Irradiation Inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in Low- and High-fat, Frozen and Refrigerated Ground Beef

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**ABSTRACT**

The influence of two levels of fat (11.1 to 13.9% [low-fat] and 27.1 to 27.9% [high-fat]) and temperature (frozen [–17 to –15°C] and refrigerated [3 to 5°C]) on gamma irradiation (60Co) inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in raw ground beef patties was investigated. Ground beef patties inoculated with stationary growth phase cells of five-strain mixtures of *L. monocytogenes* or *S. aureus* were treated with seven mean gamma irradiation doses up to 2.062 or 2.147 kGy, respectively. D_{570} values ranged from 0.507 to 0.610 kGy and 0.435 to 0.453 kGy for *L. monocytogenes* and *S. aureus*, respectively. Neither the fat content of beef nor the temperature during irradiation treatment influenced inactivation rates of the two pathogens. Regression coefficients were high for all treatment conditions, the lowest being 0.984 for *L. monocytogenes* and 0.990 for *S. aureus* in high-fat frozen beef. Based on the highest D_{570} value obtained, a dose of 2.50 kGy would theoretically kill 4.10 log_{10} *L. monocytogenes* and 5.12 log_{10} *S. aureus* per gram of ground beef. The fact that this investigation was done under commercial processing and irradiation treatment conditions, to the extent possible, makes the significance of the results more meaningful to the beef industry.

Key Words: Gamma irradiation, bacterial pathogens, ground beef.

**Listeria monocytogenes** can be isolated from vegetation (3), animals and a wide range of foods originating from these sources (13). Its ability to cause human illness and death, particularly to immunocompromised individuals and pregnant women, is of public health concern. Likewise, *S. aureus* is likewise not uncommon in the environment (10).

Gamma irradiation eliminates or greatly reduces populations of pathogenic and spoilage microorganisms in ground beef (1,12,17) and beef products (8,20). Survival of microbial cells upon treatment with irradiation depends on intrinsic factors (6) as well as extrinsic parameters such as temperature and the chemical composition of the environment (5,11). The investigation reported here was undertaken to determine if fat content of ground beef and temperature during gamma irradiation treatment have an effect on survival of *L. monocytogenes* and *S. aureus*.

**MATERIALS AND METHODS**

**Ground beef.**

Raw ground beef (2.38 mm [3/32 in.] grind) containing two levels of fat (low and high) was obtained from a commercial beef processor and stored at –18°C at the Center for Food Safety and Quality Enhancement until used, which did not exceed 5 weeks. Prior to inoculation with a five-strain mixture of *L. monocytogenes* or *S. aureus*, ground beef was thawed at 1 to 3°C over a 3-day period.

**Organisms and preparation of inocula.**

Five strains of *L. monocytogenes* and five strains of *S. aureus* were studied. Strains of *L. monocytogenes* were Scott A (human isolate), LCDC 81-861 (cabbage isolate), V7 (cow milk isolate), Brie-1 (cheese isolate) and 101M (cow milk isolate). *Staphylococcus aureus* strains, the type of enterotoxin produced and the source of isolation were FRI 100 (A, cake), FRI 472 (D, turkey salad), FRI 576 (A, source not known), FRI 798 (D, cake) and FRI 1068 (C, sheep milk). These strains were kindly supplied by Dr. Amy Wong, Food Research Institute, University of Wisconsin, Madison, WI.

Each strain of *L. monocytogenes* was cultured individually in tryptic phosphate broth (TPB, pH 7.3) (Difco Laboratories, Detroit, MI) at 33°C. *Staphylococcus aureus* strains were cultured in tryptic soy broth (TSB, 7.3) (Difco) at 37°C. Three consecutive 24-h transfers were made using loop inocula. The first two transfers were made in 10 ml of TPB or TSB (16 × 150 mm screw-cap test tubes) and the third transfer was made in 50 ml of TPB or TSB in 250-ml Erlenmeyer flasks.

**Determination of growth curves.**

Growth curves were determined for *L. monocytogenes* and *S. aureus* cultured in TPB and TSB, respectively, as described above. Samples (1.0 ml) were withdrawn from *L. monocytogenes* cultures at 4-h intervals over a 32-h incubation period, serially (1:10) diluted in 0.1% peptone (pH 7.0) and surface plated (0.1 ml) in duplicate on tryptic phosphate agar (TPB containing 1.5% agar). Colonies were counted after incubating plates for 24 h at 30°C. Growth curves for *S. aureus* cultured in TSB were similarly determined by plating diluted samples on TSA (TSB containing 1.5% agar). Colonies were counted after incubating plates for 48 h at 37°C.
**Preparation of inocula.**

Cultures incubated without agitation for 29 to 31 h (stationary growth phase) were used to prepare inocula for ground beef. Cultures were sedimented by centrifugation (3,000 g 21°C, 20 min) and resuspended in sterile 0.1% peptone. Suspensions of each bacterium containing equal populations were then combined to result in 90 ml of a five-strain mixture of L. monocytogenes and 90 ml of a five-strain mixture of S. aureus. The suspension of L. monocytogenes cells was further diluted by 100-fold in 0.1% peptone. Resulting suspensions were used to inoculate ground beef within 20 min of preparation.

**Inoculation and preparation of beef patties.**

Thawed (2 to 4°C) low- and high-fat ground beef (3,100 g) was inoculated with 40 ml of either L. monocytogenes or S. aureus cell suspension. After thorough hand mixing for 5 to 8 min, patties (100 g) (9.5 cm diameter, 1.2 cm thick) were prepared using a hamburger press (M. E. Heuck Co., Cincinnati, OH). Each patty was placed in a polyethylene stomacher bag (Seward Medical, London, UK), sealed with a Thermal Impulse Heat Sealer (Vertrad Corp., New York, NY) and labeled. Patties were placed in a freezer (-16°C) or a refrigerator (4°C) within 40 min of inoculation with test pathogens.

**Handling of inoculated patties before irradiation.**

After 20 to 22 h at -16 or 4°C, inoculated ground beef patties were sealed in polystyrene boxes which were, in turn, placed in separate freezer chests (Gott Corp., Winfield, KS), one for frozen patties and one for refrigerated patties. Frozen (-18°C) Polar Packs (Mid-Lands Chemical Corp., Omaha, NE) were placed around the polystyrene boxes before sealing the ice chests. Ground beef patties were then transported to Vindicator, Inc., Mulberry, FL and placed in a freezer (-17 to -15°C) or refrigerator (3 to 5°C). The time elapsed between removal of patties from frozen or refrigerated storage at the Center of Food Safety and Quality Enhancement and arrival at Vindicator was 5 to 6 h during which the temperature of the patties did not increase more than 3°C.

**Irradiation treatment.**

Inoculated ground beef patties were treated with gamma (60Co) irradiation 16 to 20 h after arrival at Vindicator. Eight patties (two low-fat frozen, two low-fat refrigerated, two high-fat frozen and two high-fat refrigerated) were strategically placed in a cardboard box (21.5 cm x 21.5 cm x 4.0 cm) and covered with a lid (Fig. 1). A sheet of cardboard (21.2 x 21.2 cm) was placed between the two layers of patties. gammachrome YR dosimeters (Harwell Laboratory, Atomic Energy Authority, UK) were attached at a central position on the external side of the lids of two boxes (numbers 1 and 2) and the external side (bottom) of a third box (number 3) (Fig. 1c). Each box constituted one replicate and separate dose treatments were done for each pathogen. The three stacked boxes (Fig. 1d) were centered on the top of a 10-cm thick polystyrene block placed in a turntable (2.3 rpm) approximately 220 cm from the 60Co irradiation source. Patties were exposed to target doses of 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 kGy. Actual mean doses applied were 0, 0.400, 0.612, 0.892, 1.186, 1.622 and 2.062 kGy for L. monocytogenes and 0, 0.404, 0.644, 0.930, 1.330, 2.031 and 2.147 kGy for S. aureus. The temperature of patties increased less than 4°C during irradiation treatment.

All irradiation treatments were done using a 60Co Gamma Beam 650 Facility (Nordion International, Inc., Kanata, Ontario, Canada). The dosimetry system and equipment for measuring absorbed dose in beef patties were calibrated according to standards established by the National Institute of Standards and Technology (NIST).

**Microbiological analysis.**

Frozen patties were thawed at 21 to 23°C (1 to 2 h) in preparation for microbiological analysis. Refrigerated patties were subjected to microbiological analysis without any adjustment of temperature.

Sterile 0.1% peptone (200 ml) was combined with each ground beef patty (100 g) inoculated with L. monocytogenes or S. aureus. Uninoculated (control) patties were also analyzed for the presence of these pathogens as well as for total mesophilic aerobic microorganisms. Ground beef and diluent were pummelled with a stomacher at medium speed for 1 min, serially diluted (1:10) in 0.1% peptone and surface plated (0.1 ml) in duplicate on appropriate enumeration media. Populations of each strain of L. monocytogenes and S. aureus in 29 to 31 h cultures used to prepare inocula and in five-strain mixtures of each pathogen immediately before inoculation of ground beef were also determined.

A modified Oxford medium was used to enumerate L. monocytogenes. The medium contained (L1 deionized water) 55.5 g of Listeria selective agar base (Oxoid CM856, Unipath-Oxoid U.S., Columbia, MD), 0.01 g of colistin methanesulfate (Sigma Chemical Co., St. Louis, MO), 0.2 g of Ceftazidime pentahydrate (Glaxo Group Research Ltd., Ware, Hertfordshire, UK) and 5.0 g of agar (Difco). All ingredients except Ceftazidime pentahydrate were combined and sterilized by heating at 121°C for 12 min. Ceftazidime was added to molten (50°C) agar before pouring the medium into 100-mm diameter petri dishes. Presumptive colonies of L. monocytogenes that developed on modified Oxford medium within 48 h at 30°C were counted. Confirmation was done by microscopic exami-
nation and by using appropriate biochemical tests (7).

Diluted samples (0.1 ml) were surface plated on Baird Parker agar to enumerate viable cells of S. aureus. The medium consisted of 63 g of Baird Parker agar base (Difco), 50 ml of EY tellurite enrichment (Difco) and 950 ml of deionized water. The agar base and water were sterilized by heating at 121°C for 15 min, then cooled to 47 to 50°C before adding EY tellurite enrichment and pouring into 100-mm diameter petri dishes. Presumptive colonies of S. aureus were counted after incubating plates for 48 h at 37°C. Random colonies were examined microscopically and analyzed for coagulase activity and other biochemical characteristics to confirm identity (10).

Diluted samples (0.1 ml) of uninoculated ground beef were surface-plated on plate count agar (Difco) to determine populations of mesophilic aerobic microorganisms. Plates were incubated for 48 h at 30°C before counting colonies.

### Chemical analysis.

Fat and moisture content of ground beef was measured using a CEM Fat and Moisture Analyzer (CEM Corp., Matthews, NC). Protein content was determined by the Kjeldahl method (2).

### Statistical analysis.

Each treatment combination, e.g., pathogen, fat level, temperature during irradiation and irradiation dose, was done in triplicate. Two patties subjected to each treatment combination were analyzed. Duplicate samples of each dilution were plated on recovery media. The number of survivors of L. monocytogenes and S. aureus after gamma irradiation treatment, expressed as \( \log_{10} \) CFU g\(^{-1}\) of beef, was plotted against irradiation dose. A regression line was fitted to sets of data using the REG procedure of the Statistical Analysis System (SAS) statistical package (14). Regression lines were fitted to data points that contributed to tailing or as straight-line portions of inactivation curves. Regression coefficients, slopes and 95% confidence limits were determined for all regression lines.

The irradiation resistance of each pathogen subjected to each treatment combination was assessed by calculating \( D_{10} \) values, defined as the negative reciprocal of the slope. For each pathogen, \( D_{10} \) values obtained from all treatment combinations were compared using a General Linear Model (GLM) (14). Mean \( D_{10} \) values were calculated and differences between means were determined using the Duncan’s Multiple Range Test. To determine if \( D_{10} \) values were affected by omission of data points responsible for the tailing, an Analysis of Variance (ANOVA) of the values was conducted.

### RESULTS AND DISCUSSION

#### Chemical and microbiological analyses of uninoculated beef.

The fat, protein and moisture contents of ground beef used in experiments are listed in Table 1. The amount of fat ranged from 11.1 to 13.9% in low-fat beef and 27.1 to 27.7% in high-fat beef.

Populations of aerobic, mesophilic microorganisms in uninoculated ground beef are listed in Table 2. Populations ranged from 3.78 to 7.23 \( \log_{10} \) CFU g\(^{-1}\). Coagulase-positive S. aureus (120 to 270 g\(^{-1}\)) was detected in uninoculated ground beef used for studies involving this pathogen. None of the microflora in uninoculated beef interfered with detecting or counting colonies of L. monocytogenes or S. aureus that developed from samples inoculated with these pathogens.

### TABLE 2. Populations of L. monocytogenes and S. aureus in inocula, aerobic microorganisms in uninoculated ground beef and pathogens in inoculated ground beef.

<table>
<thead>
<tr>
<th>Test pathogen</th>
<th>Inoculum (5-strain mixture, ( \log_{10} ) CFU ml(^{-1} ))</th>
<th>Ground beef (^{1} )</th>
<th>Pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \log_{10} ) CFU g(^{-1} )</td>
<td>low fat</td>
<td>high fat</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>7.19</td>
<td>4.85</td>
<td>5.43</td>
</tr>
<tr>
<td>S. aureus</td>
<td>9.25</td>
<td>3.92</td>
<td>3.78</td>
</tr>
</tbody>
</table>

\(^{1}\) Populations of aerobic mesophilic microorganisms in ground beef were determined before inoculation with pathogens. Populations of pathogens were determined 10 min after inoculating 3,100 g of ground beef with 40 ml of inoculum.

### Growth curves.

The phase of growth of bacterial cells can affect their sensitivity to gamma irradiation. Stationary phase S. aureus cells in mechanically deboned chicken meat are more resistant to irradiation than are mid-logarithmic growth phase cells (18). The irradiation resistance of Escherichia coli is highest during the lag phase of growth, decreases during the logarithmic phase and increases again during the stationary phase of growth (16). It was, therefore, desirable to know the age or physiological state of L. monocytogenes and S. aureus cells used to inoculate ground beef. Growth curves for the two pathogens are shown in Fig. 2. Listeria monocytogenes reached a stationary phase of growth within 16 h, whereas S. aureus entered a stationary phase after 12 h of incubation. Cultures (29 to 31 h) well into their stationary phase of growth were used as inocula for ground beef.

Populations of L. monocytogenes and S. aureus in inocula and in ground beef immediately after inoculation are listed in Table 2. The L. monocytogenes inoculum contained approximately 100-fold less cells than the S. aureus inoculum. This difference was not because populations of the two pathogens in 29- to 31-h cultures differed but rather because the five-strain mixture of L. monocytogenes was diluted 100-fold to prepare the inoculum. The five-strain mixture of S. aureus was not diluted before being used to inoculate ground beef. Slight decreases in populations of viable cells would be expected to occur during the holding time between inoculation and irradiation (43 to 45 h) and between irradiation and analysis (24 to 25 h). To minimize any effect on cell viability that might be
caused by transport or handling conditions, control patties (inoculated but not irradiated) were subjected to the same conditions as patties treated with gamma irradiation.

Irradiation inactivation curves.

Irradiation inactivation curves were plotted for *L. monocytogenes* (Fig. 3) and *S. aureus* (Fig. 4) subjected to all combinations of test parameters, e.g., low- and high-fat beef at -17 to -15°C and 3 to 5°C during treatment. Regression coefficients for all treatments were high, the lowest (*r* = 0.984) being obtained for *L. monocytogenes* in high-fat, frozen beef. Tailing of inactivation curves for *L. monocytogenes* was observed, whereas inactivation curves for *S. aureus* were characterized by shoulders, with the exception of the curve obtained for cells treated in high-fat frozen beef. The shaded areas in these figures represent 95% confidence limits for curves without tailing or shoulders. Huhtanen, Jenkins and Thayer (9) observed a tailing of inactivation curves for *L. monocytogenes* irradiated in mechanically deboned chicken meat. They studied the possibility of cells becoming resistant to irradiation by repeatedly selecting colonies recovered from chicken receiving the highest irradiation dose, e.g., from the tail portion of curves, and concluded that non-linearity of curves is probably not a result of a permanent change in irradiation sensitivity of cells within a population. The shape of irradiation inactivation curves for bacteria is influenced by the relative resistance of cells to increased dose (15). Convex, linear (exponential) or concave curves can result from cells in different stages of growth. Thus, the tailing and shouldering of curves observed for *L. monocytogenes* and *S. aureus* in our study may have been caused by the presence...
of cells having slightly different stages of physiological development.

$D_{10}$ values (kGy) for $L. monocytogenes$ and $S. aureus$ in low- and high-fat frozen or refrigerated ground beef are listed in Table 3. Values were calculated for inactivation curves with and without tailing and shoulders. $L. monocytogenes$ was more resistant to irradiation than was $S. aureus$. Statistical analysis of data for each pathogen revealed that there were no significant differences ($P<0.05$) in $D_{10}$ values obtained from the two types of curves. Furthermore, neither the level of fat nor the treatment temperature significantly influenced $D_{10}$ values. The lack of influence of fat content on rates of irradiation inactivation of bacterial pathogens has been observed by other researchers. Differences in fat content of mechanically deboned chicken and ground beef have been reported to not significantly alter irradiation resistance of $E. coli$ O157:H7 (18). We have also observed that fat content of ground beef does not significantly influence $D_{10}$ values of $E. coli$ O157:H7, $Campylobacter jejuni$ and $Salmonella$ (4).

$L. monocytogenes$ and $S. aureus$ were not sensitive to frozen or refrigeration temperatures when mixed with ground beef nor was their sensitivity to irradiation affected by temperature. This observation is not in agreement with those made by other researchers on pathogenic bacteria, including $S. aureus$. Thayer and Boyd (18) reported that the temperature (within a range of -10 to +20°C) at which mechanically deboned chicken meat was irradiated affects the destruction $S. aureus$. We have observed $C. jejuni$ to be more resistant to irradiation at -17 to -15°C compared to treatment at 3 to 5°C in ground beef (4). Irradiation resistance of $E. coli$ O157:H7 was unaffected by treatment at these temperatures. Thayer and Boyd (19), on the other hand, reported that $E. coli$ O157:H7 in chicken meat was more resistant to irradiation at -5°C than at +5°C. The effect of temperature on $D_{10}$ values of bacterial cells is influenced by numerous intrinsic and extrinsic factors. It does seem certain that an increase in temperature not exceeding that imposing thermal stress during irradiation treatment does not increase $D_{10}$ values.

Based on the highest $D_{10}$ values obtained for $L. monocytogenes$ and $S. aureus$ (Table 3), populations of each pathogen that would theoretically be killed in ground beef subjected to gamma irradiation doses of 0.5, 1.0, 1.5, 2.0 and 2.5 kGy were calculated (Table 4). An applied dose of 2.5 kGy would be sufficient to kill 4.1 $log_{10}$ of $L. monocytogenes$ and 5.1 $log_{10}$ of $S. aureus$ g⁻¹ of ground beef. Since such populations are considerably greater than those occasionally found naturally in contaminated beef, application of 2.5 kGy would result in a high probability of completely inactivating these pathogens. The fact that this investigation was done under commercial processing and irradiation treatment conditions, to the extent possible, makes the significance of the results more meaningful to the beef industry.

### ACKNOWLEDGMENTS

We thank the American Meat Institute, Washington, DC for financially supporting this investigation. Appreciation is also expressed to Vindicator, Inc., Mulberry, FL, for irradiating ground beef and to Brenda Nail, Kimberly Hortz, Usama Abdul-Raouf and Jerry Davis for their technical assistance at the Center for Food Safety and Quality Enhancement.

### REFERENCES


### TABLE 3. $D_{10}$ values (kGy) for $L. monocytogenes$ and $S. aureus$ as affected by fat content of ground beef and treatment temperature.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Frozen</th>
<th>Refrigerated</th>
<th>Frozen</th>
<th>Refrigerated</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L. monocytogenes$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with tailing</td>
<td>0.610  (.045)²</td>
<td>0.589 (.063)</td>
<td>0.575  (.030)</td>
<td>0.574 (.039)</td>
</tr>
<tr>
<td>without tailing</td>
<td>0.558  (.068)</td>
<td>0.578 (.043)</td>
<td>0.524  (.029)</td>
<td>0.507 (.054)</td>
</tr>
<tr>
<td>$S. aureus¹$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with shoulder/tail</td>
<td>0.451  (.038)</td>
<td>0.453 (.035)</td>
<td>0.435  (.047)</td>
<td>0.448 (.022)</td>
</tr>
<tr>
<td>without shoulder/tail</td>
<td>0.443  (.034)</td>
<td>0.437 (.029)</td>
<td>0.448  (.056)</td>
<td>0.443 (.028)</td>
</tr>
</tbody>
</table>

1. Inactivation curves in low-fat ground beef and in high-fat, refrigerated beef were characterized by slight shoulders; the inactivation curve in high-fat, frozen beef exhibited slight tailing.
2. Values in parentheses indicate ± standard deviation.

### TABLE 4. Populations of $L. monocytogenes$ and $S. aureus$ that would theoretically be killed in ground beef by treatment with 0.5, 1.0, 1.5, 2.0 and 2.5 kGy of gamma irradiation.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>0.5 kGy</th>
<th>1.0 kGy</th>
<th>1.5 kGy</th>
<th>2.0 kGy</th>
<th>2.5 kGy</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L. monocytogenes$</td>
<td>0.82</td>
<td>1.64</td>
<td>2.46</td>
<td>3.28</td>
<td>4.10</td>
</tr>
<tr>
<td>$S. aureus$</td>
<td>1.10</td>
<td>2.21</td>
<td>3.11</td>
<td>4.42</td>
<td>5.12</td>
</tr>
</tbody>
</table>
attached bacteria in a milk biofilm. J. Food Prot. 56:34-41.