

Elimination of *Escherichia coli* O157:H7 in Meats by Gamma Irradiation

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Undercooked and raw meat has been linked to outbreaks of hemorrhagic diarrhea due to the presence of *Escherichia coli* O157:H7; therefore, treatment with ionizing radiation was investigated as a potential method for the elimination of this organism. Response-surface methods were used to study the effects of irradiation dose (0 to 2.0 kGy), temperature (−20 to +20°C), and atmosphere (air and vacuum) on *E. coli* O157:H7 in mechanically deboned chicken meat. Differences in irradiation dose and temperature significantly affected the results. Ninety percent of the viable *E. coli* in chicken meat was eliminated by doses of 0.27 kGy at +5°C and 0.42 kGy at −5°C. Small, but significant, differences in radiation resistance by *E. coli* were found when finely ground lean beef rather than chicken was the substrate. Unlike nonirradiated samples, no measurable verotoxin was found in finely ground lean beef which had been inoculated with 10^{4.8} CFU of *E. coli* O157:H7 per g, irradiated at a minimum dose of 1.5 kGy, and temperature abused at 35°C for 20 h. Irradiation is an effective method to control this food-borne pathogen.

Escherichia coli O157:H7 is a rapidly emerging food-borne pathogen that can produce a clinical illness characterized by an acute grossly bloody diarrhea that is accompanied by severe, crampy abdominal pain (2, 13, 24). A few patients go on to develop hemolytic uremic syndrome or thrombotic thrombocytopenic purpura (2, 13). This pathogen has been associated primarily with undercooked and raw beef, lamb, pork, or poultry (2, 5, 13, 17). The recent approval of ionizing radiation treatments of poultry to eliminate food-borne pathogens (1, 3) makes it appropriate to determine the effect that irradiation treatments would have on this pathogen in poultry. The regulation for irradiation of poultry products from the USDA Food Safety and Inspection Service requires minimum and maximum doses of 1.5 and 3.0 kGy (1 kGy = 100 kilorads), respectively (3). Though beef is not included under the current regulation, it seemed prudent to include it in the study, since several of the outbreaks of the disease have been specifically linked to beef (2, 13) and raw beef is consumed as steak tartare. No previous studies of the effects of ionizing radiation on *E. coli* O157:H7 were found in the literature. The aims of this study were to determine the sensitivity of *E. coli* O157:H7 suspended in beef or mechanically deboned chicken meat (MDCM) to gamma radiation and also to determine the influence of processing parameters such as atmosphere or temperature on that sensitivity.

(Portions of this paper were presented at the 92nd General Meeting of the American Society for Microbiology, New Orleans, La., 26 to 30 May 1992.)

MATERIALS AND METHODS

Organisms. *E. coli* O157:H7 ATCC 43895 and *E. coli* ATCC 25922 were maintained and cloned on tryptic soy agar (Difco, Detroit, Mich.) and incubated at 35°C. *E. coli* O157:H7 ATCC 43895 was implicated in a hemorrhagic colitis outbreak involving raw hamburger meat and produces Shiga-like toxins I and II (24). *E. coli* ATCC 25922 is a

nonpathogenic clinical isolate. One milliliter from a 15- to 18-h culture of the appropriate strain incubated at 35°C in Trypticase soy broth (BBL, Cockeysville, Md.) was used to inoculate 100 ml of Trypticase soy broth in a 500-ml baffled shake flask. These cultures were incubated aerobically with shaking (150 rpm) at 35°C and harvested at 16 h for stationary-phase cells and at 4 h for mid-log-phase cells. A 10-fold cell concentrate was prepared for many studies by centrifuging the cells and resuspending them in 1/10 volume of Butterfield's phosphate (0.25 M KH₂PO₄ adjusted to pH 7.2 with NaOH).

Substrates. MDCM was obtained from a commercial manufacturer of poultry frankfurters. The mean proximate analysis of three separate lots of MDCM was 21.3% fat, 65.6% moisture, 14.0% protein, and 0.95% ash. Triple-ground lean top round beef, such as would be used in the preparation of steak tartare, was obtained from a local butcher shop. The proximate analysis for the ground beef was 2.6% fat, 73.5% moisture, 19.8% protein, and 1.4% ash. The MDCM and the ground beef were subdivided into 100 ± 0.05-g amounts and then spread thinly and vacuum sealed in Stomacher 400 (Tekmar Co., Cincinnati, Ohio) polyethylene bags. These bags were themselves vacuum sealed in Freshstuff (American National Can Company, Des Moines, Iowa) oxygen barrier pouches (oxygen transmission, 0.6 to 0.8 cm³/645 cm²/24 h at 3.2°C and 90% relative humidity). These meats were sterilized by gamma irradiation at a dose of 42 kGy at −50°C. Both sterile and nonsterile MDCM and ground beef were stored at −20°C until use.

Irradiation. The self-contained gamma radiation source was ¹³⁷Cs with a strength of approximately 134,000 Ci and a dose rate of 0.12 kGy min^{−1}. The dose rate was established by using National Physical Laboratory (Middlesex, United Kingdom) dosimeters. Variations in absorbed dose were minimized by placing thin samples (approximately 2 mm thick) within a uniform portion of the radiation field. The total mass of 5-g samples undergoing irradiation at one time during individual experiments usually did not exceed 20 g. Samples were maintained within ±0.5°C of the desired temperature by the injection of liquid nitrogen into the

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irradiation chamber. Sample temperature was monitored continuously during irradiation.

Inoculation of meat for determination of D_{10} values. Sterile meat was mixed well with an average of $10^{9.6}$ CFU of stationary-phase or $10^{9.2}$ CFU of log-phase *E. coli* O157:H7 per g, suspended in Butterfield's phosphate buffer by agitation in a stomacher for 90 s. Samples of 5.0 ± 0.05 g of the inoculated meat were aseptically transferred to sterile Stomacher 400 polyethylene bags. The inoculated meat was spread uniformly over an area of about 10 by 10 cm within the bag and heat sealed either in vacuo or with air in the bag, as appropriate. Each stomacher bag was then vacuum packaged within a Freshstuff bag to prevent oxygen absorption by vacuum-packed samples and to provide additional microbiological security for all samples during the irradiation treatment and subsequent handling.

Determination of D_{10} values. The D_{10} value is defined as the absorbed gamma radiation dose producing a 90% decrease in the number of CFU. Sterile MDCM inoculated with stationary-phase *E. coli* O157:H7 received radiation doses of 0 to 3.5 kGy in increments of 0.25 kGy at an irradiation temperature of 0°C. The meat was packaged either in vacuo or with air in the package. The analysis was repeated four times.

Sterile ground beef inoculated with stationary-phase *E. coli* O157:H7 received radiation doses of 0 to 1.75 kGy in increments of 0.25 kGy at an irradiation temperature of 0°C. The inoculated meat was packaged in vacuo. The analysis was repeated three times.

Sterile MDCM inoculated with mid-log-phase *E. coli* O157:H7 received radiation doses of 0 to 1.05 kGy in increments of 0.15 kGy at an irradiation temperature of 0°C. The analysis was repeated four times.

Effect of temperature during irradiation treatment. A modified central composite response-surface design (6) was used to determine the effect of irradiation processing temperatures from -20 to +20°C on the survival of stationary-phase *E. coli* O157:H7 in vacuum-packaged sterile ground beef. The inoculation and packaging procedures were identical to those used to determine D_{10} values. Two replicate samples were analyzed for each of the following combinations of irradiation temperature and dose: -20°C, 0 kGy; -20°C, 1.0 kGy; -20°C, 2.0 kGy; -10°C, 0.5 kGy; -10°C, 1.5 kGy; 0°C, 0 kGy; 0°C, 2.0 kGy; +10°C, 0.5 kGy; +10°C, 1.5 kGy; +20°C, 0 kGy; +20°C, 1.0 kGy; and +20°C, 2.0 kGy. Five replicate samples treated at 0°C with 1.0 kGy were analyzed.

The effect of a 1.5-kGy radiation dose on the survival of *E. coli* O157:H7 in vacuum-packaged sterile ground beef was determined at intervals of 5°C from +20 to -20°C and at 10°C intervals from -20 to -60°C. Two independent studies were completed, and duplicate samples were irradiated in each of the studies.

Sterile MDCM inoculated with stationary-phase *E. coli* O157:H7 received radiation doses of 0 to 2.0 kGy in increments of 0.25 kGy at irradiation temperatures of +5°C and -5°C. The meat was packaged in vacuo. The analysis was repeated twice.

Challenge and temperature abuse study. Sterile ground beef was inoculated with an average of $10^{4.8}$ CFU/g. Samples of 5.0 ± 0.05 g and 10.0 ± 0.05 g were vacuum packaged in sterile stomacher bags and then themselves vacuum sealed in Freshstuff bags. Two 5-g samples and one 10-g sample were irradiated for each treatment. The radiation doses were 0, 0.75, 1.50, 2.25, and 3.0 kGy; the temperature of irradiation was 0°C. One set of the 5-g samples were analyzed immediately for total surviving CFU. The second set of 5-g

samples was temperature abused at 35°C for 20 h before analysis. Each sample was also assayed for sterility by swabbing a tryptic soy agar plate with the initial dilution (10^{-1}) of the sample in Butterfield's phosphate after incubation overnight at 35°C. The 10-g samples were stored at -50°C after treatment and later analyzed for toxin. This study was repeated three times.

Microbiological analysis. Samples were assayed for CFU by standard pour-plate procedures by using tryptic soy agar with serial dilutions in sterile Butterfield's phosphate. Petri plates were incubated for 24 h at 35°C. CFU were counted with a Biotran II automated colony counter (New Brunswick Scientific, Edison, N.J.) on three petri plates containing 30 to 300 colonies; the lower detection limit was 10 CFU/g.

Toxin assay. The presence or absence of verotoxin was determined by the procedure described by Mehlman and Lovett (11) with serial dilutions made in a 96-well tissue culture plate. One 50% cytotoxic dose (CD_{50}) is the amount of toxin (titer) that produces a cytotoxic effect in 50% of the Vero cell monolayer incubated for 72 h at 37°C. Positive and negative controls were included with each assay.

Statistical analysis. Responses were expressed as the logarithm of the number of CFU per gram. These responses were converted into survival values, that is, the logarithm₁₀ of the number of CFU (N) divided by the initial number of CFU (N_0). Graphically, these results are presented as survival curves where the logarithm of N/N_0 is plotted against radiation dose. With this format the destruction of 1 log of CFU (1 D_{10}) has the value of -1.0, and D_{10} values are the negative reciprocal of the slope of the individual regression of the logarithm of N/N_0 plotted against radiation dose. Radiation D_{10} values were determined by least-squares analysis of the survival data, excluding the 0-kGy data to avoid possible shoulder effect and limiting the analysis to the linear portion of the curve, by using the REG procedure of the SAS statistical package (8, 16). Regression techniques were used to fit second-order response-surface models (6), and calculations were performed by the general linear models procedure of the SAS statistical package (8). The regressions were tested for differences by analysis of covariance.

RESULTS

Radiation D_{10} values at 0°C. The computed gamma radiation D_{10} values for stationary-phase *E. coli* O157:H7 irradiated aerobically and in vacuo at 0°C in MDCM were not significantly different, so the raw data (Fig. 1) were combined and a single value was computed (Table 1). For comparison, the D_{10} value for the wild-type *E. coli* ATCC 25922 in MDCM was determined to be 0.25 ± 0.01 kGy when irradiated in vacuo at 0°C.

The gamma radiation D_{10} value for log-phase *E. coli* O157:H7 irradiated in vacuo at 0°C in MDCM differed significantly from the value obtained for stationary-phase *E. coli* ($P < 0.0001$) (Fig. 1; Table 1). The gamma radiation D_{10} value for stationary-phase *E. coli* O157:H7 irradiated in vacuo at 0°C in finely ground lean beef differs from that obtained in MDCM ($P < 0.03$) (Table 1).

Effect of temperature during irradiation treatment. Response-surface methods revealed highly significant effects of variation in irradiation dose ($P < 0.0001$), irradiation temperature ($P < 0.0001$), and the interaction of radiation dose with irradiation temperature ($P < 0.0002$) on *E. coli* O157:H7 in MDCM. The following predictive equation was developed by the analysis of data: $\text{Log(survivors)} = -0.6410 - 1.289(\text{kGy}) + 0.002(\text{temperature}) - 0.070(\text{kGy})(\text{tempera-}$

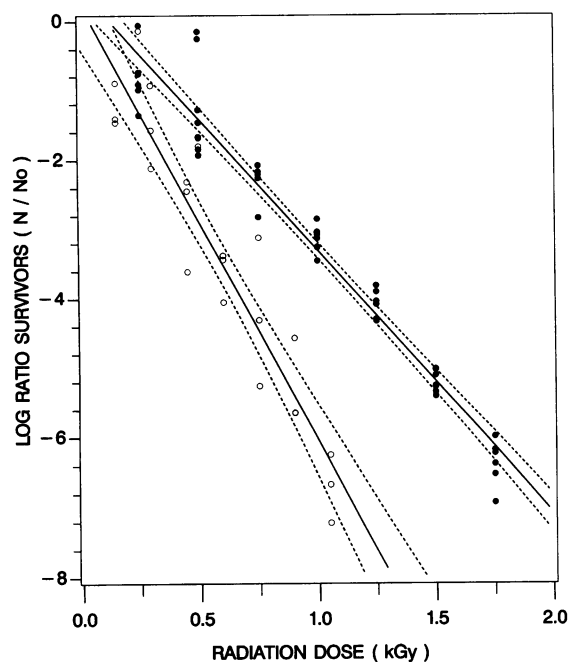


FIG. 1. Effect of gamma radiation on *E. coli* O157:H7 in MDCM. Closed circles, stationary-phase CFU g⁻¹; open circles, logarithmic-phase CFU g⁻¹; dashed lines, 95% confidence limits on mean predicted values.

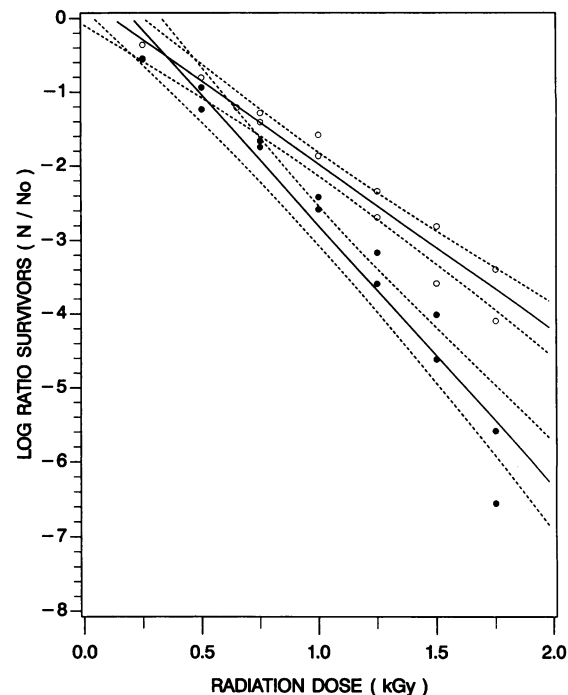


FIG. 2. Response of *E. coli* O157:H7 in finely ground lean beef to a dose of 1.5 kGy when irradiated in vacuo at temperatures of -60 to +15°C. The open circles and closed circles represent the results from two independent studies.

ture) - 1.301(kGy)² + 0.002(temperature)² ($R^2 = 0.931$). This equation predicts that this organism is extremely sensitive to the temperature of irradiation; e.g., at a dose of 1.5 kGy and a temperature of -20°C, the reduction in the log of the number of surviving CFU would be 2.64, but at 20°C the reduction would be 6.76. The equation also predicts that at a contamination level of 10⁶ CFU/g the contaminating organism would be completely eliminated by a dose of 1.5 kGy administered at 0°C. Compared with results obtained previously with *Salmonella* species (20, 23), *E. coli* O157:H7 was unusually sensitive to temperature during irradiation. Because this factor might be significant during food processing, additional studies were conducted to further define this response.

Since irradiation at 20°C to a dose of 1.5 kGy produced a 6.76-log reduction in the number of viable CFU, it was decided to expose *E. coli* O157:H7 to that dose over a wide range of closely controlled temperatures (Fig. 2). Both studies indicated that there was a large increase in the

radiation resistance of *E. coli* O157:H7 between 0°C (non-frozen) and -5°C in ground beef. Resistance to gamma radiation continued to increase to approximately -15°C; below that temperature there were only small increases in resistance down to -60°C (Fig. 2).

Because normal processing temperatures for the irradiation of meat should be close to 5°C, the D_{10} values at +5 and -5°C were determined (Fig. 3). The respective D_{10} values at +5 and -5°C were significantly different ($P < 0.0001$) (Table 1).

Challenge and temperature abuse study. An average inoculum of 10^{4.8} CFU of *E. coli* O157:H7 per g of commercial ground beef was reduced to less than 10 CFU/g in non-temperature-abused samples by a radiation dose of 0.75 kGy. No survivors (<10 CFU/g) were found in samples exposed to 1.5, 2.25, or 3.0 kGy of gamma radiation. In one of three replicate experiments, 10^{4.7} CFU/g was found in

TABLE 1. D_{10} values for *E. coli* O157:H7

Substrate	Growth phase	Temp (°C)	Atmosphere	D_{10} value ^a (kGy)	R^2
MDCM	Stationary	0	Air	0.26 ± 0.01 ^b	0.950
MDCM	Stationary	0	Vacuum	0.27 ± 0.01 ^b	0.957
MDCM (Fig. 1)	Stationary	0	Air + vacuum	0.26 ± 0.01 ^b	0.956
Beef	Stationary	0	Vacuum	0.27 ± 0.03 ^b	0.798
MDCM (Fig. 1)	Log	0	Vacuum	0.16 ± 0.01 ^c	0.925
MDCM (Fig. 3)	Stationary	+5	Vacuum	0.28 ± 0.02 ^b	0.946
MDCM (Fig. 3)	Stationary	-5	Vacuum	0.44 ± 0.03 ^b	0.949

^a Gamma radiation D_{10} value ± standard error.

^b Determined from the least-squares regression of the logarithms of survival values from 0.25 to 1.75 kGy.

^c Determined from the least-squares regression of the logarithms of survival values from 0.15 to 1.05 kGy.

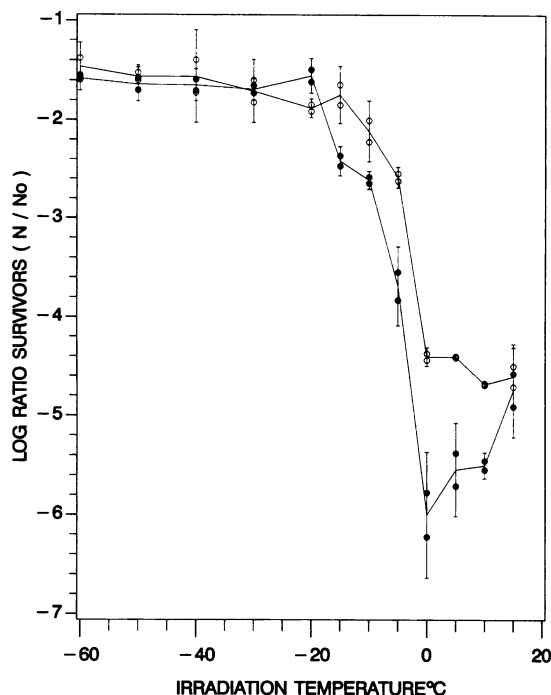


FIG. 3. Response of *E. coli* O157:H7 when irradiated in finely ground lean beef at +5 or -5°C in vacuo. Closed circles, +5°C; open circles, -5°C; dashed lines, 95% confidence limits on mean predicted values.

samples that were irradiated with 0.75 kGy following temperature abuse for 20 h at 35°C. Survivors were not detected (<10 CFU/g) in the other two samples that received 0.75 kGy or in samples that received 1.5-, 2.25-, or 3.0-kGy gamma radiation doses, even after temperature abuse for 20 h. The population of *E. coli* O157:H7 increased to 10^8 CFU/g in nonirradiated samples that were temperature abused for 20 h. Verotoxin was not detected in samples that were not temperature abused. It was detected in nonirradiated temperature-abused samples ($2,389 \pm 1,564$ 50% cytotoxic doses [mean $\pm$ standard deviation of three independent studies]) and at a trace level (mean = 4 50% cytotoxic doses) in one of the three replicate 0.75-kGy samples.

DISCUSSION

We have found *E. coli* O157:H7 to be very sensitive to gamma radiation at dosages within the range of 1.5 to 3.0 kGy, indicating that it could be very effectively controlled in poultry meat by irradiation. On the basis of the analysis of covariances, the D_{10} value of 0.26 at 0°C obtained for the combined air and vacuum analyses of the inactivation of stationary-phase cells of *E. coli* O157:H7 in MDCM is significantly different from the D_{10} value of 0.27 kGy obtained for the inactivation in finely ground lean beef. The statistically significant difference, by analysis of covariance, between these values has no practical significance, and further, the D_{10} values obtained with vacuum-packed MDCM and finely ground lean beef were identical. The relatively large differences in the fat and protein contents of these two meat products apparently did not significantly alter the toxicity of the gamma radiation for *E. coli* O157:H7. Patterson (14) reported D_{10} values of 0.27 and 0.26 kGy for

a wild-type *E. coli* strain irradiated at 10°C in chicken mince under vacuum or N_2 , respectively. We predict a D_{10} value of 0.23 kGy at 10°C for *E. coli* O157:H7 with the equation developed from the response-surface study. We obtained a value of 0.25 ± 0.01 kGy for the nonpathogenic *E. coli* strain ATCC 25922 in MDCM at 0°C and conclude that *E. coli* O157:H7 is not much more or less sensitive to gamma radiation than the nonpathogenic strains of this species.

We found no evidence for an effect of air versus vacuum packaging of the inoculated meat samples, as has been reported for *Salmonella* species (20, 23). Similarly, *Staphylococcus aureus* recently was reported as insensitive to air when irradiated on meat (22), and an oxygen effect was not detected when *Salmonella typhimurium* was irradiated on nonsterile chicken meat (21). The sensitizing effect of oxygen on *E. coli* cells irradiated in buffer solutions is well documented (7, 9, 15, 18). We know of no studies of the oxygen effect on either the wild type or *E. coli* O157:H7 in meat and postulate that any such effect was masked by the much greater mass of meat.

As expected (10), rapidly dividing logarithmic-phase cells were markedly more sensitive to gamma radiation than were stationary-phase cells.

E. coli O157:H7's greater resistance to the effects of gamma radiation at temperatures below freezing was not surprising, but the degree of its resistance at -5°C compared with its resistance at 0 or 5°C was unexpected. The D_{10} value at -5°C was 0.44 kGy, versus 0.28 kGy at +5°C, representing a 57% increase in resistance. Billen (4) estimated that water radicals (primarily $OH^\bullet$ radicals) account for at least 85% of the potential lethal X-ray damage in repair-deficient cells of *E. coli*. The G values (number of molecules changed per 100 eV of energy transferred to the system) for the formation in water of the hydrated electron (e_{aq}^-) and the hydroxyl radical ($OH^\bullet$), respectively, decrease from 2.8 and 2.7 at room temperature to 0.3 and 1.0 at -5°C (19). In addition, the movement of the $OH^\bullet$ radical is severely impeded in ice (19). Mulder (12) reported a D_{10} value of 0.58 kGy for *E. coli* K-12 on chicken skin irradiated at -18°C. The practical significance of the results reported here is that the processor must carefully consider the effects of processing temperature on the survival of this pathogen.

The failure to detect either viable *E. coli* O157:H7 or toxin in meat challenged with $10^{4.8}$ CFU/g and irradiated to 1.5 kGy at 0°C following 20 h of temperature abuse at 35°C indicates that very substantial protection can be offered to the consumer by irradiation with a minimum dose of 1.5 kGy and maximum dose of 3.0 kGy.

ACKNOWLEDGMENTS

We thank K. Felton, B. Mueller, C. Paquette, F. Schultz, and K. Snipes for technical assistance; P. Fratamico for advice and assistance on the verotoxin assay; and M. Haas, P. Fratamico, and S. C. Thayer for reviews of the manuscript.

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