantial body of literature has documented the effect of product composition on thermal inactivation of microorganisms. This work illustrates that the physical arrangement of the components within a product, in addition to the composition, affects the thermal resistance of microorganisms. In this study, *Salmonella* was significantly more heat-resistant when present in whole muscle, as opposed to in ground beef products. Therefore, thermal process validations for meat products may need to consider the physical state (whole muscle versus ground) of the particular product being manufactured.

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