

# Effects of Low-Dose, Low-Penetration Electron Beam Irradiation of Chilled Beef Carcass Surface Cuts on *Escherichia coli* O157:H7 and Meat Quality†

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## ABSTRACT

Low-dose, low-penetration electron beam (E-beam) irradiation was evaluated for potential use as an antimicrobial intervention on beef carcasses during processing. The objectives of this study were (i) to assess the efficacy of E-beam irradiation to reduce concentrations of *Escherichia coli* O157:H7 on a large beef surface and (ii) to evaluate the effect of the treatment on the sensory properties of the product. A 1-kGy dose of E-beam radiation reduced *E. coli* O157:H7 inoculated onto sections of cutaneous trunci at least 4 log CFU/cm<sup>2</sup>. In assessing organoleptic impact, flank steak was used as the model muscle. Flank steaks with various levels of penetration by radiation (5, 10, 25, 50, and 75%) were evaluated. None of the flank steak sensory attributes were affected ( $P > 0.05$ ) by any penetration treatment. Ground beef formulations consisting of 100, 50, 25, 10, 5, and 0% surface-irradiated beef were tested. A trained sensory panel did not detect any difference between the control (0%) and either the 5 or 10% treatments. These results suggest that if chilled carcasses were subjected to low-dose E-beam irradiation, aroma and flavor of ground beef would not be impacted. The data presented here indicate that low-dose, low-penetration E-beam irradiation has potential use as an antimicrobial intervention on beef carcasses during processing and minimally impacts the organoleptic qualities of the treated beef products.

*Escherichia coli* O157:H7 is the major target pathogen for control in the beef processing industry. Previous studies have indicated that multihurdle intervention strategies are the best for reducing pathogen contamination of beef carcasses during processing (2, 8, 28). Currently, processors employ a variety of intervention technologies but are still unable to eliminate contamination of the final product by pathogens such as *E. coli* O157:H7 (3, 13). Clearly, novel interventions are required to help processors minimize or eliminate such pathogens.

In several studies, irradiation significantly reduced foodborne pathogen concentrations (16, 22, 30). Consequently, irradiation has been approved and used on a wide variety of food items. Currently, ionizing radiation is approved for use in treating refrigerated or frozen uncooked meat, meat byproducts, and certain other meat food products to reduce concentrations of foodborne pathogens and to extend shelf life (34, 35). Traditionally, large lots of either nonintact cuts or ground beef are irradiated. To uniformly treat these products, high-penetration, high-energy

radiation is needed to ensure that the entire meat product, both exposed surface and internal regions, is irradiated. Such treatments may lead to the development of off-odors and can affect flavor. Recently, low-dose, low-penetration electron beam (E-beam) irradiation technology has evolved to the point where large nonuniform surface areas can be effectively treated (e.g., an entire carcass side). This approach allows for whole carcasses to be treated after chilling. With such a process, only the surface (approximately 15 mm of penetration) of each carcass side receives a significant radiation dose. Because pathogen contamination of carcasses is a surface phenomenon, this treatment is expected to dramatically lower the pathogen load without adversely affecting the organoleptic qualities of products made from the internal regions of the carcass. The present experiment was designed to simulate the effects of E-beam irradiation on pathogen concentrations and meat quality in chilled beef carcasses immediately before carcass disassembly. The objectives of this study were (i) to assess the efficacy of E-beam irradiation for reducing concentrations of *E. coli* O157:H7 on carcass surface tissues and (ii) to evaluate the effect of E-beam irradiation on product quality.

## MATERIALS AND METHODS

In these experiments, various tissues were irradiated, but the method of irradiation was the same for each experiment. Methods were designed to simulate the effect of applying E-beam irradiation to chilled beef carcass sides. Therefore, at a large-scale U.S. fed beef harvesting facility, tissues were obtained from chilled

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† Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name by U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.

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beef carcasses during the course of conventional carcass disassembly. Tissues were vacuum packaged and transported ( $-3^{\circ}\text{C}$ ) to the irradiation facility. As detailed below, tissues were unpacked, arranged on trays, and subjected to E-beam irradiation.

**E-beam irradiation.** Samples were irradiated with a 3-MeV Dynamitron (RDI, Edgewood, N.Y.) at a dosage of 1 kGy/s. Because of limited capacity of the E-beam unit used in this study, 17 irradiation batches were required to complete the tests. For each batch, two BioMax Alanine Dosimeter Films (Eastman-Kodak, Inc., Rochester, N.Y.) were used to assess the radiation delivered to the beef tissues. The free radical signal was measured with an electron paramagnetic resonance analyzer (Bruker Instruments, Inc., Billerica, Mass.). The range in delivered surface dosage was 0.98 to 1.17 kGy among the 17 batches (mean  $\pm$  standard deviation,  $1.04 \pm 0.05$  kGy). Internal dosages can be up to 1.6 times higher.

**Pathogen reduction: meat samples.** Forty cutaneous trunci pieces were used for this experiment. At the E-beam facility, the cutaneous trunci pieces were warmed to room temperature before inoculation because when carcass contamination occurs during the beef harvesting process the surface of the carcass is warm. Outlines of two 200-cm<sup>2</sup> areas were marked on each piece using edible ink and a template (10 by 20 cm); one section was treated and the other was not (control). On the control section, three areas (5 by 5 cm) were marked with edible ink. Two such areas were marked on the treated section.

**Pathogen reduction: strain.** An *E. coli* O157:H7 strain lacking both Shiga toxins (ATCC 43888) was used for all inoculations. This strain has growth characteristics similar to those of fully toxigenic *E. coli* O157:H7 strains; however, to our knowledge there have not been any studies comparing the radiation sensitivities of such strains.

**Pathogen reduction: inoculation.** *E. coli* O157:H7 (ATCC 43888) was grown overnight in tryptic soy broth (Difco, Becton Dickinson, Sparks, Md.) to approximately  $5 \times 10^8$  CFU/ml. The culture was diluted with buffered peptone water (Difco, Becton Dickinson) to  $10^8$  CFU/ml (high-concentration inoculum) and  $10^5$  CFU/ml (low-concentration inoculum). Twenty cutaneous trunci pieces were used for high-concentration inoculation (approximately  $10^6$  CFU/cm<sup>2</sup>), and 20 pieces were used for low-concentration inoculation (approximately  $10^3$  CFU/cm<sup>2</sup>). To inoculate the sections, 4 ml of the appropriate culture dilution was dispensed across the 400-cm<sup>2</sup> area and spread using a sterile spreader.

**Pathogen reduction: attachment.** After inoculation, the pieces remained at room temperature for 1 h. After 1 h, one 25-cm<sup>2</sup> piece from each control section was excised, aerobically bagged, refrigerated, and shipped overnight to the U.S. Meat Animal Research Center (MARC) for processing. These pieces were designated as time = 0 h postirradiation. The remaining sections were refrigerated overnight to simulate the chilling that occurs between the end of slaughter and carcass fabrication, i.e., between slaughter and the proposed low-dose E-beam irradiation immediately before carcass disassembly.

**Pathogen reduction: treatment.** The refrigerated sections were removed from refrigeration, and the treated and control sections were separated. The pieces to be treated were irradiated as described. Two 25-cm<sup>2</sup> sections were excised from both the control and treated sections, aerobically bagged, and shipped refrigerated to MARC for processing. These pieces were designated as time = 48 h or 120 h postirradiation. The 48- and 120-h sampling

times were designed to represent beef trimmings that experience a relatively short and a relatively long distribution and transportation period, respectively, before ground beef production.

**Pathogen reduction: detection and enumeration of *E. coli* O157:H7.** Twenty-five milliliters of buffered peptone water was added to the sample bags containing 25-cm<sup>2</sup> beef pieces. The bags were stomached (Seward Lab Blender Stomacher 400, Brinkmann Instruments, Westbury, N.Y.) for 1 min at 230 rpm, and samples were serially diluted and plated in duplicate onto sorbitol MacConkey agar (SMAC; Difco, Becton Dickinson) supplemented with cefixime (0.05 mg/liter) and potassium tellurite (2.5 mg/liter; Dynal, Lake Success, N.Y.) (ctSMAC). The remaining buffered peptone water from a subset of the treated samples was used for most-probable-number (MPN) estimation of *E. coli* O157:H7. Four aliquots (5 ml each) of the stomached samples were removed to new tubes. Forty-five milliliters of prewarmed (42°C) BAX medium (DuPont Qualicon, Wilmington, Del.) was added to the four tubes and mixed thoroughly. BAX medium (45 ml) was also directly added to the sample bag containing the remaining 5 ml and the treated section of meat. Two consecutive serial dilutions were made by transferring 5 ml from each of the previous dilutions into new tubes and adding 45 ml of BAX medium. The tubes and sample bag were incubated for 16 to 20 h at 42°C. *E. coli* O157:H7 was isolated using immunomagnetic separation and plating onto (i) ctSMAC and (ii) Rainbow agar (Biolog, Inc., Hayward, Calif.) supplemented with novobiocin (20 mg/liter; Sigma, St. Louis, Mo.) and potassium tellurite (0.8 mg/liter; Sigma) (4). Bacterial enumeration data were analyzed by one-way analysis of variance (ANOVA) in which each subcell of the incomplete variable arrangement (treatment  $\times$  inoculum level  $\times$  storage time) was considered a different level of a single factor.

**Meat quality evaluation: flank steaks.** Flank steaks were used to represent a whole muscle cut that would be exposed during whole-carcass E-beam irradiation. Thirty rough-cut flanks were used for this experiment. At the irradiation facility, 20-mm-thick flank steaks were randomly assigned to one of five treatments. The surface fat over the external side of the rectus abdominus muscle was trimmed to give five treatments of radiation penetration, assuming 15 mm penetration by the E-beam irradiation treatment: (i) 75% muscle penetration (no overlying fat tissue), (ii) 50% muscle penetration (5 mm overlying fat tissue), (iii) 25% muscle penetration (10 mm overlying fat tissue), (iv) 10% muscle penetration (13 mm overlying fat tissue), and (v) 0% penetration (untreated control).

After radiation treatments were complete, samples were vacuum packaged and shipped by air (overnight, 2°C) to MARC. At MARC, the flank steaks were stored at 5°C for an additional 12 to 14 days and then cooked and evaluated. A section (8.5 by 15 cm) was obtained from the center of the flank steak and then cut into cubes (1 by 1 by 2 cm). The cubes were stir-fried in an electric skillet (West Bend Housewares, West Bend, Wis.) at 177°C for 5.5 min. Separate skillets were used for each treatment. Samples were evaluated by a 10-member trained descriptive attribute sensory panel for six attributes: beef aroma intensity, off-aroma, tenderness, juiciness, beef flavor intensity, and off-flavor (where 8 = extremely intense, none, extremely tender, extremely juicy, extremely intense, and none, respectively; and 1 = none, intense, extremely tough, extremely dry, none, and extremely intense, respectively). Immediately after cooking, each panelist evaluated three cubes. The panel evaluated two samples of each treatment on each of three consecutive days. The first sample of each panel session was a nonexperimental warm-up sample. Flank steak sensory data were analyzed by one-way ANOVA.

TABLE 1. Effect of electron beam irradiation and time posttreatment on *E. coli* O157:H7 in beef<sup>a</sup>

Sample	High concentration inoculum			Low concentration inoculum		
	0 h	48 h	120 h	0 h	48 h	120 h
Control	7.2 A	6.6 B	5.9 C	3.9 D	2.9 E	2.6 F
Treated <sup>b</sup>		0.0 G	0.1 G		0.0 G	0.0 G

<sup>a</sup> Values are log CFU per square centimeter. Cell counts with different letters are significantly different ( $P < 0.05$ ).

<sup>b</sup> When no growth was detected, data were treated as 1 log less than the minimum level of detection, which was 10 CFU/cm<sup>2</sup>.

The remaining flank steak, after removal of the section for cooking, was cut in half horizontally to expose fresh surfaces and was allowed to bloom (convert from deoxymyoglobin to myoglobin in the presence of oxygen) at 5°C. Hunter colorimeter measurements (L\*, lightness; a\*, redness; and b\*, yellowness) were obtained in duplicate after 30 min and again after 2 h of bloom time. Flank steak colorimeter data were analyzed by ANOVA for a 5 (treatment) × 2 (bloom time) design.

**Meat quality evaluation: ground beef patties.** Boneless chuck short ribs (150 kg) were mechanically sliced into 2-cm-thick strips, vacuum packaged, and transported to the irradiation facility at -3°C. At the irradiation facility, 50 kg of trimmed short ribs was uniformly irradiated as described, and 100 kg was left untreated to serve as a control. After radiation treatments were complete, the treated and untreated short ribs were vacuum packaged and transported at -3°C to a processing facility for preparation of ground beef.

Ground beef with various percentages of irradiated versus control meat was prepared using appropriate proportions of treated short ribs blended with untreated short ribs to achieve the following proportions of treated meat in the final formulations: (i) 100%, 22.7 kg treated short ribs, (ii) 50%, 11.3 kg treated blended with 11.3 kg untreated short ribs, (iii) 25%, 5.7 kg treated blended with 17 kg untreated short ribs, (iv) 10%, 2.3 kg treated blended with 20.4 kg untreated short ribs, (v) 5%, 1.1 kg treated blended with 21.6 kg untreated short ribs, and (vi) 0%, 22.7 kg untreated short ribs. The target fat content was 20%. Proximate composition was determined by oven drying at 100°C for 24 h followed by diethyl ether Soxhlet extraction, and fat content was 23%. Ground beef formulations were formed into 113.4-g patties, blast frozen (-30°C), and packaged in plastic-lined cardboard boxes. Frozen patties were transported (-17°C) to MARC and stored at -17°C.

Ground beef patties were evaluated after 20 days (19 to 21 days) and 40 days (39 to 41 days) of frozen storage. Two samples (each sample contained two patties) from each treatment were evaluated on each of three consecutive days at each storage time. Samples were evaluated by a 10-member trained descriptive attribute sensory panel for the same six attributes: beef aroma intensity, off-aroma, tenderness, juiciness, beef flavor intensity, off-flavor (where 8 = extremely intense, none, extremely tender, extremely juicy, extremely intense, and none, respectively; and 1 = none, intense, extremely tough, extremely dry, none, and extremely intense, respectively). Patties were thawed at 5°C for 18 h and then cooked on a George Foreman grill (model GR35, Salton, Columbia, Mo.) for 3.75 min at a temperature of approximately 350°C. Cooked patties were blotted on paper towels to remove excess grease, and then each patty was cut into 12 wedges to yield 24 wedges per sample. Each panelist evaluated two wedges per sample. The panel evaluated two samples of each treatment on

TABLE 2. MPN estimates for *E. coli* O157:H7 following electron beam irradiation of beef<sup>a</sup>

MPN estimate	High concentration inoculum			Low concentration inoculum <sup>b</sup>		
	0 h	48 h	120 h	0 h	48 h	120 h
Average		11.0	11.2	0.024	0.056	
Maximum <sup>c</sup>		40	69	<0.036	0.34	

<sup>a</sup> Values are CFU per square centimeter.

<sup>b</sup> When no growth was detected (<0.036 CFU/cm<sup>2</sup>), data were treated as 0.024 CFU/cm<sup>2</sup>.

<sup>c</sup> Maximum MPN within group.

each of three consecutive days at each storage time. The first sample of each panel session was a nonexperimental warm-up sample. Ground beef sensory data were analyzed by one-way ANOVA.

At each frozen storage time, Hunter colorimeter measurements (L\*, a\*, and b\*) were obtained in duplicate for four randomly selected patties of each treatment after 18 h of thawing and bloom time at 5°C. Ground beef colorimeter data was analyzed by ANOVA for a 6 (treatment) × 2 (duration of frozen storage) design.

## RESULTS

**Pathogen reduction: direct plating.** Stomached samples were plated directly onto ctSMAC in duplicate to determine *E. coli* O157:H7 cell counts. For the low-inoculum samples, a 1.3-log reduction in cell counts for the control samples from 0 to 120 h (Table 1) was observed during storage at 4°C for 120 h. There was no *E. coli* O157:H7 growth on ctSMAC at either 48 or 120 h for the treated samples, indicating cell counts were less than 10 CFU/cm<sup>2</sup>. This is a reduction of 2.9 and 2.6 log CFU, respectively, for the 48- and 120-h treated samples compared with controls. The high-inoculum samples had a similar 1.3-log reduction in control cell counts from 0 to 120 h during storage. A 6.6-log reduction was seen for the high-inoculum treated samples at 48 h; counts for all 48-h treated samples were below the limit of detection. At 120 h, there was a 5.7-log difference between the treated and control samples, with all but two of the treated samples below the limit of detection.

**Pathogen reduction: enumeration.** After the aliquots for direct plating were removed, the stomached samples were separated into five portions and serially diluted for a 5 × 3 MPN estimation. The MPN method included an enrichment step before selective plating to allow for recovery of injured cells and had a minimum detection limit of 0.036 CFU/cm<sup>2</sup>. The results of the MPN analysis were similar to that from direct plating, indicating that the numbers of viable *E. coli* O157:H7 cells following irradiation were very low (Table 2). There were no low-inoculum samples at 48 h and only one low-inoculum sample at 120 h that had an MPN value above the limit of detection, resulting in average MPN determinations of 0.024 and 0.056 CFU/cm<sup>2</sup> for 48 and 120 h, respectively. All of the high-inoculum sam-



TABLE 3. Effect of depth of electron beam penetration on trained sensory panel ratings of flank steak

Treatment	Trained sensory panel rating <sup>a</sup>					
	Beef aroma intensity	Off-aroma	Tender-ness	Juici-ness	Beef flavor intensity	Off-flavor
Control	6.02	6.13	6.03	5.62	5.28	4.98
10% penetration	5.97	6.36	5.48	5.61	5.53	5.16
25% penetration	6.04	6.33	5.33	5.30	5.27	5.11
50% penetration	5.70	6.10	5.80	5.65	5.20	5.10
75% penetration	5.75	5.84	5.61	5.28	4.94	4.54
SEM	0.27	0.22	0.27	0.18	0.27	0.26
P value	0.84	0.48	0.40	0.38	0.65	0.46

<sup>a</sup> Six samples were evaluated for each treatment group. Ratings for the six attributes ranged from 8 (extremely intense, none, extremely tender, extremely juicy, extremely intense, and none, respectively) to 1 (none, extremely intense, extremely tough, extremely dry, extremely bland, and extremely intense, respectively).

ples were above the limit of detection, resulting in averages of 11.0 and 11.2 CFU/cm<sup>2</sup> at 48 and 120 h, respectively.

**Meat quality evaluation: flank steaks.** Split beef carcasses have thin external or surface muscles or edges of muscles that may be partially exposed from the carcass splitting process. During low-dose E-beam irradiation of carcass sides, these muscles will receive various doses of radiation depending on their location and the extent of fat cover. In this assessment for organoleptic impact, the flank steak was used as the model muscle because it is partially surface exposed, consistent in size, shape, and location, and easy to access and remove and possesses sufficient surface fat to allow appropriate trimming and surface layer molding to achieve variable penetration.

None of the flank steak sensory attributes were affected ( $P > 0.05$ ) by any penetration treatment (Table 3). All three Hunter color attributes were affected ( $P < 0.05$ ) by treatment penetration (Table 4). However, the effects on L\* and b\* were not linear or apparently dose related and thus probably are not meaningful. The effects of treatment penetration on a\* were generally linear and had a dose-related pattern, but the magnitude of the differences makes it unlikely that any treatment, with the possible exception of the 75% penetration, would impact consumer purchase decisions.

**Meat quality evaluation: ground beef patties.** Boneless chuck short ribs were utilized as the model tissue for irradiated and control muscle and fat tissue used to produce ground beef because this cut contains the appropriate lean: fat ratio for subsequent 20% fat ground beef preparations. If chilled carcasses were exposed to low-dose E-beam irradiation, at most 10% of the resulting ground beef blend would be made from the irradiated surface material. However, for the purposes of this experiment we chose to include additional blends to simulate worse-case scenarios. We also included a 100% irradiated treatment as a positive control for sensory panel evaluation.

TABLE 4. Effect of depth of electron beam penetration on color of raw flank steak<sup>a</sup>

Treatment	L*	a*	b*
Control	39.4	22.3	17.5
10% penetration	43.0	22.2	18.4
25% penetration	38.4	21.7	16.9
50% penetration	41.5	21.3	17.4
75% penetration	37.0	20.0	15.5
SEM	0.68	0.50	0.30
P value	<0.0001	<0.02	<0.0001

<sup>a</sup> Color based on Hunter attributes: L\*, lightness; a\*, redness; b\*, yellowness.

The interaction of treatment and storage time was not significant ( $P > 0.05$ ) for any trait. All ground beef patty sensory attributes were affected ( $P < 0.05$ ) by proportion of irradiated trim (Table 5). For ground beef aroma intensity and beef flavor intensity, the 100% irradiated treatment samples received less favorable ratings. This result was expected and indicates that the trained sensory panel was capable of detecting differences in aroma and flavor that could be attributed to treatment. The fact that the panel did not detect a difference between the control (0%) and either the 5 or 10% treatment samples suggests that there indeed was no difference in flavor between those samples and the control and that if chilled carcasses were subjected to low-dose E-beam irradiation, aroma and flavor of ground beef prepared from these carcasses would not be impacted. Off-flavor ratings were lowest ( $P < 0.05$ ) for the 100% irradiated samples, and both the 100% and the 50% irradiated samples had more ( $P < 0.05$ ) off-flavor and off-aroma than did all other treatment samples. Tenderness and juiciness ratings were lowest ( $P < 0.05$ ) for the 100% samples, but differences among other treatment groups were not linear or dose related, and thus it is not clear whether these effects represent meaningful differences.

Ground beef aroma and beef flavor intensities were not affected ( $P > 0.05$ ) by frozen storage time (Table 5). Off-aroma and off-flavor ratings increased (decrease in trait) ( $P < 0.05$ ), and tenderness and juiciness ratings decreased ( $P < 0.05$ ) with increased frozen storage time. The significant effects of frozen storage time are not logical and may not be of practical importance. The proportion of irradiated trim did not affect any color measurement of raw ground beef patties ( $P > 0.05$ ) (Table 6).

**DISCUSSION**

Low-dose, low-penetration E-beam irradiation has great potential as an antimicrobial intervention in the beef slaughter process. Because contamination of beef carcasses by pathogenic bacteria occurs on the external surface, a broad-spectrum antimicrobial intervention that produces large reductions in pathogen load while minimally affecting the carcass would be ideal. The objective of this study was to determine whether these criteria are met by low-dose, low-penetration E-beam irradiation.

We used direct plating to evaluate the efficacy of E-beam radiation for reducing pathogen concentrations. Di-

TABLE 5. Effect of proportion of irradiated trim and frozen storage time on trained sensory panel ratings of ground beef patties

Main effects	n	Trained sensory panel rating <sup>a</sup>					
		Beef aroma intensity	Off-aroma	Tenderness	Juiciness	Beef flavor intensity	Off-flavor
Treatment							
Control	12	5.71 A	5.78 AB	6.57 A	5.98 A	5.17 A	4.93 AB
5% irradiated	12	5.48 AB	5.65 AB	6.36 B	5.77 AB	5.11 A	4.78 AB
10% irradiated	12	5.51 AB	5.84 A	6.40 AB	5.83 AB	5.25 A	5.01 A
25% irradiated	12	5.59 A	5.85 A	6.36 B	5.73 B	5.23 A	4.87 AB
50% irradiated	12	5.41 AB	5.47 BC	6.55 A	5.84 AB	5.00 A	4.65 B
100% irradiated	12	5.19 B	5.16 C	6.13 C	5.51 C	4.56 B	4.18 C
SEM		0.11	0.12	0.07	0.07	0.12	0.12
P value		0.05	0.01	0.01	0.01	0.01	0.01
Frozen storage time							
20 days	36	5.41	5.46 B	6.63 A	5.96 A	5.10	4.62 B
40 days	36	5.55	5.79 A	6.16 B	5.59 B	5.01	4.86 A
SEM		0.07	0.07	0.04	0.04	0.07	0.07
P value		0.13	0.01	0.01	0.01	0.36	0.02

<sup>a</sup> Ratings for the six attributes ranged from 8 (extremely intense, none, extremely tender, extremely juicy, extremely intense, and none, respectively) to 1 (none, extremely intense, extremely tough, extremely dry, extremely bland, and extremely intense, respectively). Within main effect, means in same column that do not share a common letter are significantly different.

rect plating is useful for large numbers of samples and is reasonably sensitive but had two notable shortcomings in this study: (i) the limit of detection was 10 CFU/cm<sup>2</sup> and (ii) plating directly onto selective agar does not allow for recovery of injured cells and the estimated number of viable cells may be slightly lower than the actual number. For these reasons, an MPN method was used for a subset of the treated samples. The MPN method provided results with a lower limit of detection (0.036 CFU/cm<sup>2</sup>); thus, *E. coli* O157:H7 cell counts were obtained for some samples for which direct plating produced no growth.

In previous studies, E-beam radiation has been used to kill a broad spectrum of bacterial species, including *E. coli* O157:H7 (18, 19, 26, 29). The data presented here indicate that an E-beam radiation dose of approximately 1 kGy with a penetration depth of 15 mm reduced stationary-phase *E. coli* O157:H7 on the surface of beef tissue by at least 4 log CFU/cm<sup>2</sup>. However, the study was conducted using only one strain of *E. coli* O157:H7. Buchanan et al. (6, 7) found that the radiation resistance of *E. coli* O157:H7 strains can

TABLE 6. Effect of proportion of irradiated trim on color of raw ground beef patties<sup>a</sup>

Treatment	L*	a*	b*
Control	49.7	15.2	17.7
5% irradiated	48.4	14.4	16.7
10% irradiated	47.9	15.0	17.1
25% irradiated	49.5	15.2	17.7
50% irradiated	50.3	14.4	17.2
100% irradiated	49.7	15.9	18.1
SEM	0.72	0.55	0.32
P value	0.19	0.43	0.06

<sup>a</sup> Color based on Hunter attributes: L\*, lightness; a\*, redness; b\*, yellowness.

be variable, especially with respect to the level of acid tolerance (both induced and noninduced) of the particular strain. Therefore, if other strains had been incorporated into this study, the overall reduction might not have been as large. However, other *E. coli* O157:H7 strains have been used in numerous irradiation experiments, and the results were similar to those obtained here. Using a 1.5-kGy dose of gamma radiation, Fu et al. (16) obtained a 5-log CFU/g reduction of *E. coli* O157:H7 on surface-inoculated steaks. In other studies using ground beef, similar pathogen reductions have been attributed to the antimicrobial effects of irradiation. A 1-kGy dose of gamma radiation resulted in a 3- to 4-log CFU/g reduction of *E. coli* O157:H7 in frozen and refrigerated ground beef, respectively (9). Similarly, Thayer and Boyd (33) projected that *E. coli* O157:H7 contamination at 10<sup>6</sup> CFU/g in ground beef would be completely eliminated by gamma irradiation with a 1.5-kGy dose at 0°C.

Conventional antimicrobial interventions have been evaluated in several studies (10). Knife trimming and steam vacuuming, which can produce large bacterial reductions in localized areas, are useful for pathogen reduction in visibly contaminated areas or carcass regions believed to be hot-spots for contamination (e.g., hide removal pattern lines). However, these techniques cannot be used efficiently for the entire carcass. Carcass washing systems and steam pasteurization cabinets have been implemented to decontaminate whole carcasses. Hot water and organic acids are frequently used in both pre- and postvisceration carcass wash cabinets. *E. coli* O157:H7 populations have been reduced by 3.4, 4.0, and 3.5 log CFU/cm<sup>2</sup> (similar to reductions obtained in this study with E-beam irradiation) using hot water, lactic acid, and steam pasteurization, respectively (11, 12, 28). A portion of the reductions obtained in those studies could be attributed to rinsing effects, indicating that

such interventions are not necessarily completely bactericidal. In some studies, such interventions have been ineffective against bacteria attached to meat surfaces (5, 15).

Radiolytic products can cause oxidation of myoglobin and fat, leading to discoloration and rancidity or off-odors and off-flavors (23). The development of off-odors and off-flavors in irradiated meat can be affected by a number of factors, including radiation dose, dose rate, temperature, within-package environment during irradiation, postirradiation storage time, temperature, and packaging, and the condition of the meat before irradiation (27, 31). To minimize the development of objectionable odors and flavors, meat should be irradiated in a reduced-oxygen or oxygen-free atmosphere at the minimum required dose to meet safety goals (27).

A number of studies of the effect of irradiation on meat quality have been conducted on various meat products, including whole and minced chicken and chicken pieces, pork loins and chops, beef steaks, and ground turkey, pork, and beef. Results from most of these studies indicate that at low radiation doses ( $\leq 1$  kGy) no problems with odor or taste occurred. However, as dose increased to 2 kGy or higher, the frequency of off-odors and off-flavors increased (32).

In the limited number of studies specifically designed to test the effect of irradiation on sensory qualities of ground beef, the results are mixed. Weese et al. (36) studied ground beef patties irradiated at 0, 1, 3, 5, and 7 kGy and then stored at  $-18^{\circ}\text{C}$  for 6 weeks. Trained sensory panel evaluation was conducted weekly over the 6-week period. No significant differences were detected between irradiated and untreated patties for any irradiation dose of less than 7 kGy for the entire 6-week frozen storage period. Luchsinger et al. (21) studied frozen ground beef patties irradiated at 0, 2.0, or 3.5 kGy and then stored at  $-19^{\circ}\text{C}$  for 1 day. Patties were formulated at either 10 or 22% fat with either aerobic or vacuum packaging. No effect of irradiation was detected on odor or various flavor measures by a trained flavor profile panel, perhaps because storage was limited to 1 day. Lefebvre et al. (17) studied ground beef irradiated at 0, 1.0, 2.5, and 5.0 kGy and then stored for 16 days at  $4^{\circ}\text{C}$ . A 10-member nonexpert panel detected an objectionable odor in the raw irradiated product (all doses), although this effect was not detectable after cooking for the 1 kGy treatment group. These authors recommended a dosage of 1 kGy to avoid consumer acceptance problems. Fu et al. (16) studied ground beef irradiated at 0, 0.6, and 1.5 kGy and then stored for 7 days at  $7^{\circ}\text{C}$ . No effect on odor of raw product immediately after irradiation was detected by an untrained sensory panel. Using a trained sensory panel, Murano et al. (24) studied ground beef patties irradiated at 0, 2, and 5 kGy and then stored at  $-25^{\circ}\text{C}$  for 3 days. Irradiated ground beef could be distinguished from the control when samples were stored in air but not when they were stored under vacuum. No flavor differences were detected between control and irradiated ground beef samples. Lopez-Gonzalez et al. (20) reported that ground beef patties irradiated with 2 kGy by gamma radiation or E-beam at  $5^{\circ}\text{C}$ , packaged in air, and evaluated 2 days after irradiation were not different from the controls in sensory properties.

Emmerson et al. (14) reported that irradiated ground beef had higher thiobarbituric acid concentrations than did controls and, thus, greater oxidation. However, these authors concluded that antioxidants (rosemary, vitamin E, and erythorbate) reduced the effect of irradiation on thiobarbituric acid concentrations and may retard irradiation-induced oxidation. In agreement, Nam and Ahn (25) reported that antioxidants in irradiated pork patties reduced the volatile compounds that may contribute to off-odors and off-flavors.

Wheeler et al. (37) indicated that despite changes in aroma and flavor that were large enough to be detected by a trained descriptive attribute panel and were ascribed to irradiation of vacuum-packaged frozen ground beef patties at both 3.0 and 4.5 kGy, consumers rated hamburgers made with meat irradiated at all doses as some level of "fair" for taste. Individual sensitivities to various taste and smell stimuli are variable, and women generally are more sensitive than men (1). Thus, as expected, consumer ratings were variable, with a majority able to detect slight or no differences in taste of hamburgers made with patties from different irradiation treatments and small proportions of consumers rating hamburgers made with irradiated patties as better or worse than those made with control patties. Wheeler et al. (37) concluded that the irradiation-induced changes in sensory traits produced minimal effects on consumer taste ratings at the minimum irradiation dose (3.0 kGy) needed to elicit a 5-decimal kill of *E. coli* O157:H7 in 19% fat vacuum-packaged frozen ground beef patties.

*E. coli* O157:H7 contamination on beef carcasses following conventional multihurdle antimicrobial interventions is minimal, as indicated by the limited data available. Barkocy-Gallagher et al. (3) found that beef carcasses from several major processing plants had *E. coli* O157:H7 concentrations of  $<3$  CFU/100  $\text{cm}^2$  following the full complement of antimicrobial interventions. Such contamination could easily be eliminated using low-dose, low-penetration E-beam technology. E-beam treatment also has a minimal effect on the organoleptic qualities of surface-exposed beef products. Therefore, an E-beam intervention step before beef carcass fabrication would be highly effective for pathogen reduction.

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## REFERENCES

1. Amerine, M. A., R. M. Pangborn, and E. B. Roessler. 1965. Principles of sensory evaluation of food. Academic Press, New York.
2. Arthur, T. M., J. M. Bosilevac, X. Nou, S. D. Shackelford, T. L. Wheeler, M. P. Kent, D. Jaroni, B. Pauling, D. M. Allen, and M. Koohmaraie. 2004. *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae*, and *Escherichia coli*



- O157 at various steps in commercial beef processing plants. *J. Food Prot.* 67:658–665.
3. Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of Shiga toxin-producing *Escherichia coli* O157:H7 and non-O157 serotypes and *Salmonella* in commercial beef processing plants. *J. Food Prot.* 66:1978–1986.
  4. Barkocy-Gallagher, G. A., E. D. Berry, M. Rivera-Betancourt, T. M. Arthur, X. Nou, and M. Koohmaraie. 2002. Development of methods for the recovery of *Escherichia coli* O157:H7 and *Salmonella* from beef carcass sponge samples and bovine fecal and hide samples. *J. Food Prot.* 65:1527–1534.
  5. Brackett, R. E., Y. Y. Hao, and M. P. Doyle. 1994. Ineffectiveness of hot acid sprays to decontaminate *Escherichia coli* O157:H7 on beef. *J. Food Prot.* 57:198–203.
  6. Buchanan, R. L., S. G. Edelson, and G. Boyd. 1999. Effects of pH and acid resistance on the radiation resistance of enterohemorrhagic *Escherichia coli*. *J. Food Prot.* 62:219–228.
  7. Buchanan, R. L., S. G. Edelson-Mammel, G. Boyd, and B. S. Marmar. 2004. Influence of acidulant identity on the effects of pH and acid resistance on the radiation resistance of *Escherichia coli* O157:H7. *Food Microbiol.* 21:51–57.
  8. Castillo, A., L. M. Lucia, K. J. Goodson, J. W. Savell, and G. R. Acuff. 1999. Decontamination of beef carcass surface tissue by steam vacuuming alone and combined with hot water and lactic acid sprays. *J. Food Prot.* 62:146–151.
  9. Clavero, M. R. S., J. D. Monk, L. R. Beuchat, M. P. Doyle, and R. E. Brackett. 1994. Inactivation of *Escherichia coli* O157:H7, salmonellae, and *Campylobacter jejuni* in raw ground beef by gamma irradiation. *Appl. Environ. Microbiol.* 60:2069–2075.
  10. Dorsa, W. J. 1997. New and established carcass decontamination procedures commonly used in the beef processing industry. *J. Food Prot.* 60:1146–1151.
  11. Dorsa, W. J., C. N. Cutter, and G. R. Siragusa. 1997. Effects of acetic acid, lactic acid and trisodium phosphate on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157:H7, *Listeria innocua*, and *Clostridium sporogenes*. *J. Food Prot.* 60:619–624.
  12. Dorsa, W. J., C. N. Cutter, and G. R. Siragusa. 1997. Effects of steam-vacuuming and hot water spray wash on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157:H7, *Listeria innocua*, and *Clostridium sporogenes*. *J. Food Prot.* 60:114–119.
  13. Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci. USA* 97:2999–3003.
  14. Emmerson, E. P., J. G. Sebranek, and D. G. Olson. 1998. Evaluation of antioxidant effectiveness for improving quality of irradiated ground beef. *Proc. Recip. Meat Conf.* 51:177–178.
  15. Fratamico, P. M., F. J. Schultz, R. C. Benedict, R. L. Buchanan, and P. H. Cooke. 1996. Factors influencing attachment of *Escherichia coli* O157:H7 to beef tissues and removal using selected sanitizing rinses. *J. Food Prot.* 59:453–459.
  16. Fu, A., J. G. Sebranek, and E. A. Murano. 1995. Survival of *Listeria monocytogenes*, *Yersinia enterocolitica* and *Escherichia coli* O157:H7 and quality changes after irradiation of beef steaks and ground beef. *J. Food Sci.* 60:972–977.
  17. Lefebvre, N., C. Thibault, R. Charbonneau, and J.-P. G. Piette. 1994. Improvement of shelf-life and wholesomeness of ground beef by irradiation. 2. Chemical analysis and sensory evaluation. *Meat Sci.* 36:371–380.
  18. Lewis, S. J., A. Velásquez, S. L. Cuppett, and S. R. McKee. 2002. Effect of E-beam irradiation on poultry meat safety and quality. *Poult. Sci.* 81:896–903.
  19. Lopez-Gonzalez, V., P. S. Murano, R. E. Brennan, and E. A. Murano. 1999. Influence of various commercial packaging conditions on survival of *Escherichia coli* O157:H7 to irradiation by electron beam versus gamma rays. *J. Food Prot.* 62:10–15.
  20. Lopez-Gonzalez, V., P. S. Murano, R. E. Brennan, and E. A. Murano. 1999. Sensory evaluation of ground beef patties irradiated by gamma rays versus electron beam under various packaging conditions. *J. Food Qual.* 23:195–204.
  21. Luchsinger, S. E., D. H. Kropf, E. Chambers IV, C. M. Garcia Zepeda, M. C. Hunt, S. L. Stroda, M. Hollingsworth, J. L. Marsden, and C. L. Kastner. 1997. Sensory analysis of irradiated ground beef patties and whole muscle beef. *J. Sensory Stud.* 12:105–126.
  22. Molins, R. A., Y. Motarjemi, and F. K. Käferstein. 2001. Irradiation: a critical control point in ensuring the microbiological safety of raw foods. *Food Control* 12:347–356.
  23. Murano, E. A. 1995. Irradiation of fresh meats. *Food Technol.* 12:52–54.
  24. Murano, E. A., P. S. Murano, and D. G. Olson. 1997. Quality characteristics and sensory evaluation of meats irradiated under various packaging conditions, p. 276–277. In Proceedings of the 41st Annual International Congress of Meat Science and Technology, San Antonio, Tex. American Meat Sciences Association, Chicago.
  25. Nam, K. C., and D. U. Ahn. 2003. Use of antioxidants to reduce lipid oxidation and off-odor volatiles of irradiated pork homogenates and patties. *Meat Sci.* 63:1–8.
  26. Niebuhr, S. E., and J. S. Dickson. 2003. Destruction of *Bacillus anthracis* strain Sterne 34F2 spores in postal envelopes by exposure to electron beam irradiation. *Let. Appl. Microbiol.* 37:17–20.
  27. Olson, D. G. 1998. Irradiation of food. *Food Technol.* 52:56–62.
  28. Phebus, R. K., A. L. Nutsch, D. E. Schafer, R. C. Wilson, M. J. Riemann, J. D. Leising, C. L. Kastner, J. R. Wolf, and R. K. Prasai. 1997. Comparison of steam pasteurization and other methods for reduction of pathogens on surfaces of freshly slaughtered beef. *J. Food Prot.* 60:476–484.
  29. Radomyski, T., E. A. Murano, D. G. Olson, and P. S. Murano. 1994. Elimination of pathogens of significance in food by low-dose irradiation: a review. *J. Food Prot.* 57:73–86.
  30. Satin, M. 2002. Use of irradiation for microbial decontamination of meat: situation and perspectives. *Meat Sci.* 62:277–283.
  31. Thayer, D. W. 1990. Food irradiation: benefits and concerns. *J. Food Qual.* 13:147–169.
  32. Thayer, D. W. 1993. Extending shelf life of poultry and red meat by irradiation processing. *J. Food Prot.* 56:831–833, 846.
  33. Thayer, D. W., and G. Boyd. 1993. Elimination of *Escherichia coli* O157:H7 in meats by gamma irradiation. *Appl. Environ. Microbiol.* 59:1030–1034.
  34. U.S. Department of Agriculture, Food Safety and Inspection Service. 1999. Food irradiation of meat food products, final rule. *Fed. Regist.* 64:72149–72166.
  35. U.S. Food and Drug Administration. 1997. 21 CFR Part 179, Irradiation in the production, processing, and handling of food. *Fed. Regist.* 62:64107–64121.
  36. Weese, J. O., J. H. Johnson, and W. T. Roberts. 1997. Sensory changes of irradiated ground beef through six weeks of storage, p. 42. In Proceedings of the 84th Annual Meeting of the International Association of Milk, Food and Environmental Sanitarians, Inc. (Suppl. A), Orlando, Fla.
  37. Wheeler, T. L., S. D. Shackelford, and M. Koohmaraie. 1999. Trained sensory panel and consumer evaluation of the effects of gamma irradiation on palatability of vacuum-packaged frozen ground beef patties. *J. Anim. Sci.* 77:3219–3224.