Appendix 1

Journal of Food Protection, Vol. 68, No. 4, 2005, Pages 666–672

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

TERRANCE M. ARTHUR, TOMMY L. WHEELER, STEVEN D. SHACKELFORD, JOSEPH M. BOSILEVAC, XIANGWU NOU,‡ AND MOHAMMAD KOOHMARAIE*

U.S. Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska 68933-0166, USA

MS 04-449: Received 29 September 2004/Accepted 3 December 2004

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^2. 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

* Author for correspondence. Tel: 402-762-4221; Fax: 402-762-4149; E-mail: koohmaraie@email.marc.usda.gov.

† 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.

‡ Present address: USDA, ARS, Animal and Natural Resource Institute, Building 201, BARC-East, 10300 Baltimore Avenue, Beltsville, MD 20705-2350, USA.

beef carcasses during the course of conventional carcass disassembly. Tissues were vacuum packaged and transported (−3°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 ± standard deviation, 1.04 ± 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^2 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 5×10^8 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^2), and 20 pieces were used for low-concentration inoculation (approximately 10^3 CFU/cm^2). To inoculate the sections, 4 ml of the appropriate culture dilution was dispensed across the 400-cm^2 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^2 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^2 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^2 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 × inoculum level × 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*[a]

	High concentration inoculum			Low concentration inoculum		
Sample	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[b]		0.0 G	0.1 G		0.0 G	0.0 G

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

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

TABLE 2. *MPN estimates for* E. coli *O157:H7 following electron beam irradiation of beef*[a]

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

[a] Values are CFU per square centimeter.

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

[c] Maximum MPN within group.

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 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^2. 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^2. 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^2 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[a]					
	Beef aroma intensity	Off-aroma	Tenderness	Juiciness	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

[a] 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^2 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*[a]

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

[a] 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[a]					
		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

[a] 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^2 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^2); 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^2. 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 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 10^6 CFU/g in ground beef would be completely eliminated by gamma irradiation with a 1.5-kGy dose at 0°C.

TABLE 6. *Effect of proportion of irradiated trim on color of raw ground beef patties*[a]

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

[a] Color based on Hunter attributes: L*, lightness; a*, redness; b*, yellowness.

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 hotspots 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 postevisceration carcass wash cabinets. *E. coli* O157:H7 populations have been reduced by 3.4, 4.0, and 3.5 log CFU/cm^2 (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°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°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°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°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°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°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 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.

ACKNOWLEDGMENTS

This project was funded in part by beef and veal producers and importers through their $1-per-head checkoff and was produced for the Cattlemen's Beef Board and state beef councils by the National Cattlemen's Beef Association and by the American Meat Institute Foundation. We thank Julie Dyer, Bruce Jasch, Kathy Mihm, Frank Reno, Greg Smith, and Pat Tammen for technical support and Carol Grummert for secretarial support. We also thank Carrie Thomas (Tyson Fresh Meats, Inc.) and John Logar, Richard Galloway, and Chip Colonna (IBA) for their assistance in conducting these experiments.

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. Marmer. 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. *Lett. 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.

SILLIKER
Assuring Quality Worldwide

Research Report

July 19, 2004

Shelf-life Study of the Impact of Low Dose, Low Penetration Electron Beam Surface Treatment of Beef

Prepared for

Randy Huffman
American Meat Institute Foundation

rhuffman@meatami.com

Prepared by

Erdogan Ceylan, Ph.D.
Operations Manager

Ann Marie McNamara, Sc. D.
Vice President
Food Safety and Scientific Affairs

Objective:
The objective of this study was to evaluate the effects of a carcass irradiation treatment on the shelf-life of rough-cut navel plates.

Protocol:

Twelve 'rough-cut' plates were removed from chilled beef carcasses at ~24-hr after initial post-slaughter chill and split into equal halves, individually bagged (poly liner) and labeled with an appropriate numbering system and randomized for treatment or control groups.

Six plates were designated as 'air-exposed'. Three were irradiated and three were controls. These plates remained as 'rough-cuts' with no additional trimming throughout the shelf-life period.

Six plates were designated as 'vac-pac'. Three were irradiated and three were control. All vac-pac plates were trimmed to industry standard finished product specifications (export plates) and then vacuum packaged and fresh chilled stored throughout shelf-life.

After bagging, all plates were shipped via refrigerated truck to the RDI test facility in Long Island, NY for irradiation treatments. All irradiation treatments for shelf-life were performed on the same day as well as subsequent packaging and chill storage initiation. Plates were irradiated with a 1 kGy surface treatment with an ~15mm penetration (1 pass).

After irradiation treatments, treatment and control plates were randomly subdivided into four equal segments. Each segment then was allocated into a time-slot designated below for shelf-life assessment.

- Air Exposed Shelf-life (days post irradiation)
  - 1, 3, 6 and 9 days
- Vac-Pac Shelf-life (days post irradiation)
  - 1, 10, 20 and 30 days

Treated and control samples were handled and processed equally to simulate normal handling and prevent biased sampling. Product temperatures were maintained at 35-45°F during fabrication, transportation, irradiation and final packaging. Storage temperature during shelf-life were maintained at 40°F.

Samples for thiobarbituric acid (TBA) analysis and various microbiology analyses were acquired by tissue excision (~100cm^2 x 1/8" thick). Each sample were analyzed for total aerobic plate count, hetero- and homo-lactics, total coliforms and Biotype I *E. coli*. The methods of analysis are outlined in Table 1.

Sample Analyses:

Table 1. Methods used for microbiological and chemical (thiobarbituric acid) analyses of rough-cut navel plates.

Test	Medium	Time, Temperature and Atmosphere
Total aerobic plate count	Tryptone Glucose Yeast Agar	35°C, 48 hr, aerobic
Hetero and homo lactics	DeMan Rogosa Sharpe Agar with overlay	30°C, 5 days, microaerobic
Total coliforms	Most Probable Number Method (<3 MPN/cm^2)	35°C,24 - 48 hr, aerobic
Biotype I *E. coli*	Most Probable Number Method (<3 MPN/cm^2)	35-45°C, 24- 48 hr, aerobic

Test	Method
TBA	JAOCS 37(1):44

Results and Discussion

The effect of the irradiation treatment was evaluated by comparing the levels of total aerobic plate count (APC), hetero- and homo-lactics (LAB), total coliforms (TC) and Biotype I *E. coli* (EC) in the untreated and treated samples during storage at 40°F. Results are shown as the average log CFU/cm^2 of three independent samples and the standard deviations at each time period.

Figures 1 through 4 show the effects of irradiation on the microbial flora of air-exposed beef navels during four days of storage at 40°F. For APC and LAB, irradiation resulted in the recovery of fewer bacteria throughout shelf-life. At day 9, APC and LAB counts were 1.3 log cfu/cm^2 and 1.8 log cfu/cm^2 higher, respectively, for non-irradiated versus irradiated plates. This data shows that irradiated samples had nearly caught up to the controls at the end of shelf-life. No TC or EC was recovered on either irradiated or non-irradiated beef navels, except at day 9 when low numbers of TC were detected. This suggests that sporadic, low numbers of TC may occur at the end of shelf-life or the data may represent sampling contamination.

Figures 5 through 8 show the effects of irradiation on the microflora of vacuum-packed beef navels during thirty days of storage at 40°F. Similar to the results for irradiated air-exposed beef navels, irradiation of vacuum-packed beef navels resulted in the recovery of fewer APC and LAB throughout shelf-life in the irradiated versus non-irradiated samples. APC and LAB remained within 1.5 logs of each other throughout shelf-life. It appears that an error occurred in the collection, processing, or laboratory analysis of day 1 samples for APC and LAB.

Two out of three irradiated samples at day 1 had APC counts greater than 100 CFU/cm^2. This result was inconsistent with bacterial levels recovered in other data sets and suggests experimental error. However, this inconsistent result did not appear to influence the results of the overall process since the trends for these samples are consistent with other experimental test results in which bacterial numbers are lowest at day 1 and continue to increase over shelf-life. Sporadic microcolonies of TC (days 1 and 20) and EC (day 20) were recovered on two sampling days for irradiated beef navels; otherwise all sampling dates were negative for both irradiated and non-irradiated beef navels. As shown by the standard deviations on the figures, these results are low-level spurious results that could have been zero and represent low-level recovery on one out of three navel samples by Most Probable Number method.

TBA results can be analyzed according to the interpretation guidelines provided in Table 2 (Sofos, 2004). In general, TBA results were slightly higher in irradiated samples versus non-irradiated samples, although the results generally fall within the standard deviations of non-irradiated samples (Figures 9 and 10). TBA levels were lower in vacuum-packed beef navels versus air-exposed beef navels. These results are in agreement with those of Murano et al. (1998). These researchers exposed ground beef to an irradiation treatment of 2 kGy and studied the effects of subsequent storage under various packaging conditions. While beef patties irradiated in air had a higher degree of lipid oxidation than those irradiated under vacuum or nonirradiated controls, irradiated patties had TBA values that were the same as controls after one week of storage at 4^{o}C.

Vacuum packed beef navels (Figure 9) ranged from limited, tolerably oxidized to somewhat oxidized over 30 days of shelf-life. Air-exposed navels (Figure 10) ranged from limited, tolerably oxidized at day 2 of shelf-life to oxidized at day 9 of shelf life. All samples were below the range of rancidity.

Conclusion

When measured by the shelf-life indicators of APC and LAB, irradiation resulted in the recovery of fewer bacteria throughout shelf-life. APC and LAB counts in irradiated samples increased over shelf-life and had nearly approximated the controls at the end of shelf-life (results within approximately 1.5 logs).

The TBA results were all below rancidity for both the irradiated samples and the nonirradiated controls. In general, TBA results were slightly higher in irradiated samples versus non-irradiated samples. The data would suggest that the irradiated samples would turn rancid slightly before the nonirradiated samples.

References

Murano, P.S., Murano, E. A., and Olson, D.G. (1998). Irradiated ground beef: Sensory and quality changes during storage under various packaging conditions. J. Food Sci. 63:548-551.

Sofos J.N., 2004. Colorado State University. Personal communication

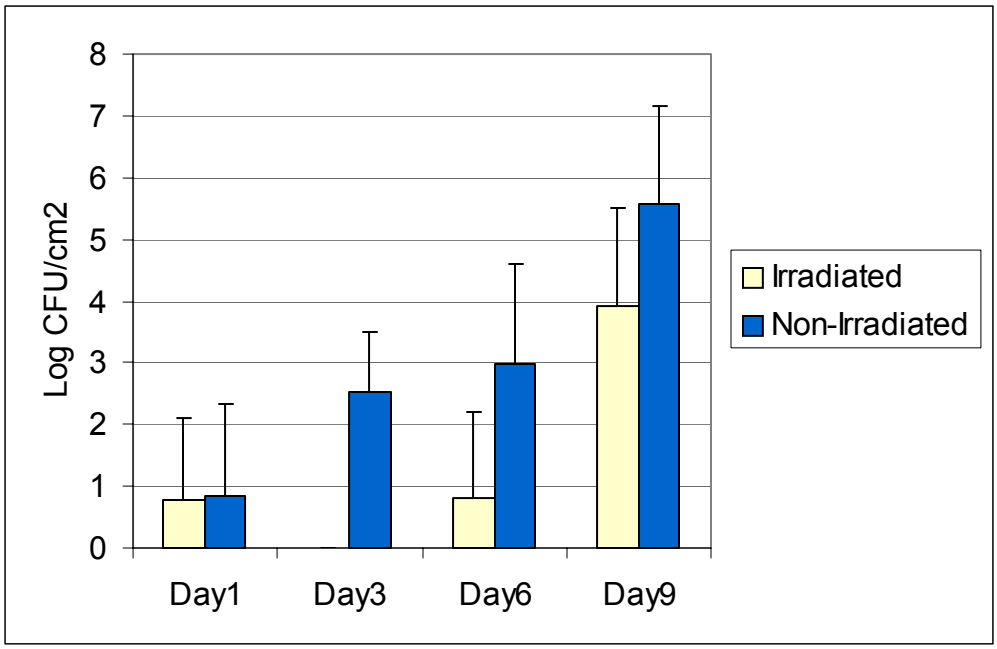

Figure 1. Aerobic plate counts of air-exposed beef navels during storage at 40°F.

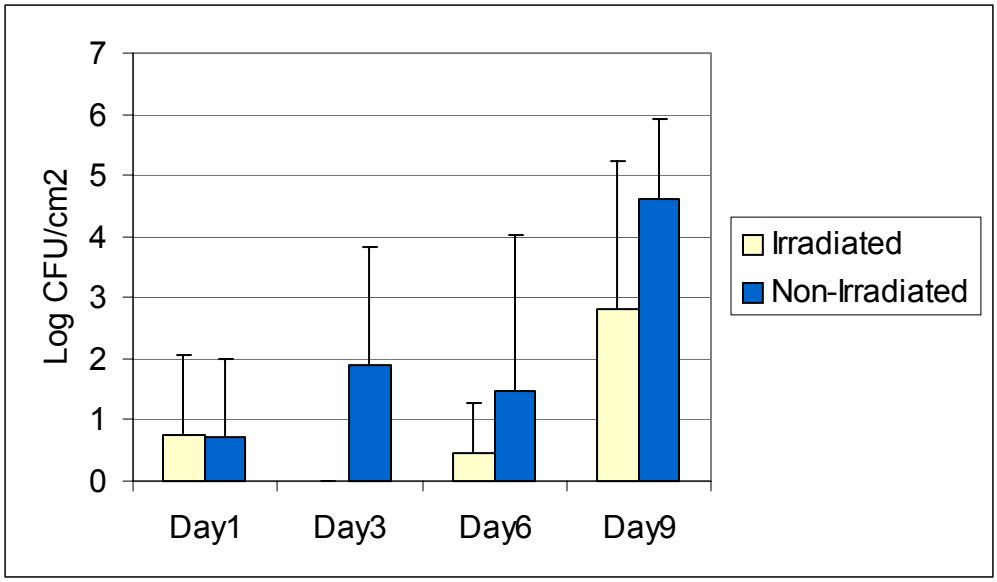

Figure 2. Lactic acid bacteria counts of air-exposed beef navels during storage at 40°F.

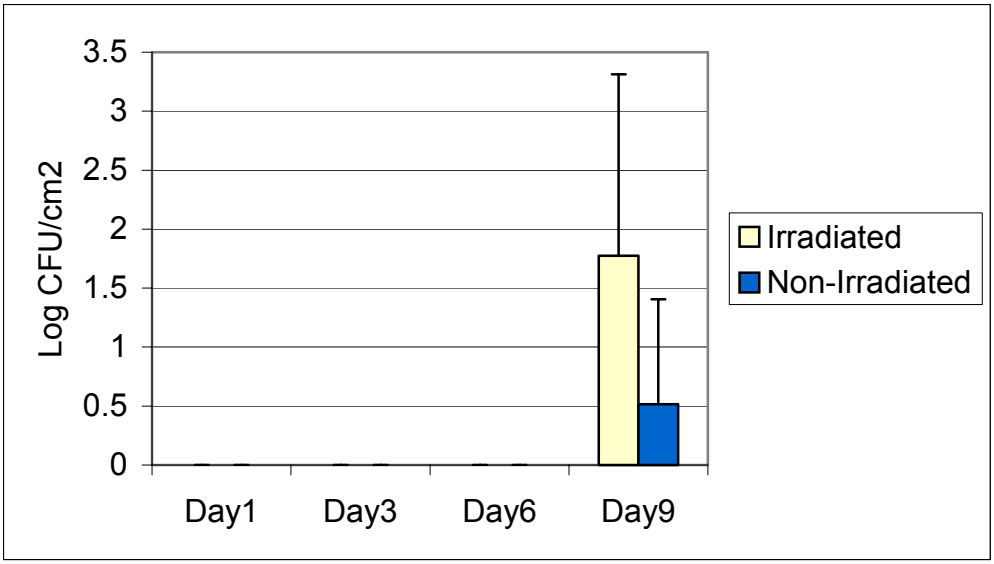

Figure 3. Total coliform counts of air-exposed beef navels during storage at 40°F.

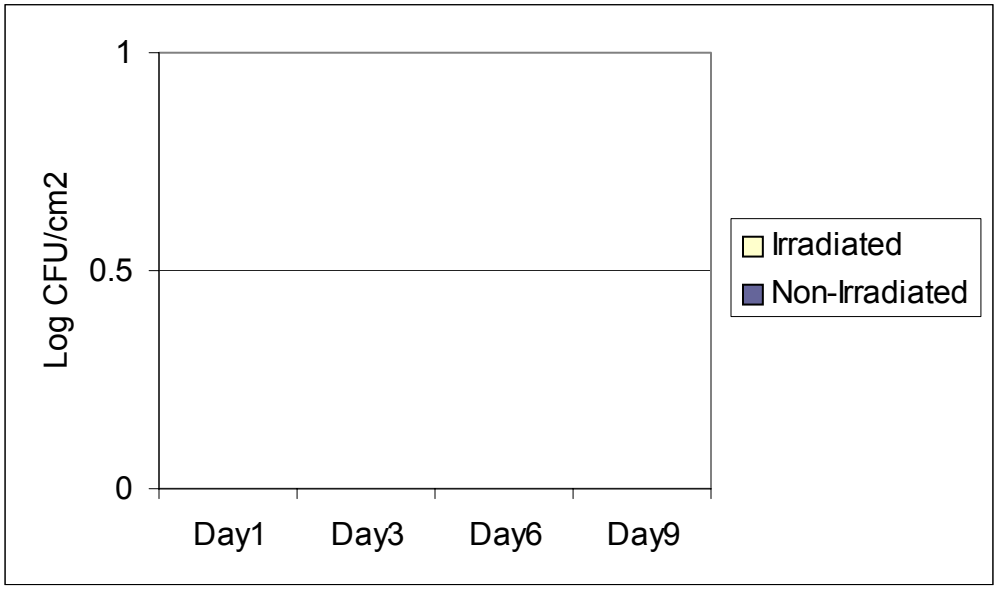

Figure 4. *Escherichia coli* counts of air-exposed beef navels during storage at 40°F.

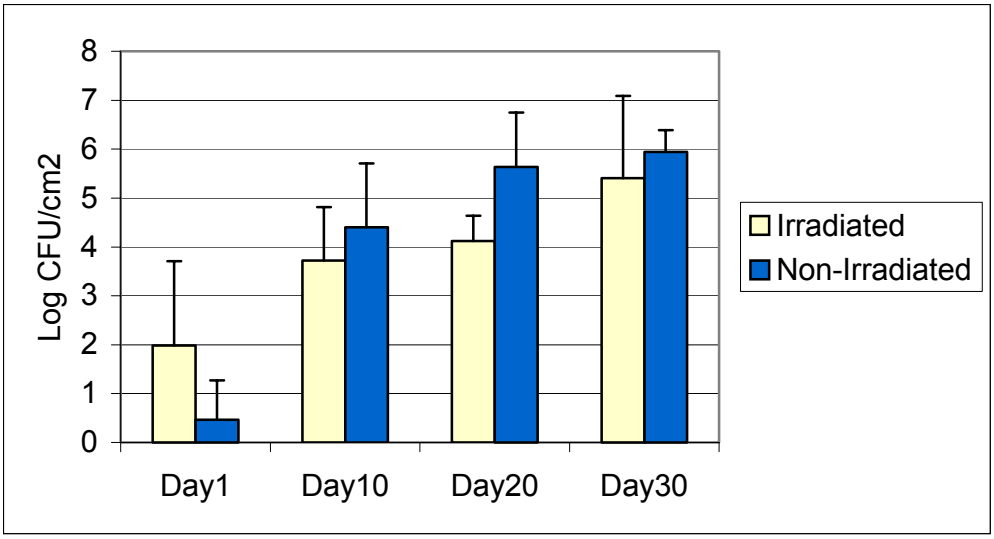

Figure 5. Aerobic plate counts of vacuum packed beef navels during storage at 40°F.

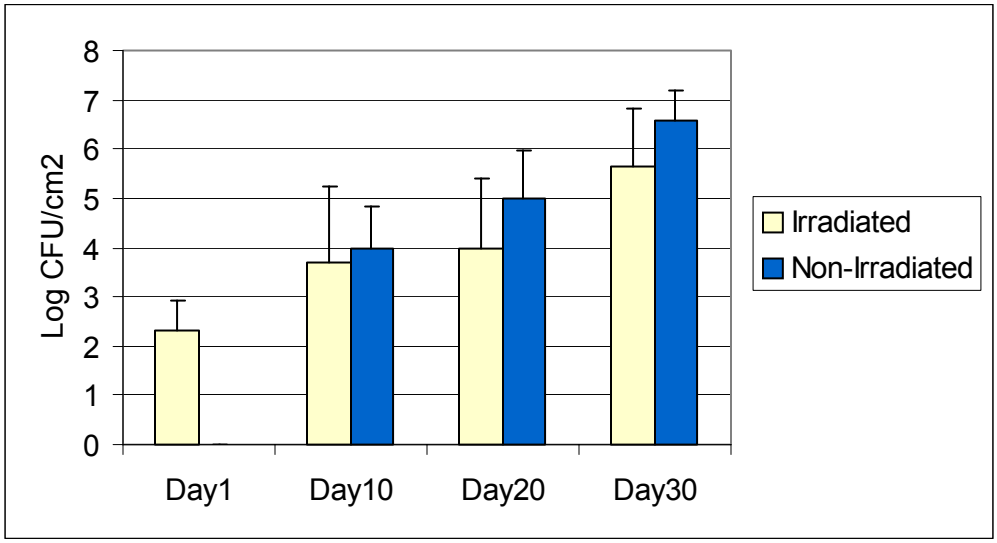

Figure 6. Lactic acid bacteria counts of vacuum packed beef navels during storage at 40°F.

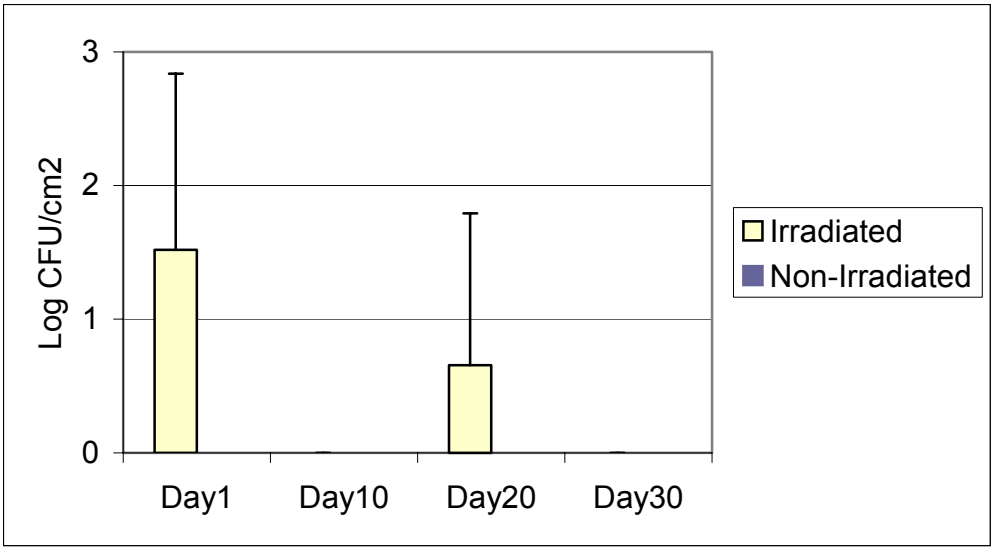

Figure 7. Coliforms counts of vacuum packed beef navels during storage at 40°F.

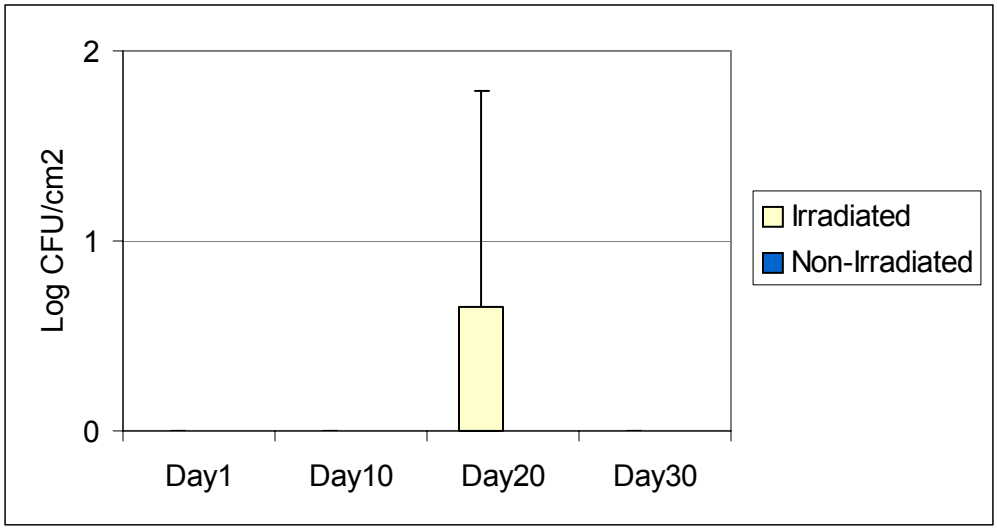

Figure 8. *Escherichia coli* counts of vacuum packed beef navels during storage at 40°F.

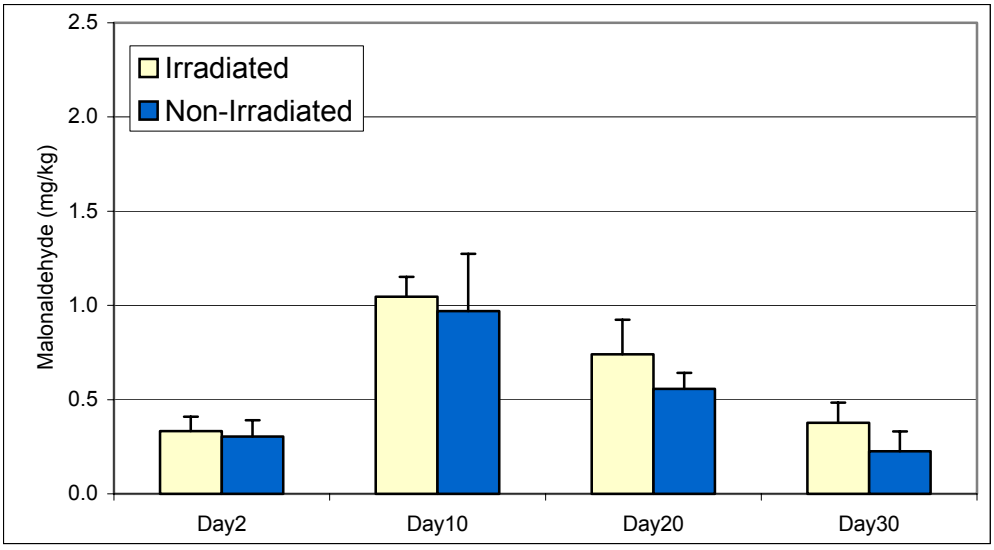

Figure 9. Thiobarbituric Acid (TBA: malonaldehyde mg/kg) values of vacuum packaged beef navels stored at 4°C.

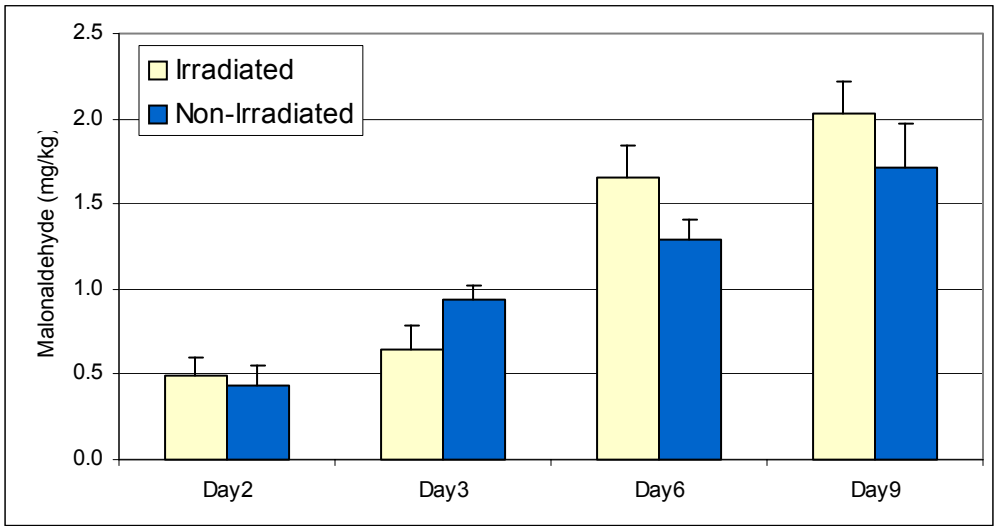

Figure 10. Thiobarbituric Acid (TBA; malonaldehyde mg/kg) values of air exposed beef navels stored at 4°C.

Table 2. Interpretations of Thiobarbituric Acid Reactive Substances (TBARS) values as mg of malonaldehyde per kg sample

TBARS	Interpretation
< 0.2	Good
0.2-0.5	Limited, tolerable
0.5-1.5	Somewhat oxidized
1.5-5.0	Oxidized
> 5.0	Rancid

Literature Review and Analysis of The Effects of Beef Carcass Surface Irradiation on Micro- and Macro-Nutrients

Donald W. Thayer, Ph.D.

274 Almwch Place, Lower Gwynedd, PA 19002-2018

Phone/fax 215-641-9412; dwscthayer@aol.com; dwschome@verizon.net

October 4, 2004

Prepared for: AMI Foundation, 1700 North Moore Street, Suite 1600, Arlington, VA 22209

I. Objective

The objective of this report is to provide an evaluation of the impact on macro- and micro-nutrients of a surface treatment of chilled beef carcasses with a very low penetration (≤20 mm) and very low dosage (≤1.0 kGy) electron beam.

The following reviews should be consulted for an overview of the effects of ionizing radiation on foods: Diehl (1995), Diehl and Josephson (1994), Joint FAO/IAEA/WHO Study Group on High-Dose Irradiation (1999), Murray (1983), Skala and others (1987), Thayer (1990), and Thayer (1994).

II. Effects of ionizing radiation on macro-nutrients (proximates)

Large scale nutritional, genetic, teratogenic, and multigenerational feeding studies of irradiation-sterilized, enzyme-inactivated chicken meat were initiated by the U.S. Army in 1976 by contract to Raltech Scientific Services of St. Louis, Missouri (Thayer and others 1987). The study required 298,568 lbs of chicken and compared the effects of frozen storage, thermal sterilization, and both electron and gamma-sterilization doses of 46 to 68 kGy at -25°C ± 15°C. The scale of this study provides a unique opportunity to assess the effects of high-dose radiation treatments and, by extrapolation, low-dose effects on both the micro- and macro-nutrients of chicken and other meats based on the proven concept of chemiclearance (Merritt and Taub 1983). There were no changes in the water, protein, fat, and ash levels of the chicken meat as a result of either gamma- or electron-sterilization, nor were there changes in the pH (Wierbicki 1985). Protein-efficiency ratios for rats fed the irradiation-sterilized chicken were not adversely affected (Thayer and others 1987). Josephson (1983) reported that on the basis of the

concentrations of 18 amino acids there was no adverse effect on the protein of gamma- or electron-irradiation sterilized beef.

No significant differences occurred in the peroxide and iodine values of lipids following irradiation (up to 10 kGy) of the *m. longissimus dorsi* from pork, lamb, and beef; nor were any changes detected in turkey leg and breast muscle. There were no significant changes following irradiation in the malonaldehyde concentrations in pork, lamb, and beef *m. longissimus dorsi* (Hampson and others 1996).

III. Effects of ionizing radiation on micro-nutrients

A. Free fatty acids, individual fatty acids

No significant differences were identified in the means of the percentages of individual fatty acids, free fatty acid, and crude fat in enzyme-inactivated chicken meat as a result of having been gamma- or electron-irradiated at -25 ± 15°C to an absorbed dose of 46-68 kGy (Thayer 1990). Maxwell and Rady (1989) analyzed the effects of ionizing radiation at doses of 0 to 10 kGy at 2-5°C on the fatty acid profiles of neutral and polar lipids of skin and muscle tissue of chicken. Only minor changes were identified in the fatty acid profiles of the neutral and polar lipids of muscles and skin, and there were no statistically significant changes in the totals of saturated and unsaturated acids up to a dose of 10 kGy.

B. Amino acids

No significant differences were identified in the means for the percentages of the individual amino acids in enzyme-inactivated chicken meat as a result of having been gamma- or electron-irradiated at -25 ± 15°C to an absorbed dose of 46-68 kGy (Thayer 1990).

C. Vitamins

There have been many studies and reviews on effects of ionizing radiation on vitamins. The following reviews should be consulted for an overview of the effects of ionizing radiation on vitamins: Diehl (1995), Diehl and Josephson (1994), Joint FAO/IAEA/WHO Study Group on High-Dose Irradiation (1999), Kilcast (1994), Murray (1983), Skala and others (1987), Sheffner and Spector (1957), Thayer and others (1991), and Tobback 1977. Of the water soluble vitamins, thiamin appears to be the most sensitive to ionizing radiation with riboflavin being much less sensitive. Vitamins E and A are the most sensitive of the fat soluble vitamins. Product composition, temperature, and the partial pressure of oxygen are known to influence vitamin loss. This discussion will be limited to the effects of ionizing radiation on vitamins in beef; however, other studies will be cited when direct data is lacking on beef. The validity of extrapolating results from one species to another is based upon the observation that the effects of low-dose ionizing-irradiation on vitamins are predictable and the same or very similar regardless of species (Fox and others 1995, Lakritz and others1995, Lakritz and others 1998, and Merritt and Taub 1983).

Several authors studied the effects of sterilization doses of gamma radiation on vitamins in ground beef from which we may estimate the effect of a 1-kGy dose. Such extrapolations should, however, be viewed with care because of the large differences between the absorbed doses, temperatures, and atmospheres from those under consideration in this report. In general, one can expect freezing to significantly protect vitamins during irradiation. Alexander and others (1956) sealed raw ground beef in cans then froze and irradiated them to an absorbed dose of 3.0 megarep (27.9 kGy). However, the actual temperature during irradiation was not stated.

The means of the losses in thiamin, riboflavin, pyridoxine, niacin, choline, and folacin were 63, 10, 25, 0, 0, and 0%, respectively. These results would lead to predictions of losses of 2.3, 0.36, and 0.90% per kGy of thiamin, riboflavin, and niacin, respectively. Day and others (1957a) sealed raw ground beef in cans, froze it, and irradiated it to an absorbed dose of 3.0 to 3.2 megarep (27.9 to 29.8 kGy). The treatment temperature was not stated. The means of the losses in riboflavin, pyridoxine, niacin, and inositol were 10, 24, 0, and 0%, respectively. The loss per kGy for riboflavin and pyridoxine would be ~ 0.36 and 0.86% per kGy, respectively. Day and others (1957b) sealed fresh raw ground beef in cans, froze it, and irradiated it to an absorbed dose of 3.0 to 3.2 megarep (27.9 to 29.8 kGy). The treatment temperature was not stated. The means of the radiation destruction of thiamin in the ground beef from biological, microbiological, and chemical determinations were 66, 64, and 61%, respectively, and correspond to values of ~ 2.36, 2.29, and 2.19% per kGy, respectively.

Thayer (1990) reported the effects of radiation sterilization at -25 ± 15°C to an absorbed dose of 46-68 kGy on the nutrients of enzyme-inactivated chicken meat. Losses in thiamin of 32.0 and 14.3% were found in the gamma- and electron-irradiated meat, respectively, and would correspond to losses of 0.70 and 0.31% per kGy. The loss of thiamin from the electron-irradiated meat was not statistically significant. The percentages of riboflavin and folic acid were significantly higher in the electron-irradiated meat than in the frozen control. The percentages of niacin, pyridoxine, pantothenic acid, biotin, choline, vitamin A, vitamin D, vitamin K, and vitamin B_{12} were not significantly altered by radiation sterilization. Thomas and others (1981) also reported less loss of thiamin in electron-irradiated meat than in gamma-irradiated meat.

Ground beef was either packed under nitrogen or oxygen in cans and irradiated above freezing to a dose of 9.3 kGy (Groninger and others 1956). Under oxygen, losses of 24, 10, and 0% were observed in B_1, B_2, and niacin, respectively. These values correspond to losses of 2.58 and 1.07% in thiamin and riboflavin, respectively. Under nitrogen the losses were 51, and 20% for B_1 and B_2, respectively. The finding of a decreased loss under oxygen is surprising.

Fox and others (1989) determined the effects of gamma radiation doses from 0 to 6.65 kGy at temperatures from -20 to +20°C on thiamin (B_1), riboflavin (B_2), niacin, pyridoxine (B_6), and cobalamin (B_{12}) in raw and cooked pork chops and chicken breasts. The experimental samples were packaged in oxygen-permeable pouches and the temperature was monitored and controlled during irradiation. From this study we can predict that following a 1 kGy radiation dose at a temperature of 5°C (41°F) the loss of thiamin, riboflavin, niacin, pyridoxine, and cobalamin in pork would be 10.6%, 0%, 0%, 0%, and 0%, respectively. The destruction of thiamin was directly related to the temperature during irradiation and if, for example, the product were irradiated at -5°C (23°F), the loss in thiamin would be 7.90%.

Fox and others (1993) determined that a 1-kGy dose of gamma irradiation at 5° (41°F) would produce an 11.5% loss of thiamin from ground-beef gluteus maximus muscle tissue.

Fox and others (1995) and Lakritz and others (1995) compared the rates of loss of thiamin, riboflavin, and α-tocopherol in beef, lamb, and pork longissimus dorsi muscle and also in turkey leg and breast meats under identical conditions of preparation and irradiation. Rate constants were determined from the concentrations in the finely-ground meats packaged in oxygen-permeable pouches following exposure to eight doses of gamma radiation at 5 ± 0.5°C between 0 and 9.4 kGy. The average for all meats was an 11% loss for thiamin and a 2.5% loss,

above 3 kGy, for riboflavin. The rate of thiamin loss in beef was significantly higher than in other meats and averaged 16.8 ± 2.3% per kGy absorbed dose at 5°C (41°F). Loss of riboflavin was not different among species. There was no statistically significant difference in the rate of loss of α-tocopherol due to species except in turkey breast meat. The loss of α-tocopherol from beef averaged 30.8% per kGy. The results support the concept of chemiclearance, allowing extrapolation of results to include similar tissues of other animals.

Lakritz and others (1998) compared the rates of loss of thiamin, riboflavin, and α-tocopherol in alligator, caiman, bison, and ostrich meats under identical conditions of preparation and irradiation. Rate constants were determined from their concentrations in the finely-ground meats packaged in oxygen-permeable pouches following exposure to nine doses of gamma radiation at 5 ± 0.5°C between 0 and 10.0 kGy. Thiamin, riboflavin, and α-tocopherol losses from bison top-round meat were 8.65, 1.39, and 16.1% per kGy. The results supported the concept of chemiclearance.

IV. Assessment of the overall impact on macro- and micro-nutrients of a surface treatment of chilled beef carcasses with a very low penetration (≤20 mm) and very low dosage (≤1.0 kGy) electron beam.

A. Macro-nutrients

There will be no measurable effects on either the macro-nutrients (protein, lipid, and minerals (ash)) or on the protein-efficiency ratio of beef that receives an absorbed dose of ionizing radiation ≤1.0 kGy.

B. Free fatty acids, individual fatty acids, and amino acids

There will be no measurable effects on the concentrations of free fatty acids, individual

fatty acids and amino acids of beef that receives an absorbed dose of ionizing radiation ≤1.0 kGy.

C. Vitamins

As indicated above, the radiation sensitivity of the water-soluble vitamins in beef is in the order thiamin (-16.8 ± 2.3% per kGy @ 5° C) > riboflavin (at absorbed doses > 3kGy, -2.5% per kGy @ 5° C) > pyridoxine > niacin > vitamin B_{12} > choline > inositol > folacin. In several studies, the percentages of niacin, vitamin B_{12}, choline, inositol, and folacin were unaltered even by sterilization doses of ionizing radiation. Among the fat soluble vitamins of importance to man in beef, α-tocopherol (-30.8% per kGy @ 5° C) appears to be relatively sensitive to ionizing radiation. Thus, at an absorbed dose of electron- or gamma-radiation of 1 kGy at about 5°C, only thiamin and α-tocopherol should be significantly decreased by the treatment. The impact can be judged from the following: A 1-quarter pound serving of raw hamburger, 80% lean meat / 20% fat, provides 0.049 mg of thiamin (4.1% of the RDA [1.2 mg/d] for men > 14 years of age) and 0.35 mg of α-tocopherol (2.3% of the RDA [15 mg/d] for men > 14 years of age) (U.S. Department of Agriculture, Agricultural Research Service 2004). The loss in terms of the RDA for thiamin, as the result of an absorbed dose of 1.0 kGy, would range from 0.60 to 0.78%. The loss in terms of the RDA for α-tocopherol, as the result of an absorbed dose of 1.0 kGy, would be approximately 0.95%. These figures can be further adjusted to reflect the estimated contribution of hamburger to the % of total thiamin in the US diet from the NHANES II survey, 1976 - 1980, which was 2.71% (Block and others 1985). Using this figure, the loss in RDA for thiamin would be 0.016 to 0.021%. Block and others (1985) do not provide an equivalent figure for α-tocopherol, but one can estimate, from the relative contributions to the RDA, that the

equivalent figure for α-tocopherol would be 1.52%. Thus, the predicted loss in RDA for α-tocopherol would be 0.014% from the U. S. diet.

One should also consider that it's extremely unlikely that 100% of the meat used to produce hamburger will be irradiated, so the above values should be adjusted downward to reflect the percentage of irradiated to unirradiated meat.

D. Conclusion

The conclusion is that beef carcass surface, low dosage (≤1.0 kGy) electron beam irradiation will not produce significant losses of either micro- or macro-nutrients from the U. S. diet.

REFERENCES

Alexander, H. D., E. J. Day, H. E. Sauberlich, and W. D. Salmon. 1956. Radiation effects on water soluble vitamins in raw beef. *Feder. Proc*. 15:921-923.

Block, G., C. M. Dresser, A. M. Hartman, and M. D. Carroll. 1985. Nutrient sources in the American diet: Quantitative data from the NHANES II survey 1. vitamins and minerals. *Am. J. Epidemiol*. 122:13-26.

Day, E. J., H. D. Alexander, H. E. Sauberlich, and W. D. Salmon. 1957a. Effects of gamma radiation on certain water-soluble vitamins in raw ground beef. *J. Nutrition*. 62:27-38.

Day, E .J., H. E. Sauberlich, H. D. Alexander, and W. D. Salmon. 1957b. The bioassay of thiamine in beef exposed to gamma radiation. *J. Nutrition*. 62:107-118.

Diehl, J. F. 1995. Safety of Irradiated Foods., 2nd ed. Marcel Dekker, Inc. New York.

Diehl, J. F. and E. S. Josephson. 1994. Assessment of wholesomeness of irradiated foods. *Acta*

Alimentaria 23:195-214.

Fox, J. B Jr., L. Lakritz, J. Hampson, R. Richardson, K. Ward, and D. W. Thayer.1995. Gamma irradiation effects on thiamin and riboflavin in beef, lamb, pork, and turkey. *J. Food Sci*. 60:596-598 & 603.

Fox, J. B. Jr., L. Lakritz, and D. W. Thayer. 1993. Effect of reductant level in skeletal muscle and liver on the rate of loss of thiamin due to γ-radiation. *Int. J. Radiat. Biol*. 64:305-309.

Fox, J. B Jr., D. W. Thayer, R. K. Jenkins, J. G. Phillips, S. A. Ackerman, G. R. Beecher, J. M. Holden, F. D. Morrow, and D. M. Quirbach. 1989. *Int. J. Radiat*. Biol. 55:689-703.

Groninger, H. S., A. L. Tappel, and F. W. Knapp. 1956. Some chemical and organoleptic changes in gamma irradiated meats. *Food Res.* 21:555-564.

Hampson, J. W., J. B. Fox, L. Lakritz, and D. W. Thayer. 1996. Effect of low dose gamma radiation on lipids in five different meats. *Meat Sci*. 42:271-276.

Joint FAO/IAEA/WHO Study Group on high-dose irradiation. 1999. High-dose irradiation: Wholesomeness of food irradiated with doses above 10 kGy. WHO Technical Report Series 890. Geneva.

Josephson, E. S. 1983. Rdappertization of meat, poultry, finfish, shellfish, and special diets., p. 231-251. *In* E. S. Josephson and M. S. Peterson (ed), Preservation of food by ionizing radiation, vol. 3. CRC Press, Boca Raton, Fla.

Kilcast, D. 1994. Effect of irradiation on vitamins. *Food Chem*. 49:157-164.

Lakritz, L., J. B. Fox Jr., J. Hampson, R. Richardson, K. Kohout, and D. W. Thayer. 1995. Effect of gamma radiation on levels of α-tocopherol in red meats and turkey. *Meat Sci*. 41:261-

271.

Lakritz, L.,J. B. Fox, and D. W. Thayer. 1998. Thiamin, riboflavin, and α-tocopherol content of exotic meats and loss due to gamma radiation. *J. Food Prot*. 61:1681-1683.

Maxwell, R. J. and A. H. Rady 1989. Effect of gamma irradiation at various temperatures on air and vacuum packed chicken tissues II. Fatty acid profiles of neutral and polar lipids separated from muscle and skin irradiated at 2-5°C. *Radiat. Phys. Chem.* 34:791-796.

Merritt Jr., C., and I. A. Taub. 1983. Commonality and predictability of radiolytic products in irradiated meats. p. 27-57 *In* P. S. Elias and A. J. Cohen (ed.), Recent advances in food irradiation. Elsevier Biomedical Press, Amsterdam.

Murray, T. K. 1983. Nutritional aspects of food irradiation, p. 203-216. *In* P.S. Elias and A. J. Cohen (ed.), Recent advances in food irradiation. Elsevier Biomedical Press, Amsterdam.

Sheffner, A. L. and H. Spector. 1957. Action of ionizing radiations on vitamins, sterols, hormones and other physiologically active compounds. p. 159-168. *In* S. D. Bailey, J. M. Davies, B. H. Morgan, R. Pomerantz, R. G. H. Siu, and R. G. Tischer (ed.) Radiation Preservation of Food. The United States Army Quartermaster Corps, U. S. Government Printing Office, Washington, D. C.

Skala, J. H., E. L. McGown, and P. P. Waring. 1987. Wholesomeness of irradiated foods. *J. Food Prot*. 50:150-160.

Thayer, D. W. 1990. Food irradiation: benefits and concerns. *J. Food Quality*. 13:147-169.

Thayer, D. W. 1994. Wholesomeness of irradiated foods. Food Technol.48(5):132, 134, 136.

Thayer, D. W., J. P. Christopher, L. A. Campbell, D. C. Ronning, R. R. Dahlgren, G. M. Thomson, and E. Wierbicki. 1987. Toxicology studies of irradiation-sterilized chicken. *J.*

Food Prot. 50:278-288.

Thayer, D. W., J. B. Fox Jr., and L. Lakritz. 1991. Effects of ionizing radiation on vitamins, p. 285-325. *In* S. Thorne (ed.), Food Irradiation, Elsevier Applied Science Publishers, London.

Thomas M. H., B. M. Atwood, E. Wierbicki, and I. A. Taub. 1981. Effect of radiation and conventional processing on the thiamin content of pork. *J. Food Sci*. 46:824-828.

Tobback, P. P. 1977. Radiation chemistry of vitamins, p. 187-220. P. S. Elias and A. I. Cohen (ed.), Radiation chemistry of major food components. Elsevier Scientific Publishing Company, Amsterdam.

U.S. Department of Agriculture, Agricultural Research Service. 2004. USDA National Nutrient Database for Standard Reference, Release 17. Nutrient Data Laboratory Home Page. Available at: http://www.nal.usda.gov/fnic/foodcomp.

Wierbicki, E. 1985. Technological and irradiation conditions for radappertization of chicken products used in the United States Army Raltech toxicology study, p. 79-99. *In* Food Irradiation Processing, Proceedings of an International Symposium, Washington, DC., 4 to 8 March 1985.