



July 8, 2005

**Hand Delivered**

Mr. Philip Derfler  
Assistant Administrator  
Office of Policy, Program and Employee Development  
Food Safety and Inspection Service  
Room 350 Administration Building  
U.S. Department of Agriculture  
Washington, D.C. 20250-3700

**Re: Citizens Petition To Recognize the Use of E-Beam on Carcasses  
as a Processing Aid**

Dear Mr. Derfler:

The American Meat Institute respectfully submits this citizen's petition regarding the use of low dose, low-penetration electron beam (e-beam) applied to the outermost surfaces of chilled beef carcasses.

The American Meat Institute (AMI) represents the interests of packers and processors of beef, pork, lamb, veal and turkey products and their suppliers throughout North America. Together, AMI's members produce 95 percent of the beef, pork, lamb and veal products and 70 percent of the turkey products in the U.S. Headquartered in Washington, DC, the Institute provides legislative, regulatory, public relations, technical, scientific and educational services to the industry. Its affiliate, the AMI Foundation, is a separate 501(c)3 organization that conducts research, education and information projects for the industry.

**Action Requested**

The Food Safety and Inspection Service (FSIS) officially recognizes low dose, low-penetration electron beam (e-beam) applied to the surface of chilled beef carcasses as a "processing aid" and accordingly that this process need not be labeled on any products derived from the carcass.

## Statement of Grounds

FSIS food ingredient regulations, 9 CFR § 424.22(c), permit irradiation of beef products in accordance with Food and Drug Administration (FDA) regulations. FDA regulations permit the irradiation of refrigerated or frozen, uncooked products that are meat within the meaning of 9 CFR 301.2(rr), meat byproducts within the meaning of 9 CFR 301.2(tt) and [certain] meat food products within the meaning of 9 CFR 301.2(uu). Hence, the question is not whether the e-beam processing of the chilled carcasses is consistent with the regulations; it is. Instead, the question is whether this low dose, low-penetrating application of electron beam to the outermost surface of the carcass needs to appear on the labels of meat derived from the carcasses.

The general rule is that a food ingredient must be declared on the label of any product regardless of whether it was directly added or it was an ingredient of a component. However, there are exceptions. In the context of this petition, we respectfully direct the agency's attention to its policy concerning the labeling of processing aids. With regard to this request, FSIS has defined "processing aids" as:

Substances that are added to a food for their technical or functional effect in the processing but are present in the finished food at insignificant levels and do not have any technical or functional effect in that food<sup>1</sup>.

The information being provided in this petition demonstrates that the process is being used as a processing aid. It is effective as an antimicrobial on the carcass but in **no** case has FSIS ever required the labeling of the ingredient merely because of its antimicrobial properties at time of treatment. In the case of this process, it has no other technical or functional effect on the carcass nor on the products derived from the carcass. This petition demonstrates the process has no significant effect on the organoleptic properties, shelf life or nutritional properties of the carcass or products derived there from. Moreover, we submit that this lack of any technical effect demonstrates that the process is insignificant in terms of the products of the carcass. Low dose, low penetration e-beam application results in an insignificant portion of the carcass receiving e-beam exposure, and most of the edible portion of the carcass would not receive any e-beam exposure. The external surface of the carcass is largely used in ground beef manufacture where it constitutes about 5% of the ground beef blend. Much of the carcass surface is covered by adipose tissue which is inherently self-limiting as a component of ground beef blends. Indeed, we submit it would be misleading

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<sup>1</sup> FSIS Directive 7,120.1, Attachment 2, Question 2, c.

to mandate the labeling of the process or any beef derived from the carcass since those products would evidence no characteristics of irradiated products.

A series of controlled research studies were designed and conducted to develop the scientific basis for this request. These studies address questions that were reasonably anticipated to be requested by FSIS, specifically:

- Does the proposed e-beam application result in a significant reduction in pathogenic bacteria (specifically *E. coli* O157:H7) on chilled beef?
- Does the proposed e-beam application result in significant organoleptic changes to the resulting beef products?
- Does the proposed e-beam application result in a lasting effect on shelf life of the resulting beef products?
- Does the proposed e-beam application result in a significant reduction in macro or micro nutrients of beef, such that it will result in a significant impact on human dietary intake of these nutrients?

To address these important questions, research studies were commissioned with experts in the field of meat microbiology, food irradiation, sensory analysis, and biochemistry of meat products. In summary, this series of research projects provide the scientific evidence to demonstrate that this process:

1. Is exceptionally effective at reducing levels of *E. coli* O157:H7,
2. Does not have any effect on organoleptic properties or appearance,
3. Does not have any lasting effect on shelf life, and
4. Does not produce significant losses of either macro- or micro-nutrients.

1. The Process is Exceptionally Effective

Research conducted by the USDA Agricultural Research Service's Meat Animal Research Center (MARC) focused, in part, on the effectiveness of the intervention on reduction of *E. coli* O157:H7 (Appendix #1, *J. Food Protection*. 2005. 68:4 666-672). Briefly, portions of beef cutaneous trunci muscle were selected to represent the carcass surface since this muscle, which covers portions of the beef plate and beef flank, is an outermost surface muscle that approximates the surface matrix of a beef carcass. Forty cutaneous trunci pieces were inoculated with *E. coli* O157:H7; twenty with a high concentration of 6 log cfu/cm<sup>2</sup> (high inoculation), and 20 with a low concentration of 3 log cfu/cm<sup>2</sup> (low inoculation). After inoculation, the beef samples were left at room temperature for one hour to allow bacterial attachment and then refrigerated to simulate chilling.

After chilling, the forty samples were cut into two equal portions (for a total of eighty samples) designated as "treated" and "control." One half was treated with low-level e-beam irradiation with a targeted surface dosage of 1 kGy/s, which results in a slightly higher sub-surface dosage in the range of

about 1.6 kGy/s. The other half was not treated and served as the control. Analytical units were removed at zero, 48 and 120 hours for the control and at 48 and 120 hours for the treated samples. After treatment, samples were held refrigerated for 48 and 120 hours to represent beef trimmings destined for ground beef production that are held for relatively short and relatively long periods of time in distribution. Samples were microbiologically analyzed for detection and enumeration of *E. coli* O157:H7 by using the most current and reliable detection methods available (see Appendix #1, p. 667).

Results for direct cell count plating indicate that while the *E. coli* O157:H7 contamination of the control product remained around the high<sup>2</sup> and low<sup>3</sup> inoculation level, the samples exposed to the treatment were undetectable for *E. coli* O157:H7 at the high inoculation after 48 hours and approximately 0.1 log after 120 hours. For the low inoculation level, e-beam treatment resulted in a reduction of 2.9 and 2.6 log CFU, respectively, for the 48- and 120 hour post-treatment time periods. For the high inoculation level, e-beam treatment resulted in a reduction of 6.6, and 5.7 log CFU, respectively, for the 48- and 120 hour post treatment time periods. In addition to direct plating, researchers conducted enumeration of positive samples using the most probable number (MPN) technique, which further illustrated effectiveness of the treatment. The MPN results were similar to direct plating, confirming that even after an enrichment step in the MPN procedure, the number of viable *E. coli* O157:H7 cells were very low.

Based on these data, we respectfully submit that a low dose, low penetration surface e-beam process will produce a significant surface reduction of *E. coli* O157:H7 on chilled beef.

## 2. The Process Does Not Have Any Affect on Quality or Appearance

The MARC study (Appendix #1) also addressed effects of low dose, low penetration e-beam process on organoleptic properties of treated product. Researchers choose to use thin, surface exposed muscles (flank steak) and boneless short ribs for use in ground beef. These two approaches approximate the potential treatment effects on both whole muscle cuts and ground beef products.

### Whole muscle cut evaluation

A beef carcass includes certain surface muscles that are thin, on the edges of the carcass and are likely to receive the greatest degree of variable e-beam dose treatment due to their location and the extent of fat covering, therefore, one of these muscles, *rectus abdominus*, was determined to be appropriate for assessment of organoleptic properties. This muscle cut was chosen because it

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<sup>2</sup> Approximately 7 logs after attachment; 6.5 logs at 48 hours and 5.85 logs at 120 hours

<sup>3</sup> Approximately 3.8 logs after attachment, 2.9 logs after 48 and 2.55 logs after 120 hours

is partially surface exposed, consistent in size, shape and location, is easy to access and remove, and possesses sufficient surface fat to allow trimming and surface layer molding to achieve variable e-beam penetration.

Forty-two flank steaks were chosen from a commercial beef processing facility; 30 of which were used for color and sensory analysis and 12 were used for sensory panel training. To assess the impact, the overlying fat was trimmed to vary its thickness over a 20 mm flank steak. The overlying fat varied from 13 mm (resulting in a 10% penetration into the meat) to no overlying fat (resulting in a 75% penetration). A non-exposed (0% penetration) treatment was used as a control.

Sensory analysis was conducted using a ten member trained descriptive attribute sensory panel. Panelists assess beef aroma intensity, off-aroma, tenderness, juiciness, beef flavor intensity, and off-flavor. None of the flank steak sensory attributes were significantly affected ( $P > 0.05$ ) by any e-beam penetration treatment.

Hunter Color measurement indicated some treatment effects, however the effects on  $L^*$  (lightness) and  $b^*$  (yellowness) were not linear with dose, and thus were deemed not meaningful treatment related differences. The effects of treatment on the  $a^*$  (redness) values were linear, however the magnitude of the effect appears slight and would likely have no impact on consumer acceptance.

#### Ground beef evaluation

Boneless short ribs were used as the model beef tissue for evaluating e-beam treatments because this cut is comprised of the appropriate lean:fat ratio for preparing 20% fat ground beef blends. This experiment was designed to evaluate the full range of possible scenarios for the amount of ground beef material which had received exposure to e-beam, including 100% exposure, allowing the experiment to have a positive control with the 100% treatment. In reality, it is anticipated that at most 10% of the ground beef blend would contain tissue that has received e-beam exposure.

Fifty kg of beef short rib pieces were exposed to e-beam and 100 kg of beef short rib pieces were untreated and used for blending and negative control. The treated and untreated portions were combined in appropriate ratios to provide the following treatments: 100%, 50%, 25%, 10%, 5%, and 0% of e-beam exposed product. Each treatment was formulated to contain approximately 20% fat.

The ground beef was formed into patties and held 20 and 40 days in frozen storage, at which point the patties were removed for testing. The patties were then presented to a 10 member trained descriptive attribute sensory panel as described for the whole muscle cut evaluation.

Results of the sensory panel indicate that the panel was able to determine a difference in the positive control of 100% e-beam treated blend, indicating the trained panel was capable of discerning a difference due to treatment. Given this result, the data indicate that measures of aroma and flavor were not different between the non-treated control and the 5%, 10% and 25% treatment. Values for tenderness were affected by treatment, but not in a linear fashion and it does not appear that the differences noted are meaningful.

The proportion of e-beam exposed trim did not affect any of the Hunter color measurements of the ground beef patties.

### 3. The Process Does Not Have an Effect on Shelf Life

The study on the effects of low dose, low-penetration e-beam surface exposure process on the shelf life of beef was performed by Silliker Inc. (Appendix #2).

Twelve chilled beef plates from a commercial beef slaughter facility were removed from beef carcasses and transported to a commercial irradiation facility. Six beef plates were designated “air-exposed” and left untrimmed. Three of these six were treated with low level, low penetration surface e-beam (1 kGy surface treatment with a maximum sub-surface dosage of 1.6 kGy). The other three were left untreated as controls.

The other group of six, designated as “vac-pac,” were trimmed and placed in vacuum packaging for the duration of shelf life study. As with the air-exposed plates, three of the vac-pac plates were treated as described above, and three were un-treated and held as controls. The groups of three, air-exposed/vac-pac and control/treated were randomly subdivided into four groups each and maintained at 40°F during storage. Air exposed product was tested at the following shelf life time periods to approximate the total life of non-vacuum packaged product: 1, 3, 6, and 9 day post treatment. The vacuum packaged product, which has a longer shelf life, was tested at: 1, 10, 20, and 30 days post treatment. All products were held at 40°F throughout the storage period.

The following microbiological tests were performed at each measurement time: total aerobic plate count (APC), hetero- and homo-lactic acid bacteria (LAB), total coliforms, and Biotype I *E. coli*. To provide a measure of oxidative rancidity, analysis was completed to measure thiobarbituric acid (TBA) throughout shelf life.

Based on the results of this study, it is evident that the initial antimicrobial effects of the treatment are minimal and over the course of shelf life the APC and LAB counts on the surface e-beam treated product had increased to the

point that quantitative levels nearly approximated the non-treated controls at the end of the storage period. In addition, one of the principal measurements of shelf life and product spoilage – rancidity – as measured by TBA, indicated that the treated samples would turn rancid slightly before the non-treated controls. These data clearly indicate that the e-beam surface treatment of beef plates does **not** have a lasting effect on the product shelf-life.

#### 4. The Process Does Not Produce Significant Losses of Nutrients

The fourth issue addressed in this series of studies concerns whether the low-dose, low penetration surface e-beam process will have an effect on the nutrient profile of the product. In this regard, Dr. Donald W. Thayer, retired USDA – ARS researcher, has conducted an exhaustive review of the scientific literature on the issue (Appendix #3). According to Dr. Thayer:

- There will be no “no measurable effects” on macro-nutrients.
- There will be “no measurable effects” on the concentrations of free fatty acids, individual fatty acids, and amino acids.
- The water soluble vitamins in beef (niacin, vitamin B12, choline, inositol and folacin) will be “unaltered.”
- One water soluble and one fat soluble vitamin (thiamin and tocopherol) would likely be decreased.

For these two vitamins, Dr. Thayer estimates, worse case, that the maximum net decrease in the U.S. diet would be only 0.021% for thiamin and 0.014% for tocopherol." We respectfully submit that this would not be significant in terms of the total diet.

Based on the above, Dr. Thayer concluded that “beef carcass surface, low dosage ( $\leq 1.0$  kGy) electron beam irradiation will not produce a significant loss of either micro- or macro-nutrients from the U.S. diet.”

### **Environmental Impact**

Since the e-beam process would operate in compliance with existing requirements, we respectfully submit no new environmental issues are raised by this petition.

## **Economic Impact**

Since the use of the process is and would remain voluntary, we do not anticipate an economic impact of more than \$100 million.

## **Conclusion**

In summary, based upon the above referenced data and analysis we submit that the proposed process of using e-beam to treat the surface of a chilled beef carcass would meet the USDA-FSIS definition of a processing aid and would result in a significant reduction in pathogens such as *E. coli* O157:H7, while causing no meaningful change in the organoleptic properties, the shelf life, or the nutritional profile of products produced from the beef carcass. We appreciate your prompt attention to this petition and look forward to a favorable response. If you have any questions or desire additional information, please do not hesitate to contact us.

Sincerely,

A handwritten signature in black ink that reads "Randall Huffman". The signature is written in a cursive style with a large initial "R".

Randall Huffman, Ph.D.  
Vice President, Scientific Affairs

Cc: J. Patrick Boyle  
Mark Dopp  
Jim Hodges  
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Skip Seward

# 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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Research Report

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

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**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<sup>2</sup> 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 <sup>2</sup> )	35°C, 24 - 48 hr, aerobic
Biotype I <i>E. coli</i>	Most Probable Number Method (<3 MPN/cm <sup>2</sup> )	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<sup>2</sup> 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<sup>2</sup> and 1.8 log cfu/cm<sup>2</sup> 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<sup>2</sup>. 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°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.

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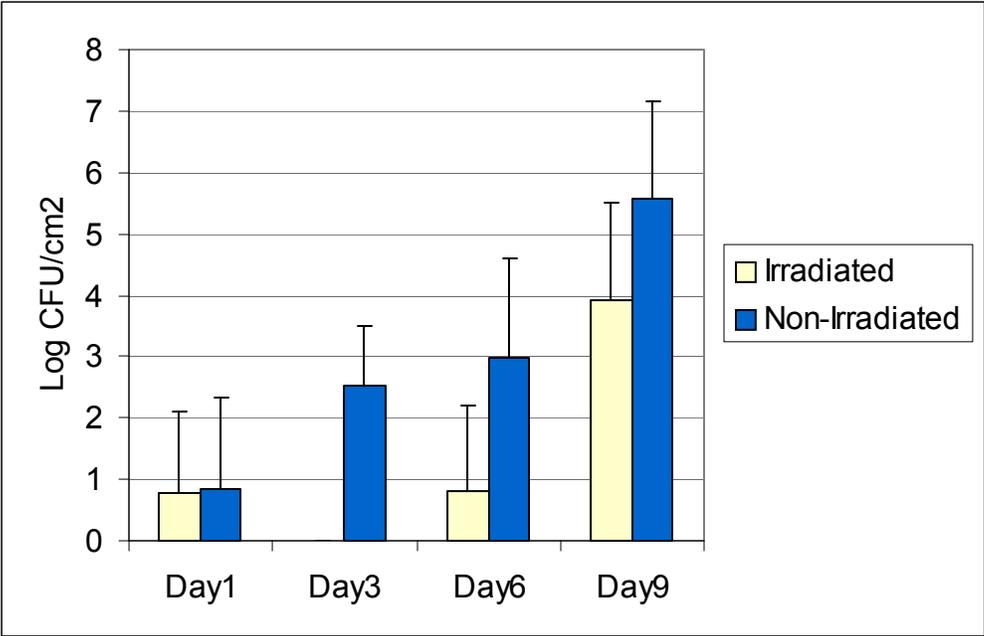


Figure 1. Aerobic plate counts of air-exposed beef navels during storage at 40°F.

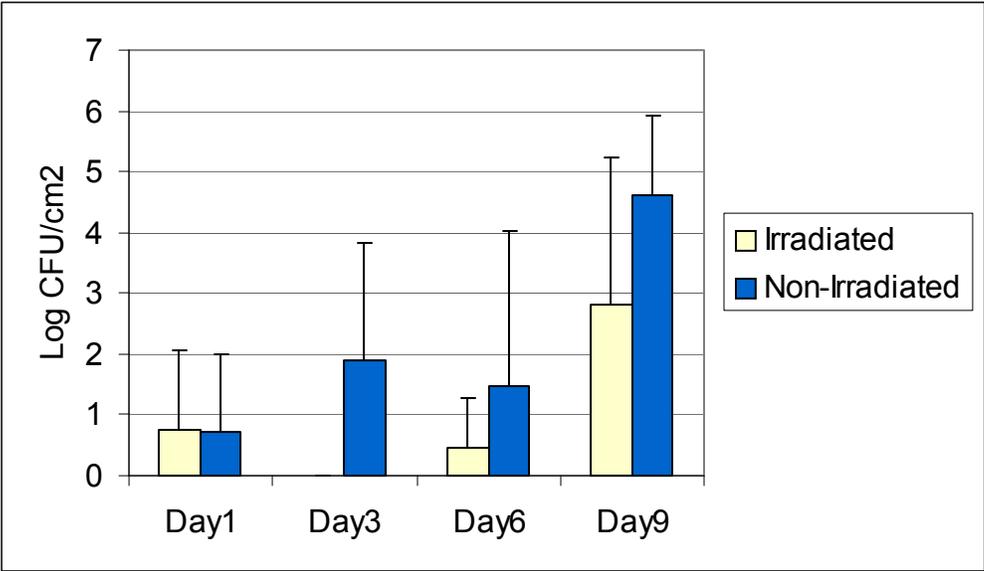


Figure 2. Lactic acid bacteria counts of air-exposed beef navels during storage at 40°F.

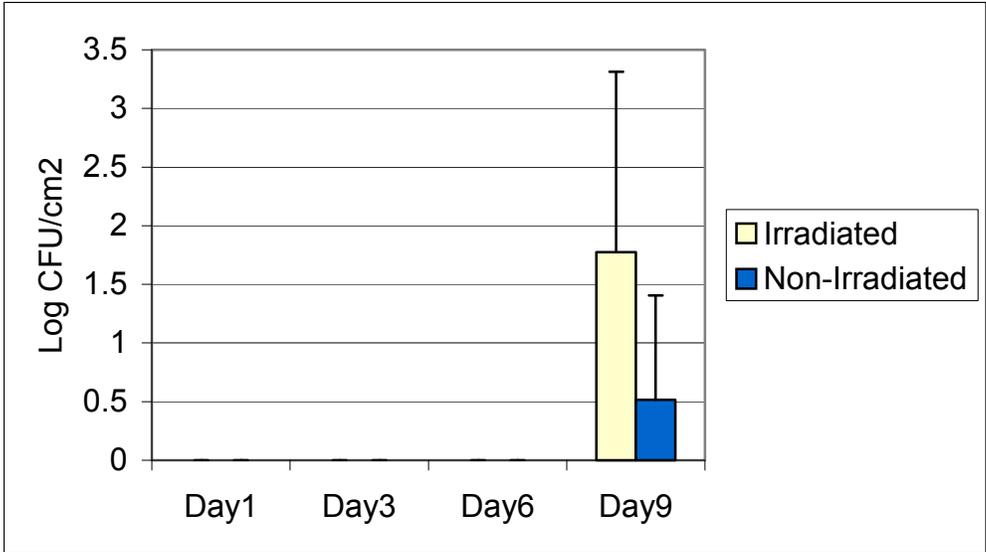


Figure 3. Total coliform counts of air-exposed beef navels during storage at 40°F.

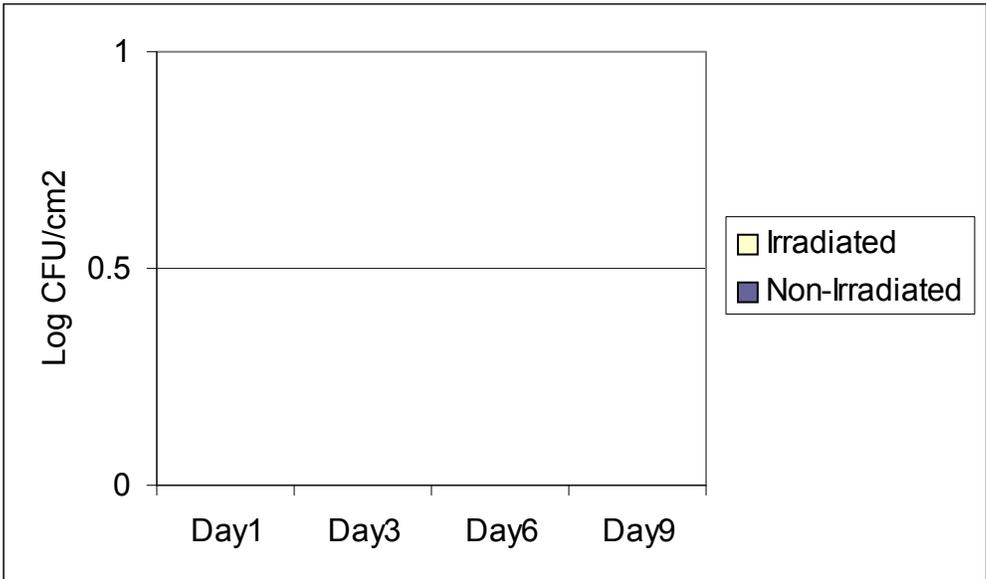


Figure 4. *Escherichia coli* counts of air-exposed beef navels during storage at 40°F.

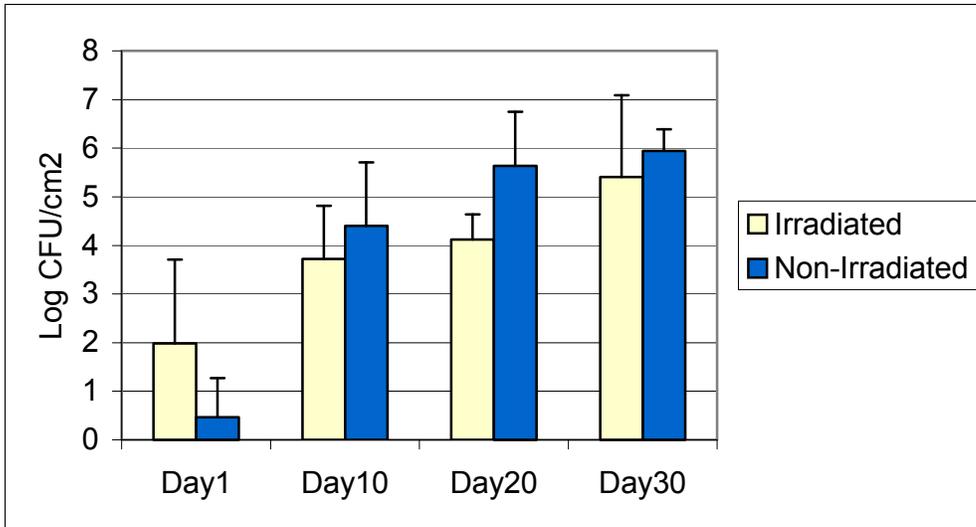


Figure 5. Aerobic plate counts of vacuum packed beef navels during storage at 40°F.

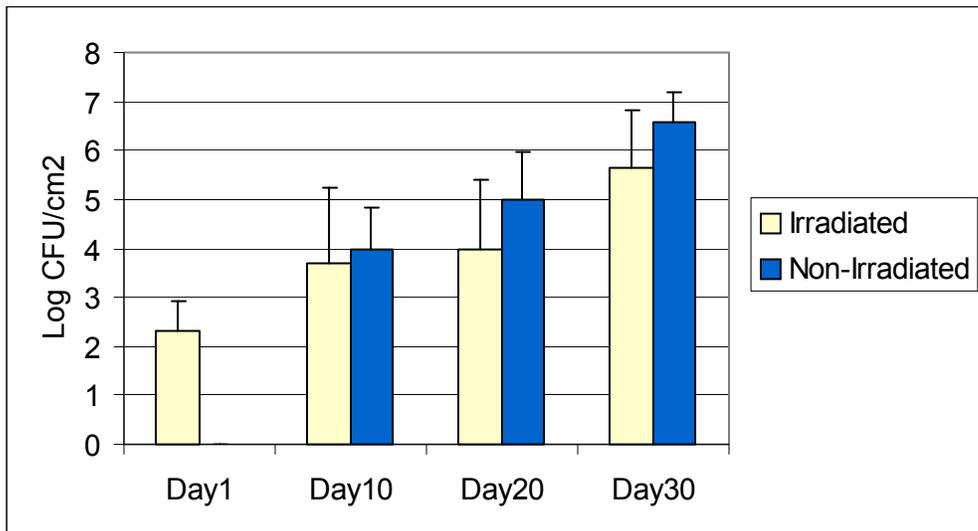


Figure 6. Lactic acid bacteria counts of vacuum packed beef navels during storage at 40°F.

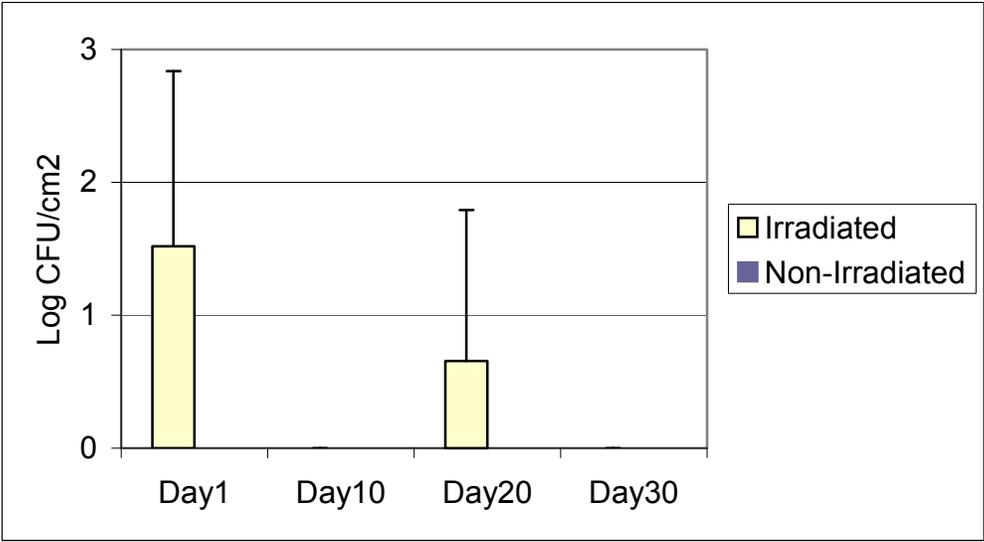


Figure 7. Coliforms counts of vacuum packed beef navels during storage at 40°F.

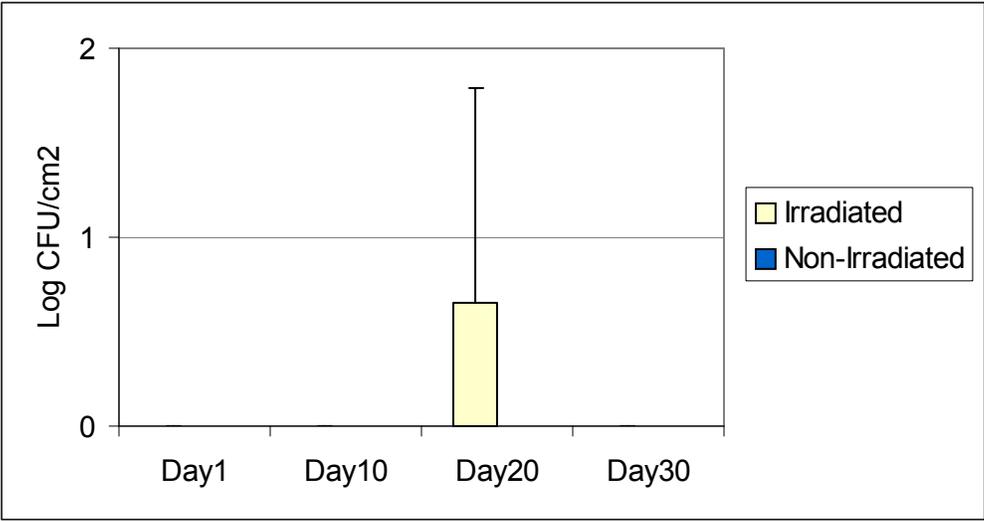


Figure 8. *Escherichia coli* counts of vacuum packed beef navels during storage at 40°F.

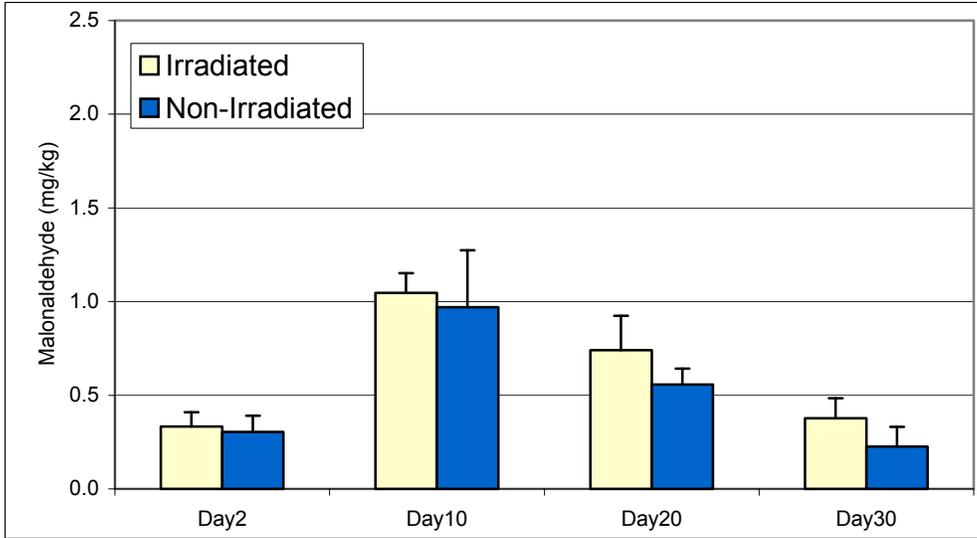


Figure 9. Thiobarbituric Acid (TBA: malonaldehyde mg/kg) values of vacuum packaged beef navels stored at 4°C.

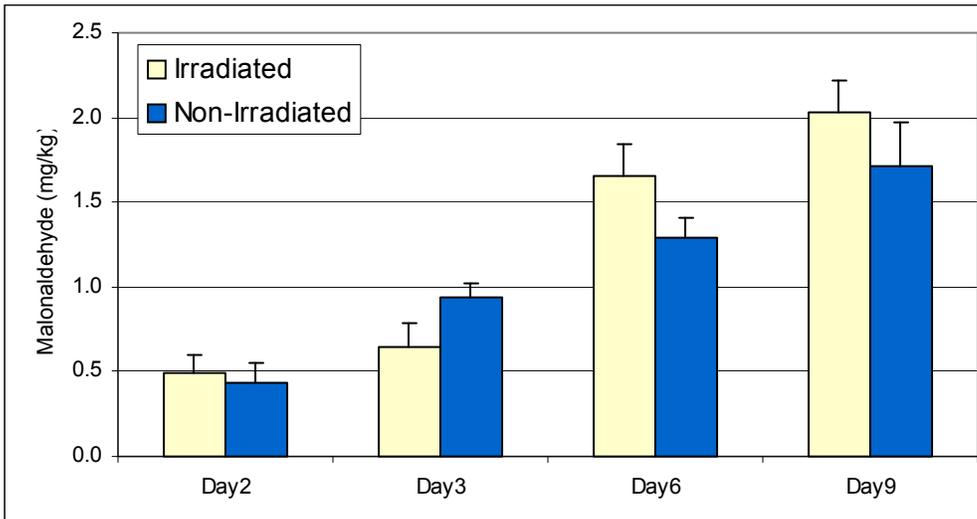


Figure 10. Thiobarbituric Acid (TBA; malonaldehyde mg/kg) values of air exposed beef navels stored at 4°C.

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**

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## **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 ( $\leq 20$  mm) and very low dosage ( $\leq 1.0$  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  $-25^{\circ}\text{C} \pm 15^{\circ}\text{C}$ . 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  $-25 \pm 15^{\circ}\text{C}$  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  $2-5^{\circ}\text{C}$  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  $-25 \pm 15^{\circ}\text{C}$  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 Tობback 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 others 1995, 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  $-25 \pm 15^{\circ}\text{C}$  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<sub>12</sub> 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<sub>1</sub>, B<sub>2</sub>, 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<sub>1</sub> and B<sub>2</sub>, 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<sub>1</sub>), riboflavin (B<sub>2</sub>), niacin, pyridoxine (B<sub>6</sub>), and cobalamin (B<sub>12</sub>) 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  $\alpha$ -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  $5 \pm 0.5^\circ\text{C}$  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  $16.8 \pm 2.3\%$  per kGy absorbed dose at  $5^{\circ}\text{C}$  ( $41^{\circ}\text{F}$ ). Loss of riboflavin was not different among species. There was no statistically significant difference in the rate of loss of  $\alpha$ -tocopherol due to species except in turkey breast meat. The loss of  $\alpha$ -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  $\alpha$ -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  $5 \pm 0.5^{\circ}\text{C}$  between 0 and 10.0 kGy. Thiamin, riboflavin, and  $\alpha$ -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 ( $\leq 20$  mm) and very low dosage ( $\leq 1.0$  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  $\leq 1.0$  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  $\leq 1.0$  kGy.

### C. Vitamins

As indicated above, the radiation sensitivity of the water-soluble vitamins in beef is in the order thiamin ( $-16.8 \pm 2.3\%$  per kGy @  $5^\circ\text{C}$ ) > riboflavin (at absorbed doses > 3kGy,  $-2.5\%$  per kGy @  $5^\circ\text{C}$ ) > pyridoxine > niacin > vitamin B<sub>12</sub> > choline > inositol > folacin. In several studies, the percentages of niacin, vitamin B<sub>12</sub>, choline, inositol, and folacin were unaltered even by sterilization doses of ionizing radiation. Among the fat soluble vitamins of importance to man in beef,  $\alpha$ -tocopherol ( $-30.8\%$  per kGy @  $5^\circ\text{C}$ ) appears to be relatively sensitive to ionizing radiation. Thus, at an absorbed dose of electron- or gamma-radiation of 1 kGy at about  $5^\circ\text{C}$ , only thiamin and  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -tocopherol, but one can estimate, from the relative contributions to the RDA, that the

equivalent figure for  $\alpha$ -tocopherol would be 1.52%. Thus, the predicted loss in RDA for  $\alpha$ -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 ( $\leq 1.0$  kGy) electron beam irradiation will not produce significant losses of either micro- or macro-nutrients from the U. S. diet.

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