Reduction and Survival of *Listeria monocytogenes* in Ready-to-Eat Meats after Irradiation

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ABSTRACT

A five-strain *Listeria monocytogenes* culture was inoculated onto six different types of ready-to-eat (RTE) meats (frankfurters, ham, roast beef, bologna, smoked turkey with lactate, and smoked turkey without lactate). The meats were vacuum packed and stored at 4°C for 24 h prior to irradiation. Populations of *L. monocytogenes* were recovered by surface plating on nonselective and selective media. The margins of safety studied include 3-log (3D) and 5-log (5D) reduction of pathogenic bacteria to achieve an optimal level of reduction while retaining organoleptic qualities of the meats. A 3-log reduction of *L. monocytogenes* was obtained at 1.5 kGy when nonselective plating medium was used. The dosages for 3-log reduction were 1.5 kGy for bologna, roast beef, and both types of turkey and 2.0 kGy for frankfurters and ham on the basis of use of selective medium. The $D_{10}$-values ranged from 0.42 to 0.44 kGy. A 5-log reduction of *L. monocytogenes* was obtained at 2.5 kGy with nonselective medium. With selective medium, the dosages were 2.5 kGy for bologna, roast beef, and both types of turkey and 3.0 kGy for frankfurters and ham. Survival of *L. monocytogenes* in the same RTE meat types after irradiation was also studied. Meats were inoculated with 5 log *L. monocytogenes* per g and irradiated at doses of 2.0 and 4.0 kGy. Recovery of the surviving organisms was observed during storage at temperatures of 4 and 10°C for 12 weeks. Preliminary results showed no growth in meats irradiated at 4.0 kGy. Survivors were observed for irradiated meats at 2.0 kGy stored at 10°C after the second week. No growth was observed in samples irradiated at 2.0 kGy stored at 4°C until the fifth week.

*Listeria monocytogenes* is considered a foodborne pathogen of great public health significance (3, 5, 16). *L. monocytogenes* has been incriminated in numerous foodborne illness outbreaks associated with ready-to-eat (RTE) meats. A significant outbreak occurred with frankfurters in 1998 and 1999, which resulted in 21 fatalities and approximately 100 reported cases of listeriosis (1). In 2002, another notable outbreak occurred in the northeastern United States, resulting in 10 deaths from the consumption of sliced turkey deli meat (2, 4). The U.S. Department of Agriculture (USDA) established a “zero tolerance” policy for *L. monocytogenes* in RTE processed meats, and this bacterium is considered to be a postprocessing contaminant (7, 9, 17). *Listeria* is well known for its resistance to several environmental stresses, such as refrigeration or freezing temperatures, heat, low pH, desiccation, and high salt and nitrite concentrations (12, 18). Of particular interest is its ability to grow at refrigeration temperatures and in environments with increased salt concentrations, which depict conditions commonly associated with RTE meats (14). RTE meats, by definition, are often consumed without a final thermal process by the consumers (8).

Many technologies have been developed to curb this problem of postprocessing contamination. One such method is use of low-dose irradiation (6, 11). A study group from the World Health Organization (WHO) concluded that food irradiated to any dose “appropriate to achieve the intended technological objective” is both safe to consume and nutritionally adequate (19). The microbiological objectives are to improve safety, reduce initial population, and extend shelf life. Decimal reduction values, or $D_{10}$-values, estimate the radiation dose (in kGy) needed to reduce bacterial numbers by 90%, or a 1-log reduction (13, 14). *L. monocytogenes* is fairly susceptible to irradiation. Various $D_{10}$-values have been reported because they are dependent on strain, substrate, irradiation type, and plating medium (12, 15). However, only a few published investigations have described the effects of irradiation on processed meats. High-dose irradiation has a negative effect on the organoleptic qualities of the meat (10, 14). Therefore, an optimal dose is required for a margin of safety while preserving the quality of meat. Low-dose gamma irradiation (<5 kGy) was speculated to eliminate pathogens while maintaining sensory qualities of frankfurters (14). With the lower doses, there might be a chance for *L. monocytogenes* to survive and grow. In order to look at this issue further, an experiment was conducted to study the survival and growth of this pathogen on selected RTE meats stored at refrigeration temperatures.

This investigation was conducted (i) to determine the irradiation doses required for a 3- and 5-log reduction of *L. monocytogenes* on selected RTE meats (frankfurters, sliced bologna, sliced chopped ham, sliced roast beef, sliced smoked turkey with lactate, and sliced smoked turkey without lactate), (ii) to determine the $D_{10}$-values for *L. monocytogenes* with the use of an electron beam source on the...
same RTE processed meats indicated, and (iii) to study the postirradiation survival and growth of *L. monocytogenes* in RTE meats (frankfurters, bologna, turkey ham, and roast beef). Two varieties of smoked turkey were used, with or without lactate, which is known to be an antilisterial processing aid. A comparison was done to observe whether a difference in irradiation dose is required to reduce the number of cells.

**MATERIALS AND METHODS**

**Culture conditions.** A five-strain culture of *L. monocytogenes* [Scott A (FSRL culture collection), 12a H7764, 4b H7969, 4b H7962, and OB90393] was used in this experiment. Each strain was grown individually in 10 ml tryptic soy broth containing 0.6% yeast extract (TSBYE; Becton Dickinson and Company, Sparks, Md.) for 24 h at 37°C. The strains were then combined by adding 1 ml of each to 500 ml of TSBYE and incubating at 37°C (100 rpm) with agitation for 14 h. These cells were harvested, washed twice, and suspended into 50 ml of Butterfield’s phosphate solution made from potassium phosphate monobasic (Fisher Scientific, Fair Lawn, N.J.), resulting in an inoculation cocktail of approximately $10^{10}$ cells per ml. The experiment was independently replicated three times.

**Preparation of RTE meats.** Background flora of *L. monocytogenes* was tested and found to be negligible (data not shown) on the selected commercially available pieces/slices of meats (frankfurters, chopped ham, bologna, turkey ham, roast beef, smoked turkey with lactate, and smoked turkey without lactate).

In order to determine the $D_{10}$-values, these meats were individually transferred to sterile stomacher bags with filters and inoculated with 1 ml of the *L. monocytogenes* cocktail. These bags were vacuum packed and stored at 4°C for 24 h prior to irradiation at the appropriate doses. For the survival and growth study, 25 g of the meat samples were weighed into sterile stomacher bags and then surface inoculated with 1 ml of a previously diluted solution containing *L. monocytogenes* at approximately $10^5$ CFU/g. Samples were then vacuum packed and stored at 4°C for 24 h prior to irradiation. Nonirradiated samples were used as controls.

**Irradiation.** Irradiation was carried out at the Iowa State University Linear Accelerator Facility with an electron beam source. The accelerator is a MeV Circe III linear electron accelerator (Thomson-CSF LINAC Parc Technologique—Geminii II, Saint Aubin, France). The accelerator was operated at a power level of 10 kilowatts (kw) with a beam energy of 10 million electron volts (MeV). Dosimetry was conducted with alanine pellets and a Brucker electron paramagnetic spin resonance analyzer (Brucker BioSpin, Rheinsetten/Karlsruhe, Germany). Doses selected for the irradiation reduction and the $D_{10}$-value experiments ranged from 0 to 4 kGy with 0.5 increments. For the study of the survival and growth of *L. monocytogenes* after irradiation, the product doses selected were 2 and 4 kGy, and these meats were stored at 4 and 10°C for 12 weeks.

**Sampling and enumeration.** For the irradiation reduction and $D_{10}$-value experiments, 20 ml of Butterfield’s phosphate solution was added and meat samples were homogenized in the Stomacher 400 Lab Blender (Tekmar Company, Cincinnati, Ohio).
TABLE 2. *L. monocytogenes* (log CFU/ml) recovered from meats irradiated at specific doses, providing estimated doses required for specific reductions on the basis of the selective medium modified Oxford agar

<table>
<thead>
<tr>
<th>Dose (kGy)</th>
<th>Frankfurter</th>
<th>Bologna</th>
<th>Ham</th>
<th>Roast beef</th>
<th>Smoked turkey with lactate</th>
<th>Smoked turkey without lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.84 ± 0.96</td>
<td>9.32 ± 0.06</td>
<td>8.49 ± 0.50</td>
<td>8.99 ± 0.25</td>
<td>9.85 ± 0.04</td>
<td>9.88 ± 0.06</td>
</tr>
<tr>
<td>0.5</td>
<td>7.82 ± 0.82</td>
<td>7.47 ± 0.17</td>
<td>7.40 ± 0.43</td>
<td>7.52 ± 0.28</td>
<td>8.50 ± 0.08</td>
<td>8.04 ± 0.13</td>
</tr>
<tr>
<td>1</td>
<td>7.15 ± 0.70</td>
<td>6.94 ± 0.15</td>
<td>6.85 ± 0.26</td>
<td>6.38 ± 0.18</td>
<td>7.89 ± 0.06</td>
<td>7.10 ± 0.05</td>
</tr>
<tr>
<td>1.5</td>
<td>6.02 ± 0.57</td>
<td>6.33 ± 0.13</td>
<td>5.91 ± 0.05</td>
<td>5.36 ± 0.52</td>
<td>6.77 ± 0.05</td>
<td>5.98 ± 0.06</td>
</tr>
<tr>
<td>2</td>
<td>5.57 ± 0.87</td>
<td>5.55 ± 0.23</td>
<td>5.54 ± 0.15</td>
<td>4.96 ± 0.26</td>
<td>5.64 ± 0.03</td>
<td>4.99 ± 0.05</td>
</tr>
<tr>
<td>2.5</td>
<td>4.62 ± 0.58</td>
<td>4.28 ± 0.10</td>
<td>4.21 ± 0.12</td>
<td>4.01 ± 0.67</td>
<td>4.36 ± 0.07</td>
<td>4.72 ± 0.06</td>
</tr>
<tr>
<td>3</td>
<td>3.76 ± 0.47</td>
<td>3.72 ± 0.12</td>
<td>3.40 ± 0.19</td>
<td>2.70 ± 0.27</td>
<td>3.76 ± 0.07</td>
<td>3.51 ± 0.21</td>
</tr>
<tr>
<td>3.5</td>
<td>0</td>
<td>2.30 ± 0.00</td>
<td>0</td>
<td>2.45 ± 0.15</td>
<td>3.40 ± 0.02</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Mean and standard error of the mean (SEM) of *L. monocytogenes* (log CFU/ml) recovered from meats irradiated at specific doses.*

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for 2 min. Appropriate dilutions were made and surface plated with the Spiral Plater onto tryptic soy agar with 0.6% yeast extract (TSAYE) and modified Oxford agar in duplicate. Plates were incubated at 37°C for 48 h to enumerate total plate counts. For the survival study, inoculated and irradiated meat samples were stored at 4 and 10°C and sampled once a week for 12 weeks. The reported results are the average of three independent replications. For the different bags with meat samples, 225 ml of 0.1% sterile peptone water was added to the 25-g sample and homogenized for 2 min in the stomacher. Appropriate dilutions were made and surface plated in duplicate onto TSAYE and modified Oxford agar. Plates were incubated at 37°C for 48 h and then enumerated with the Synoptics Ltd. (Cambridge, UK) ProtoCOL (model 60000) automated plate counter.

**Calculation of D_{10}-values.** *D_{10}-values* were determined by plotting the irradiation dose (kGy) against the average log CFU/g from each dose in the duplicate samples of three independent

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**FIGURE 3. Survival and growth of *L. monocytogenes* on frankfurters.**
FIGURE 4. Survival and growth of *L.* monocytogenes on bologna.

trials. Linear regression curves were generated with statistical software, JMP (SAS Institute Inc., Cary, N.C.), where the $D_{10}$-values were determined as the absolute value of the reciprocal of the slope of the curve.

RESULTS AND DISCUSSION

Irradiation reduction. A petition for the approval of the irradiation of processed meats has been filed with the U.S. Food and Drug Administration, and is pending for approval. *L.* monocytogenes postprocessing contamination from the environment might not achieve high counts that would require high-dose irradiation for safety. Approximately 3- or 5-log reduction with lower doses might be sufficient to achieve a margin of safety for processed meats. Tables 1 and 2 illustrate estimated doses required for specific reductions on the basis of a nonselective medium (TSAYE) and on a selective medium (modified Oxford agar). A 3-log reduction of *L.* monocytogenes was obtained at 1.5 kGy (actual doses: 1.89 ± 0.12 kGy for frankfurters; 1.60 ± 0.09 kGy for sliced meats) when a nonselective medium was used for plating. On a selective medium, the doses were 1.5 kGy (actual dose: 1.60 ± 0.09 kGy) for bologna, roast beef, and both types of smoked turkey and 2.0 kGy for frankfurters (actual dose: 2.43 ± 0.13 kGy) and ham (actual dose: 2.07 ± 0.14 kGy). A 5-log reduction of *L.* monocytogenes on the nonselective medium was obtained at 2.5 kGy (actual doses: 3.20 ± 0.16 kGy for frankfurters; 2.70 ± 0.24 kGy for sliced meats). On a selective medium, the doses were 2.5 kGy (actual dose: 2.70 ± 0.24 kGy) for bologna, roast beef, and both types of smoked turkey and 3.0 kGy for frankfurters (actual dose: 3.96 ± 0.09 kGy) and ham (actual dose: 3.30 ± 0.25 kGy). The 0.5-kGy difference for frankfurters from the selective media could be due to the thickness (~1.5 cm); a higher irradiation dose is generally required for effective penetration of electrons into frankfurters compared to the sliced meats. As for ham, the meat and fat were finely chopped and not blended well, which might have affected the higher dose required. The irradiation doses found from this experiment were below that reported (3.55 kGy) for a 5-log reduction of *L.* monocytogenes from gamma-irradiated frankfurters (14).

$D_{10}$-value. By the irradiation reduction procedure, the $D_{10}$-values for the meats could be obtained by plotting the values on a straight line and calculating the $D_{10}$-values from the slope. The $D_{10}$-values for frankfurters, bologna, ham, and roast beef ranged from 0.42 to 0.44 kGy, with an average of 0.44 kGy, with the use of the nonselective medium TSAYE (Fig. 1). These doses were lower than previously reported by Sommers and Thayer (14), which ranged from 0.49 to 0.71 kGy, with an average of 0.61 kGy, on the basis
of surface-inoculated frankfurters, vacuum packed, and gamma irradiated.

**Survival and growth.** Survival and growth of *L. monocytogenes* in the selected RTE meats after irradiation at doses of 2 and 4 kGy were studied during storage at temperatures of 4 and 10°C for 12 weeks. Figure 2, with the use of the USDA-ARS Pathogen Modeling Program (PMP), version 6.1 (http://www.arserrc.gov/mfs/PMP6_Start.htm), shows the projected growth of *L. monocytogenes* on RTE processed meats stored at 10°C. No survivors were observed in samples irradiated at 4 kGy. A dose of 2 kGy was successful in reducing the numbers of *L. monocytogenes*, and storage at 4°C proved to be effective in suppressing the growth of the organism for about 5 weeks after irradiation. As expected, storage at 10°C allowed the growth of higher numbers of *L. monocytogenes* in all the selected RTE meats. Figures 3 through 5 illustrate the survival and growth of *L. monocytogenes* on the selected RTE meats stored at 4 and 10°C.

Prolonged lag phases after the RTE meats were irradiated are shown in Figures 3 through 5. An extended lag phase was observed with selected processed meats irradiated at 2 kGy, especially for the ones stored at 4°C. When bologna and turkey ham were irradiated at 2 kGy and stored at 4°C, the lag phase increased 4 weeks and 2 weeks, respectively. This extended shelf life is important and means that the irradiated meats can be stored for that much longer time. Increase in lag phase of *L. monocytogenes* corresponds with an increase in irradiation dose but is less pronounced with temperature increase. This might be a result of high irradiation dose injury of the cells and the longer recovery from damage it would take at lower temperatures (12). Patterson et al. (12) conducted a similar study with gamma-irradiated cooked poultry meat and found the lag phase was extended to 18 d for meat irradiated at 2.5 kGy compared to 1 d for nonirradiated meat when stored at 6°C. *L. monocytogenes* on roast beef was found to be more sensitive to radiation earlier in this investigation (data not shown). Product formulation and processing might play a role in the effect of irradiation on foodborne pathogens (14). In this experiment, *L. monocytogenes* exhibited minimal or no growth when examined in roast beef samples. Therefore, only data for the survivors growing on bologna, frankfurters, and turkey ham are presented (Figs. 3 through 5).

No apparent difference in generation time was observed. No apparent difference was found in the maximum population densities (although 2-kGy samples consistently had lower maximum population densities).
CONCLUSIONS

Obtaining an optimal irradiation dose to achieve a margin of safety in processed meats is essential. Having this information would help meat processors obtain a safe product and at the same time maintain product quality. Estimated dose for a 3-log reduction of *L. monocytogenes* on selected RTE meats is 1.5 kGy and for a 5-log reduction is 2.5 kGy (data from nonselective medium). No apparent difference was observed between the irradiation $D_{10}$-values in the smoked turkey with lactate and without lactate. The counts obtained from the experiment were comparable. The average $D_{10}$-value for *L. monocytogenes* on the selected meats (frankfurters, bologna, ham [pork and turkey], roast beef, and both types of smoked turkey) is 0.44 kGy. These estimates provide information as to the approximate irradiation dose required for securing the safety of processed meats. Depending on the initial inoculum or contamination level, the irradiation doses can be adjusted to be sufficient to eliminate this pathogen while preserving the qualities of the meats. As for the survival and growth of *L. monocytogenes* on selected processed meats, no recoverable bacteria was found in samples irradiated at 4 kGy and stored at either temperature (4 or 10°C). The results from this study provide RTE meat processors a better idea on the use and effects of irradiation as part of the unit operations in securing the safety of RTE meats that do not have a final heat kill step before consumption.

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REFERENCES