Irradiation Inactivation of *Listeria monocytogenes* in Low-Fat Ground Pork at Freezing and Refrigeration Temperatures

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ABSTRACT

Gamma radiation effectively controls *Listeria monocytogenes* in uncooked and in ready-to-eat foods. This study was conducted to determine if gamma radiation could be used to control *L. monocytogenes* in ground pork. Ground pork was contaminated with *L. monocytogenes*, kept at refrigeration (4°C), chilling (0°C), and freezing (—18°C) temperatures overnight, exposed to gamma radiation and stored at 4°C for 7 days, and at 0 and —18°C for 60 days. Following irradiation, the meat was assayed for *L. monocytogenes* viable counts and lipid oxidation. A triangle test was performed to determine if sausage made from the irradiated and nonirradiated ground pork differed in sensory quality. It was observed that a 5-log reduction of *L. monocytogenes* viable counts would require a 3.0-kGy radiation dose. The results of a 60-day storage study of ground pork inoculated with 10⁵ to 10⁶ CFU of *L. monocytogenes* per gram indicated that counts for nonirradiated meat remained fairly constant at refrigeration, chilling, and freezing temperatures. However, irradiation of ground pork at 3.0 kGy could inactivate *L. monocytogenes* totally in ground pork subsequently held at all the temperatures used in this study. Lipid oxidation measurements, as determined by the thiobarbituric acid–reactive substance assay, ranged from 0.16 nmol/g for nonirradiated ground pork and 0.20 nmol/g for meat irradiated at 3.0 kGy. Sensory panelists could distinguish between irradiated and nonirradiated sausage but were divided on whether irradiation adversely affected the sausage quality. Our results suggest that gamma radiation could be useful to control *L. monocytogenes* in ground pork and improve the safety of ground pork products.

*Listeria monocytogenes* can be isolated from vegetables (2), 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 great public health concern (2, 13). *L. monocytogenes* causes an estimated 2,500 cases of foodborne illness annually, with a mortality rate of approximately 20% in immunocompromised or other susceptible individuals in the United States (14). It is capable to grow at refrigeration temperatures, can survive in high-salt environments, and is readily isolated from retail meat products (14). A significant outbreak occurred with frankfurters in 1998 through 1999, which resulted in 21 fatalities and approximately 100 reported cases of listeriosis (3). In 2000 and 2002, outbreaks occurred in the northeast of the United States, resulting in 10 deaths from the consumption of sliced turkey deli meat (4, 5).

Ionizing radiation has been shown to effectively eliminate *L. monocytogenes* in processed meat products. While *L. monocytogenes* is susceptible to radiation, high-dose (>5 kGy) radiation has a negative effect on the organoleptic qualities of the meat (9, 15). Therefore, an optimal dose is required for a margin of safety while preserving the quality of meat. Low-dose gamma radiation (<5 kGy) was speculated to eliminate pathogens while maintaining sensory qualities of frankfurters (15). With the lower doses, there might be an opportunity 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 ground pork products stored at freezing, chilling, and refrigeration temperatures.

This investigation was conducted to (i) determine the radiation doses required for a 5-log reduction of *L. monocytogenes* on ground pork, (ii) study the postradiation survival and growth of *L. monocytogenes* in ground pork, and (iii) determine the thiobarbituric acid–reactive substance (TBARS) values in irradiated ground pork stored at refrigeration and freezing temperatures and the acceptance of cooked sausage prepared using irradiated ground pork.

MATERIALS AND METHODS

Test strains. The rifampin-resistant mutants of *L. monocytogenes* ATCC 43256 (from Mexican-style cheese) and ATCC 49594 (derived from *L. monocytogenes* strain Scott A) (American Type Culture Collection, Manassas, Va.), and JCM 7676 (from roast beef), JCM 7672 (from salami sausage), and JCM 7671 (from lax ham) (Japan Collection of Microorganisms) were used in this study. All test strains of *L. monocytogenes* were grown in tryptose phosphate broth (TPB, pH 7.0; Difco, Becton Dickinson, Sparks, Md.) containing 50 μg of rifampicin per ml before their use as an inoculum. Plating on media containing rifampin greatly minimized interference with colony development by naturally occurring microorganisms, thus facilitating detection of the test pathogen on recovery media. Validation of the radiation resistance of the native strains versus that of the rifampin- and naladixic acid-resistant strains was conducted to determine if gamma radiation could be used to control *L. monocytogenes* in ground pork.
resistant mutant derivatives used in this study revealed only minor, statistically irrelevant, differences between the survival of the bacteria when suspended in raw ground pork (data not shown).

**Preparation of inocula.** Each strain of *L. monocytogenes* was cultured in TPB, containing 50 μg of rifampin per ml, at 37°C for three successive 24-h intervals, immediately before use as inocula. Cells of each strain were collected by centrifugation (3,000 × g for 10 min at 20°C) and resuspended in 5 ml of phosphate-buffered saline (PBS; pH 7.2). Equal volumes of cell suspensions (approximately 8.0 log CFU/ml) of the five strains were combined to give approximately equal populations of each strain. The inoculum was maintained at 21 ± 1°C and applied to the test ground pork within 1 h of preparation.

**Inoculation procedure.** Boneless fresh ground pork (2.66% fat) were obtained from PRIMA Ham Co. Ltd. (Ibaraki, Japan) and stored at freezing temperature (−18°C) for a maximum of 2 to 3 days before being used in the experiments. Before inoculation, the commercial pack of meat samples (4 kg) was thawed in water for 2 h, opened, and a 1,000-g meat sample was aseptically transferred into a stainless steel dish. Fifty milliliters of cell suspension, containing about 10⁶ CFU/ml, was inoculated into the meat sample and thoroughly mixed. The inoculated meat sample was then packed (25 g of each), sealed, and kept at 4, 0, and −18°C for 24 h. The next day, the samples were irradiated at different doses maintaining the respective temperatures.

**Irradiation.** The inoculated samples were treated with 0.0 (control), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 kGy. For each temperature, triplicate samples were used. These samples were run concurrently. All irradiation runs on a given day were conducted at a single temperature to maintain consistency in the temperature control. The samples were irradiated at a dose rate of 1.4 kGy/h from a cobalt-60 gamma source of 16,841 Ci (gamma Cell-220, Nordion International, Inc., Kanata, Ontario, Canada). The absorbed dose was confirmed utilizing cellulose triacetate (CTA) film dosimeters (FTR-125; Fuji Photo Film Co. Ltd., Tokyo, Japan), attached to the surface of the plastic bag, according to McLaughlin et al. (10). After irradiation, the samples were kept at 4°C for 7 days and at 0°C and −18°C for 60 days and their microbiological parameters were evaluated on days 1, 3, 5, and 7 for 4°C and days 1, 5, 10, 15, 30, 60 for 0°C and −18°C post-irradiation. A batch of noninoculated irradiated and nonirradiated samples was kept at the same temperature as the experimental samples and their natural aerobic, coliform, and yeast and mold counts were determined.

Our irradiation facility lacks the ability to control the temperature during the process. However, in order to maintain meat in the frozen state during processing, we used ice cube with salt to maintain 0°C and dry ice to maintain temperature at or below −18°C. Simultaneously, a temperature logger was set outside the radiation chamber to record the temperature.

**Microbiological analyses.** Twenty-five grams of irradiated, nonirradiated, or postirradiated samples was aseptically transferred to stomacher bags and 225 ml of 0.1% peptone water was added to each bag. The bag contents were homogenized for 90 s in a stomacher (ILU Instrument, model CE-97, Barcelona, Spain) at medium speed. Serial decimal dilutions were prepared with 0.1% peptone water and were surface plated (0.1 ml, in duplicate) on tryptose soy agar (TSA) supplemented with 50 μg/ml rifampin (TSAR) and modified Oxford medium (Oxoid) supplemented with 50 μg/ml rifampin (MOXR). The MOXR medium contains 55.5 g of *Listeria*-selective agar base (Oxoid CM 856, Unipath-Oxoid, Basingstoke, Hants, England), 0.01 g of colistin methanesulfate (Sigma Chemical Co., St. Louis, Mo.), 0.02 g of ceftazidime pentahydrate (Glaxo Group Research Ltd, Ware, Hertfordshire, UK), 50 mg of nalidixic acid, and 5.0 g of agar (Difco) per liter of deionized water. All ingredients except ceftazidime pentahydrate and rifampin were combined and sterilized by autoclaving. Ceftazidime pentahydrate solution and rifampin were added to the molten agar before pouring the medium into petri plates. The plates were incubated at 37°C for 24 to 48 h before presumptive colonies of each pathogen were counted. At least five randomly picked presumptive colonies of *L. monocytogenes* per replication were confirmed with API *Listeria* diagnostic kits.

**Lipid oxidation.** Lipid oxidation in irradiated and nonirradiated noninoculated meat samples stored at 4, 0, and −18°C was measured by a modified version of the thiobarbituric acid (TBA) assays of Hodges et al. (8) and Zipser and Watts (18). Briefly, 10 g of meat sample was homogenized with 25 ml of 0.5 M phosphate buffer (pH 2.5), containing 0.08% sulfanilamide and 0.01% butylated hydroxytoluene (BHT), using a homogenizer (model T25 basic 1, IKA-Werke GmbH & Co. KG, Staufen, Germany) at speed 70 for 2 min. The homogenate was then centrifuged at 6,700 × g for 10 min at 5°C and filtered through 70-μm nylon mesh (Becton Dickinson Co., Bedford, Mass.). A 1.6 ml aliquot of the supernatant was added to a test tube containing 1.6 ml of either −TBA solution (20% [wt/vol] trichloroacetic acid and 0.01% BHT) or +TBA solution (20% [wt/vol] trichloroacetic acid, 0.01% BHT, and 0.65% TBA). Samples were then vigorously mixed, heated to 95°C in a water bath for 25 min, cooled, and filtered through 0.45-μm-pore-size Millipore filters. The absorbance was measured at 440, 532, and 600 nm with a spectrophotometer. The TBA values were expressed as malondialdehyde (MDA) equivalents and calculated by the formulas developed by Hodges et al. (8):

\[
\frac{([\text{Abs}532_{-\text{TBA}} - \text{Abs}600_{-\text{TBA}}])}{([\text{Abs}440_{-\text{TBA}} - \text{Abs}600_{-\text{TBA}}])} = A \\
\frac{([\text{Abs}532_{-\text{TBA}} - \text{Abs}600_{-\text{TBA}}])}{([\text{Abs}440_{-\text{TBA}} - \text{Abs}600_{-\text{TBA}}])} = B
\]

MDA (nmol/g) = \[A \times B + 157.000\] 10⁶

**Sensory analysis.** A triangle test was used to determine if panelists could distinguish between noninoculated irradiated and nonirradiated ground pork. For this analysis, meat samples were irradiated at 4.0 kGy and kept at −18°C for 24 h. On the day of testing, frozen irradiated and nonirradiated meat sample were thawed for 2 h and sausages were prepared using casings and boiled for 5 min without adding salt or any other seasonings. The sausages were cooled to room temperature, cut into small pieces, placed on paper plates, and presented to panelists.

Testing was conducted in the sensory laboratory at the National Food Research Institute. The panelists, 33 volunteers, were informed about the nature of the study. The test area was free of extraneous odors and sound and panelists were instructed not to talk during testing.

Panelists evaluated samples and marked score sheets in individual booths. Preliminary work had shown that panelists could distinguish the sausages made from irradiated ground pork from those made from nonirradiated meat by taste. Three sausage samples (without condiments) coded with three-digit random numbers were presented to each panelist, along with unsalted crackers and water. Panelists were instructed to evaluate samples in the order of presentation and to clear their palates between samples with the crackers and water. The presentation order of the samples was balanced among panelists to avoid a position error bias. Panelists
were informed that two of the samples were identical and were asked to pick the “odd” sample. Some panelists swallowed the samples during their evaluation; others tasted but did not consume the samples. Score sheets were compared with a key containing code numbers for each of the sausage treatments. Panelists’ responses were noted on score sheets as “C” (correct) or “NC” (incorrect) (6).

**Statistical analysis.** The experiments were done two times with triplicate samples being analyzed at each sampling time. L. monocytogenes levels were expressed as the log CFU per gram recovered by direct plating count. Statistical analysis was performed with the statistics package in Microsoft Excel 2000. A single-factor analysis of variance was used to determine if L. monocytogenes levels differed significantly between the irradiated and nonirradiated meat samples or between the time intervals during storage after irradiation. For sensory analysis, the number of correct responses was compared with the critical number given in Table T8 of Meilgaard et al. (11) at the 95% confidence level.

**RESULTS**

**Meat composition.** A proximate analysis of the meat from the Nippon Ham Co. Ltd. gave the following results: ash, 3.95%; fat, 2.66%; moisture, 68.34%; protein, 24.44%; and mean water activity, 0.984.

**Natural microflora.** The initial populations of natural microflora of nonirradiated and irradiated samples are shown in Figure 1. The levels of aerobic and coliform bacteria in nonirradiated samples stored at 4°C increased gradually and at day 7 the mesophilic aerobic and coliform counts were 4.1 log CFU/g and 1.18 log CFU/g, respectively. However, the level of yeasts and molds was below 1.0 log CFU/g and remained constant up to 7 days of storage. No L. monocytogenes was detected in noninoculated samples. Aerobic mesophilic bacteria, coliform, and yeast and mold counts were below the detection level in meat irradiated at 3.0 kGy throughout the postirradiation storage period (Fig. 1A).

At the chilling temperature (0°C), the levels of aerobic and coliform bacterial levels were 5.22 log CFU/g and 2.06 log CFU/g, respectively, at day 15 in nonirradiated meat samples, and had also increased further by day 30 and day 60. Yeast and mold levels also increased accordingly. However, all the bacterial counts were below the detection level in meat irradiated at 3.0 kGy throughout the postirradiation storage period (Fig. 1B).

At the freezing temperature (−18°C), the levels of aerobic and coliform bacteria and yeast and mold level in nonirradiated meat samples were fairly constant throughout the 60-day incubation period. However, in the irradiated samples (3.0 kGy), the level of these organisms was below the detection level throughout the storage period (Fig. 1C).

**Refrigeration storage.** To determine if L. monocytogenes levels change during refrigeration storage after gamma radiation, L. monocytogenes levels were assayed at 1, 3, 5, and 7 days after exposure to doses ranging from 0.5 to 3.0 kGy (Fig. 2). The viable counts for L. monocytogenes in the nonirradiated meat remained fairly constant up to 7 days. However, increasing radiation doses gradually decreased the number of bacteria and at the 3.0-kGy dose of radiation, no viable counts were observed in either selective or nonselective medium.

**Chilling storage.** The level of L. monocytogenes in irradiated ground pork stored postirradiation at 0°C was assayed on days 1, 5, 10, 15, 30, and 60. The viable counts for L. monocytogenes in nonirradiated meat decreased slightly within 10 days and were constant throughout the incubation period. However, at 3.0-kGy radiation dose, no viable counts of L. monocytogenes were found (Fig. 3).

**Freezing storage.** Similarly, ground pork was contaminated with L. monocytogenes, exposed to gamma radiation, and kept at −18°C temperature for 60 days. The level of L. monocytogenes in irradiated ground pork stored postirradiation at 0°C was assayed on days 1, 5, 10, 15, 30, and 60. The viable count of L. monocytogenes in the nonirradiated meat decreased slightly within 5 days and remained approximately constant thereafter. However, at 3.0-kGy radiation doses, no viable counts of L. monocytogenes were detected (Fig. 4).
FIGURE 2. Survival of Listeria monocytogenes artificially inoculated in ground pork and stored at 4°C for 7 days with or without irradiation treatment. (A) TSAR medium, (B) MOXR medium. Each data point is the average of triplicate samples. Bars indicate the standard errors.

Lipid oxidation. Lipid oxidation measurements as determined by the TBARS assay ranged from 0.16 nmol/g for nonirradiated ground pork to 0.20 nmol/g for meat irradiated at 3.0 kGy (Table 1). The small but statistically insignificant increase in lipid oxidation was dependent on the radiation dose and storage temperature, as determined by Duncan's multiple-range test ($n = 3$, $\alpha = 0.05$).

Sensory analysis. Sensory panelists were able to differentiate sausages made from nonirradiated meat from those made using ground pork irradiated at 4 kGy ($P > 0.001$). The number of correct responses (21) was compared with the critical number in table T8 of Meilgaard et al. (11). In the present study ($n = 33$), the critical value at the 95% confidence level was 15. Since we had 21 correct responses, which exceeded the critical value, the assumption of no difference was rejected. Although panelists could detect a difference between irradiated and nonirradiated sausages, not all panelists disliked the radiated sausages. Of the 21 panelists who responded correctly, 6 preferred the irradiated sample, 7 expressed no preference, and 8 preferred the nonirradiated sample. Comments made by panelists who preferred the irradiated sausages indicated that the sausages had more flavor and that these panelists found the nonirradiated sausages bland. Panelists who preferred the nonirradiated sausages found that the irradiated sausages had a sharp, more processed "chemical" or "refrigerator" taste. Some panelists noticed differences between the two types of samples, but were not specific in describing those differences. For example, one panelist noted that the nonirradiated sample was "good," and others simply stated

FIGURE 3. Survival of Listeria monocytogenes artificially inoculated in ground pork and stored at 0°C for 60 days with or without irradiation treatment. (A) TSAR medium, (B) MOXR medium. Each data point is the average of triplicate samples. Bars indicate the standard errors.

FIGURE 4. Survival of Listeria monocytogenes artificially inoculated in ground pork and stored at −18°C for 60 days with or without irradiation treatment. (A) TSAR medium, (B) MOXR medium. Each data point is the average of triplicate samples. Bars indicate the standard errors.
that the difference between samples was in the texture. One possibility for future research would be a hedonic study comparing irradiated foods with nonirradiated controls. A hedonic study could help food manufacturers determine if their consumers like or dislike irradiated foods which could help manufacturers to determine whether irradiation would affect their consumers' purchase intent.

**DISCUSSION**

In our previous study (1) with *Escherichia coli* O157: H7, we have shown that traditional selective media do not support colony development by a portion of *E. coli* O157: H7 exposed to some of the chemical and irradiation treatments. Therefore, in this study, both nonselective TSAR and selective MOXR were used for the enumeration of *L. monocytogenes* on treated and untreated meat. However, the data presented in the “Results” section were from nonselective medium. Regardless of meat conditions or treatments, higher populations of *L. monocytogenes* were recovered on TSAR than on MOXR. *L. monocytogenes* counts were 0.91 to 1.25 log CFU/g higher when samples from inoculated meat sample (control) were plated on TSAR compared with selective medium.

The U.S. Food and Drug Administration has approved the use of irradiation up to 4.5 kGy and 7 kGy to control bacterial pathogens on refrigerated meat and frozen meat, respectively (7). Thayer et al. (17) found that *D_10*-values for *L. monocytogenes* ranged from 0.45 to 0.50 kGy in uncooked pork, beef, lamb, and turkey at 5°C. Thayer and Boyd (16) investigated the effect of the temperature on resistance to gamma radiation and found that *D_10*-values for *L. monocytogenes* on beef increased sharply as the temperature decreased from 0 to -5°C. These investigators found that the *D_10*-value increased nearly threefold as the temperature decreased from 0 to -20°C; they observed smaller increases in the *D_10*-value below -20°C. However, Monk et al. (12) reported that *L. monocytogenes* and *Staphylococcus aureus* were not sensitive to freezing or refrigeration temperatures when mixed with ground beef, nor was their sensitivity to radiation affected by temperature. In our experiments, we also did not find much influence of the temperature on irradiation of *L. monocytogenes* in low-fat ground pork.

The sensory parameters, biochemical parameters, along with the microbiological parameters, suggested that a radiation dose of 3.0 kGy would be sufficient to eliminate approximately 5.0 log CFU/g of *L. monocytogenes* from refrigerated, chilled, and frozen low-fat ground pork without significantly affecting their overall quality.

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