

Effect of Beef Product Physical Structure on *Salmonella* Thermal Inactivation

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ABSTRACT: Numerous studies have assessed thermal inactivation of *Salmonella* in beef. However, the impact of muscle structure has been considered only recently, with several studies reporting enhanced thermal resistance in whole-muscle as compared to ground meat. The functional relationship between meat product physical structure and *Salmonella* thermal resistance has not been reported; therefore, it is not known whether thermal resistance is affected by the degree of grinding (that is, size of resulting particles). The objective of this study was to evaluate the relationship between thermal resistance of *Salmonella* and degree of grinding (whole-muscle, coarsely ground, finely ground, and beef puree). Each of the 4 product types was irradiated to sterility and inoculated with a marinade containing an 8-serovar *Salmonella* cocktail to achieve approximately $10^{7.8}$ CFU/g. Samples (5 g each) were packed into sterile brass tubes, which were sealed, held at 60 °C in a water bath, and removed at 30 s intervals. Samples were then serially diluted and plated on Petrifilm™ aerobic count plates to enumerate surviving salmonellae. All samples had the same composition, thermal history, and initial *Salmonella* counts; therefore, differences in thermal resistance were due entirely to the degree of grinding. Overall, thermal resistance of *Salmonella* was highest ($P < 0.0001$) in whole-muscle ($D = 2.7$ min), but there were no differences among the 3 ground products ($D_{\text{mean}} = 1.2$ min). Therefore, it would be prudent for *Salmonella* thermal inactivation models to consider whether a product is whole-muscle or ground, but not necessarily the degree of grinding.

Practical Application: The results of this study suggest that thermal process validations for ready-to-eat meat products should also consider the structure of the product (which in this study was changed by the physical act of grinding). *Salmonella* was more resistant to heat in whole-muscle beef than in ground products; however, the degree of grinding did not affect the resistance.

Keywords: heat, lethality, meat, microbial modeling, *Salmonella*

Introduction

Whole-muscle meat is primarily surface-contaminated and therefore less likely than ground meat to contain bacterial pathogens, with more meat-related outbreaks traced back to the latter (Doyle and Mazzotta 2000). Although the interior of intact, whole-muscle products has long been assumed to be sterile (Elmossalami and Wassef 1971), contamination and survival of pathogens have been reported (Elmossalami and Wassef 1971; Gill and Penney 1982; Jay 2000). The few studies that have tested migration of *Salmonella* into whole-muscle products have found that the pathogen does migrate and survive inside the muscle (Elmossalami and Wassef 1971; Warsaw 2003; Velasquez 2006).

After meat products are contaminated, the thermal inactivation rate for *Salmonella* varies based on strain, product, and environmental factors (Doyle and Mazzotta 2000). *Salmonella* thermal inactivation is affected by total solid content (Blackburn and others 1997; Doyle and Mazzotta 2000), pH (Blackburn and others 1997; Doyle and Mazzotta 2000), water activity (Doyle and Mazzotta 2000; Carlson and others 2005), fat content (Smith and others 2001), some food additives (Doyle and Mazzotta 2000), and redox potential (Lihono and others 2001). Also, survival of *Salmonella* in meats depends on meat species (Ghazala and others 1995; Veeramuthu

and others 1998). The pathogen location or attachment site also plays a role; when attached to meat, *Salmonella* exhibits greater thermal resistance than when unattached or suspended in liquid media (Thomson and others 1979; Humphrey and others 1995; Doyle and Mazzotta 2000).

For all fully cooked, ready-to-eat meat products, *Salmonella* lethality is the basis for the USDA-FSIS performance standards, which require a 6.5- \log_{10} reduction in *Salmonella* spp. for cooked beef products (USFSIS 2001). However, commonly used tools to calculate process lethality do not account for all factors that affect pathogen survival (Guo and Marks 2005). One factor that has not been included in the existing models for calculating process lethality is the physical structure of the meat product (that is, the size of grind or size of resulting particles).

Previous studies have demonstrated that *Salmonella* has a higher thermal resistance in whole-muscle beef, pork, and turkey than in equivalent ground products (Orta-Ramirez and others 2005; Velasquez 2006; Tuntivanich and others 2008). However, those studies tested only whole-muscle and finely ground products, and did not explain the cause of the observed effects or model the relationship between product structure and *Salmonella* inactivation. Therefore, the objective of this study was to evaluate the relationship between thermal resistance of *Salmonella* and degree of grinding (whole-muscle, coarsely ground, finely ground, and beef puree).

Materials and Methods

The overall experimental design for this study consisted of preparing and inoculating 4 different types of product

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(whole-muscle, coarsely ground, finely ground, and pureed beef) from the same original lot of beef (Figure 1). The details of each step are described in the following sections.

Meat preparation

Beef chuck, shoulder clods (114C, according to NAMP 1997) were obtained from Packerland-Plainwell, Inc. (Plainwell, Mich., U.S.A.) 48 h after slaughter. Cubic pieces (approximately 280 g) containing the *triceps brachii long head* were removed from the shoulder clods, vacuum-packaged at the Michigan State Univ. (MSU) meat laboratory, and held at -29°C . All samples were transported on dry ice to CFC Logistics (Quakertown, Pa., U.S.A.), where they were irradiated (approximately 10 kGy average dose) to eliminate background flora. Efficacy of the irradiation treatment was verified by diluting the samples (1:5) in peptone water (Difco, Becton, Dickinson and Co., Sparks, Md., U.S.A.) and plating on Petrifilm™ aerobic count plates (3M, St. Paul, Minn., U.S.A.) for 48 h at 37°C .

Moisture and fat contents were determined in quadruplicate from 4 different beef shoulder clods using AOAC methods 950.46B and 991.36, respectively (AOAC 1996).

Whole-muscle. Whole-muscle cylinders (5.00 ± 0.05 g; approximately 6 to 8 cm long) were obtained from the irradiated beef muscle using a sterile coring device (1.27 cm diameter, G.R. Electrical Mfg. Co., Manhattan, Kans., U.S.A.)

Coarsely ground and finely ground beef. Irradiated beef was ground in a sterile grinder (Model 5126, Toledo Chopper, Toledo, Ohio, U.S.A.), by passing the beef sample twice through a plate with 16 or 6 mm holes to obtain coarsely ground and finely ground samples, respectively.

Beef puree. Irradiated beef was comminuted in a sterile grinder, passing 5 times through a plate with 6-mm dia holes as previously described, and then blended at puree speed for 1 min in a sterilized Osterizer blender (Model 6641, Oster[®], Mexico).

Bacterial cultures

As in previous *Salmonella* thermal inactivation studies by Orta-Ramirez and others (2005), the following 8 *Salmonella* serovars of moderate to high thermal resistance (Juneja and others 2001b), previously obtained from Dr. V.K. Juneja (USDA-ARS, Eastern Regional Research Center, Wyndmoor, Pa., U.S.A.), were used: *S. Montev-*

ideo FSIS 051 (beef isolate); *S. Thompson* FSIS 120 (chicken isolate); *S. Enteritidis* H3527 (chicken isolate, phage type 13A); *S. Enteritidis* H3502 (chicken isolate, phage type 4); *S. Hadar* MF60404 (turkey isolate); *S. Copenhagen* 8457 (pork isolate), *S. Typhimurium* DT104 H3380 (human isolate); and *S. Heidelberg* F5038BG1 (human isolate). Each strain was stored at -80°C in tryptic soy broth (TSB) (Difco) containing 20% glycerol. In preparation for use, the 8 serovars were each subjected to 2 transfers after 24-h incubations in TSB at 37°C .

Marinade preparation

A typical marinade used by the meat industry was prepared to contain 96% filtered and deionized water, 3.2% NaCl (J.T. Baker, Philipsburg, N.J., U.S.A.), and 0.8% potassium phosphate solution (Butcher and Packer Supply Co., Detroit, Mich., U.S.A.). After combining and totally dispersing the components, the marinade was poured into glass bottles (520 mL each), autoclaved (121°C for 20 min), and stored at room temperature.

Inoculation

Immediately before inoculating the beef samples, the *Salmonella* cocktail was prepared by combining equal volumes (9 mL) of each serovar in a centrifuge bottle. After centrifugation ($6000 \times g$, 20 min at 4°C), the pellet was resuspended in 520 mL of marinade to yield approximately 10^8 CFU/mL. The concentration of *Salmonella* in the marinade was verified by serial dilution in 0.1% peptone water, followed by plating in duplicate on Petrifilm aerobic count plates.

Several methods for inoculating the 4 different products were evaluated to determine the effects on initial counts and subsequent thermal resistance. The methods used in this study are described below for each of the 4 products.

Whole-muscle. Whole-muscle cylinders were immersed in the inoculated marinade for 20 min, which resulted in a marinade uptake of approximately 0.15 g marinade/g beef.

Coarsely ground and finely ground beef. Coarsely ground and finely ground samples were inoculated dropwise after the 1st grind (Figure 1), in the same proportion as the uptake of the whole-muscle samples (0.15 g marinade/g beef), and then passed through the grinder a 2nd time.

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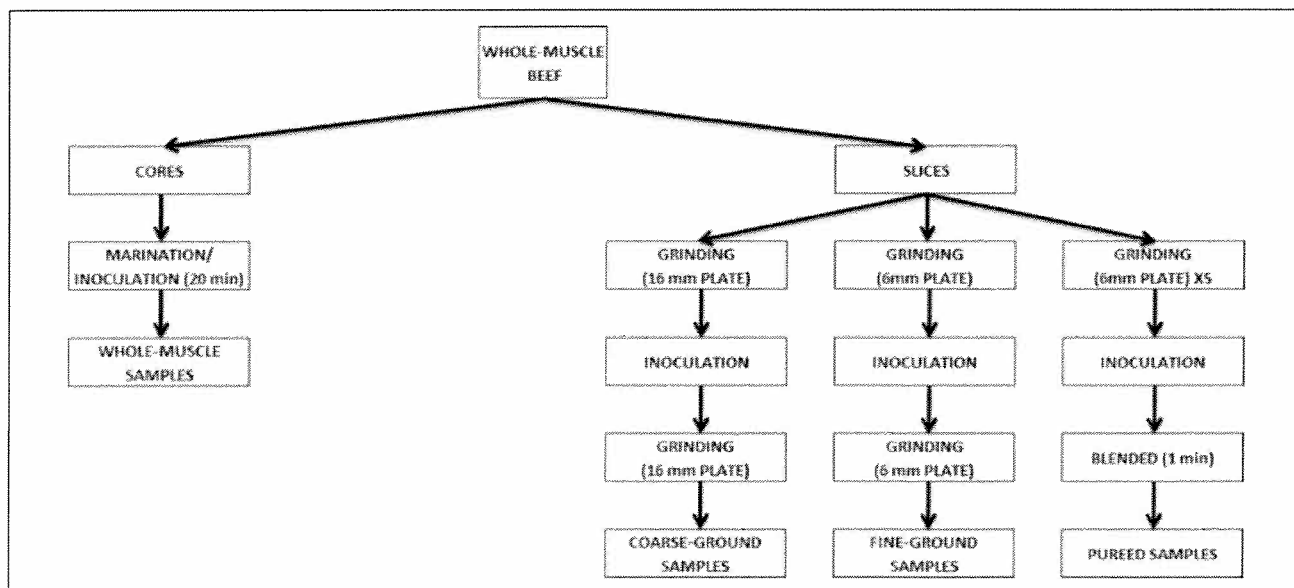


Figure 1 – Overall flow of experimental protocols for sample preparation and inoculation prior to thermal treatment.

Beef puree. The samples to become puree were inoculated dropwise (0.15 g marinade/g beef) after grinding 5 times through the 6 mm plate and before the blending step.

The moisture content of each product (whole-muscle, coarsely ground, finely ground, and beef puree) was also determined in duplicate after marination, using AOAC method 950.46B.

Thermal inactivation

Whole-muscle (5.00 ± 0.05 g), coarsely ground, finely ground, and pureed beef samples (each 5.00 ± 0.01 g) were aseptically packed into sterile brass tubes (12.7-mm dia, 10 cm long), which were sealed with sterile rubber stoppers wrapped with Teflon[®] tape, and held temporarily at approximately 4 °C. Samples were then heated isothermally in an agitated, 60.5 °C water bath (NESLAB Instruments Inc., Newington, N.H., U.S.A.) with the sample temperature monitored using a thermocouple probe (Type T, 1 mm, Omega Engineering, Stamford, Conn., U.S.A.) inserted into the center of the sample.

The 1st tube was removed from the water bath after the thermal come-up time, defined as the time when the sample attained the target temperature (60 °C). After removing the 1st sample, subsequent samples were removed every 30 s and placed in an ice bath. The whole-muscle sample treatments continued for 5 min ($n = 11$), and the coarsely ground, finely ground, and pureed sample treatments for 4.5 ($n = 10$), 3.5 ($n = 7$), and 5 ($n = 11$) min, respectively. Whole-muscle, coarsely ground, and finely ground samples were each tested in duplicate ($m = 2$), and the beef puree samples were tested in triplicate ($m = 3$), yielding 22, 20, 14, and 33 total observations for whole-muscle, coarsely ground, finely ground, and pureed samples, respectively. Surviving salmonellae in the chilled samples were enumerated within 30 min.

Salmonella enumeration

The chilled samples were diluted (1:10) in 0.1% peptone water, homogenized for 180 s in a masticator (Model 0410, IUL Instruments USA, Inc., Cincinnati, Ohio, U.S.A.), serially diluted in peptone water, and plated on Petrifilm aerobic count plates (48 h, 37 °C). Because the uninoculated, irradiated beef samples contained no detectable background microflora as determined from plating 1:5 dilutions on Petrifilm aerobic count plates, all colonies developing after incubation were counted as *Salmonella*.

Statistical analysis

Salmonella survivor curves at 60 °C were determined for all samples by plotting the logarithm of the survival ratio compared with time. The k values were calculated as the slope from linear regression of $\ln(N/N_0)$ compared with t for each replicate test series for a given product, where N/N_0 was the survival ratio, and t was the time (min). The D values for each sample were then calculated as $2.303/k$ (that is, the equivalent to $-1/\text{slope}$ for the linear regression of $\log_{10}(N/N_0)$ compared with time). The thermal come-up times, the initial counts after the come-up time, and the k values for each product type were compared using the Tukey-HSD multiple means test ($\alpha = 0.05$). All statistical analyses were conducted using JMP (v.7, SAS Inst. Inc., Cary, N.C., U.S.A.).

Results and Discussion

Proximate analysis

The raw beef contained $73.0 \pm 0.93\%$ moisture and $4.5 \pm 0.07\%$ fat. The marinated whole-muscle, coarsely ground, finely ground, and pureed samples contained $75.93 \pm 0.99\%$, $71.28 \pm 0.98\%$, $73.85 \pm 0.99\%$, and $75.01 \pm 0.01\%$ moisture, respectively.

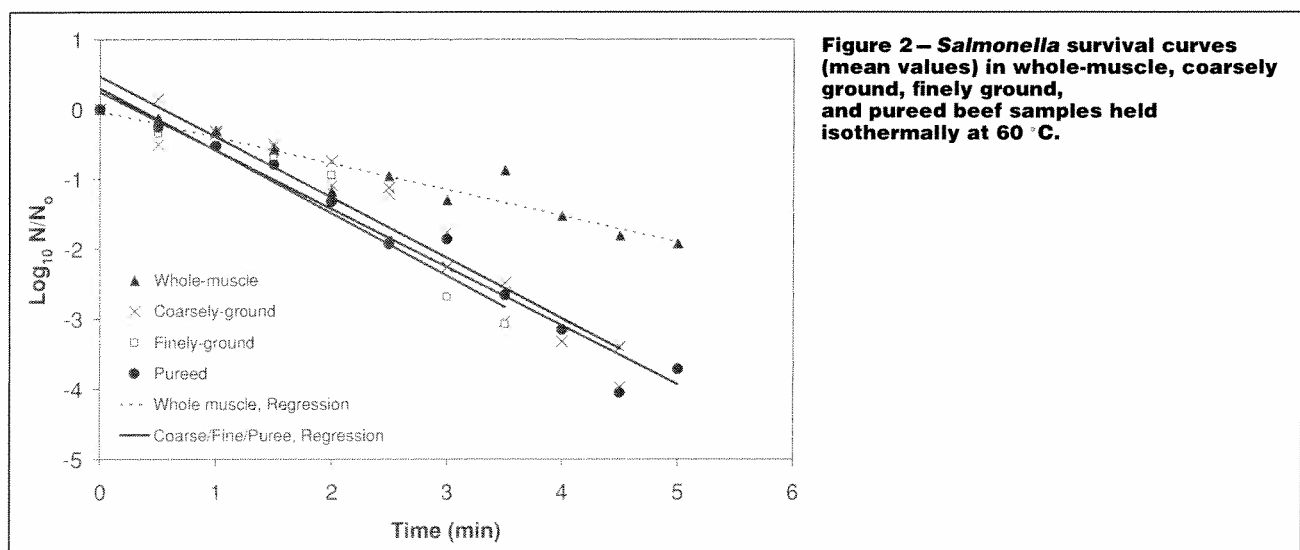
Inoculation of marinade and samples

The marinade was inoculated to obtain approximately 10^8 *Salmonella* CFU/mL. Before each experiment, the inoculated marinade was plated to determine the *Salmonella* concentration, which averaged $8.7 \pm 0.2 \log_{10}$ CFU/mL. The uptake/addition of 0.15 g of marinade per gram of beef yielded a target initial concentration of approximately $7.8 \log_{10}$ CFU/g of product.

Thermal inactivation results

The mean thermal come-up times for whole-muscle, coarsely ground, finely ground, and pureed beef were 2.8 ± 0.14 , 2.1 ± 0.40 , 2.2 ± 0.45 , and 2.5 ± 0.05 min, respectively, with no significant differences ($\alpha = 0.05$) among these values.

After the come-up time, *Salmonella* counts for the t_0 samples of whole-muscle, coarsely ground, finely ground, and pureed beef were 6.9 ± 0.22 , 7.6 ± 0.18 , 7.3 ± 0.01 , and $7.4 \pm 0.36 \log_{10}$ CFU/g, respectively, with no significant differences ($\alpha = 0.05$) among these values. At all subsequent sampling times, the overall standard deviation among replicate survivor counts (that is, replication error across all treatments) was $0.38 \log_{10}$ CFU/g.



Thermal resistance of *Salmonella* was higher ($P < 0.0001$) in whole-muscle than in coarsely ground, finely ground, and pureed samples (Figure 2, Table 1; Note: k values are reported for $\ln(N/N_0)$ compared with time, to be consistent with 1st-order kinetics, and Figure 2 is reported as $\log_{10}(N/N_0)$ compared with time for ease of interpretation by the reader). Based on a Tukey-HSD multiple means test ($\alpha = 0.05$), the rate of *Salmonella* inactivation in whole-muscle was lower ($P < 0.05$) than in the other 3 sample types, which all showed similar inactivation rates ($P > 0.05$).

All products came from the same original lot of beef and had the same thermal history and initial *Salmonella* counts; therefore, differences in thermal resistance were due to the degree of grinding (that is, meat structure or particle size). *Salmonella* exhibited greater heat resistance in whole-muscle compared to coarsely ground, finely ground, and pureed beef.

Even though the purpose of this study was not to understand the mechanism by which *Salmonella* exhibits more heat resistance in whole-muscle compared to the identical product when comminuted, some insights into this phenomenon can be gained from previously published findings.

Orta-Ramirez and others (2005) reported the same trend when comparing whole-muscle beef and ground beef, stating that the physical arrangement of the food components within the food matrix might cause a difference in bacterial thermal resistance. According to Doyle and Mazzotta (2000), the location of *Salmonella* in the food may also have an effect on the organism's thermal resistance.

Previous research has shown that the heat resistance of bacteria in meat increases with increasing fat content (Hansen and Riemann 1963; Stumbo 1973; Ahmed and others 1995; Juneja and others 2001a; Smith and others 2001). It is possible that bacterial attachment to segregated fat tissue in the whole-muscle leads to enhanced thermal protection. Such a protective effect might be lost in the ground and pureed products (of equivalent proximate composition), due to the homogenous distribution of the fat (Orta-Ramirez and others 2005).

The status of water in meat may also affect thermal resistance of *Salmonella*. Perhaps the chemical potential of water in whole-muscle is lower than in the ground product. Even if the moisture content is identical, some limited osmotic potential across muscle cell membranes might increase *Salmonella* thermal resistance. It has been shown that the thermal resistance of *Salmonella* is significantly greater when the water activity of meat is reduced (Carlson and others 2005).

Other researchers have suggested that *Salmonella* has a higher thermal resistance when attached to meat than when suspended in broth (Thomson and others 1979; Humphrey and others 1995; Murphy and others 1999). In muscle, bacteria may have a better opportunity to attach than in ground product. Therefore, salmonellae in ground and pureed beef may be suspended in the liquid component of the food, making the organism more susceptible to thermal inactivation.

Table 1 – First-order inactivation constant, k , and D values (mean \pm SD) at 60 °C, calculated by linear regression for *Salmonella* in whole-muscle, coarsely ground, finely ground, and pureed beef.

Beef structure	$k_{60^\circ\text{C}}$ (per min)	$D_{60^\circ\text{C}}$ value (min)	R^2
Whole-muscle	0.87 \pm 0.20	2.73 \pm 0.64	0.73
Coarsely ground	1.99 \pm 0.10	1.16 \pm 0.06	0.92
Finely ground	2.06 \pm 0.29	1.12 \pm 0.20	0.90
Puree	1.87 \pm 0.11	1.26 \pm 0.07	0.91

The results of this study suggest that thermal process validations should also consider the structure of beef (which in this study was due to the physical act of grinding), which appears to affect the inactivation of *Salmonella*.

Conclusions

Previous studies have shown the importance of product composition on thermal resistance of *Salmonella*, but the distribution of the components has not been considered. In this study, the physical structure due to grinding of beef products influenced *Salmonella* thermal resistance. However, no significant difference in thermal resistance was seen between coarsely ground, finely ground, and pureed samples. Therefore, it would be prudent for *Salmonella* thermal inactivation models to consider whether a product is whole-muscle or ground, but not necessarily the degree of grinding.

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