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Inactivation of *Escherichia coli* O157:H7, Salmonellae, and *Campylobacter jejuni* in Raw Ground Beef by Gamma Irradiation

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Raw ground beef patties inoculated with stationary-phase cells of *Escherichia coli* O157:H7, salmonellae, or *Campylobacter jejuni* were subjected to gamma irradiation (^{60}Co) treatment, with doses ranging from 0 to 2.52 kGy. The influence of two levels of fat (8 to 14% [low fat] and 27 to 28% [high fat]) and temperature (frozen [−17 to −15°C] and refrigerated [3 to 5°C]) on the inactivation of each pathogen by irradiation was investigated. In ascending order of irradiation resistance, the D_{10} values ranged from 0.175 to 0.235 kGy (*C. jejuni*), from 0.241 to 0.307 kGy (*E. coli* O157:H7), and from 0.618 to 0.800 kGy (salmonellae). Statistical analysis revealed that *E. coli* O157:H7 had a significantly ($P < 0.05$) higher D_{10} value when irradiated at −17 to −15°C than when irradiated at 3 to 5°C. Regardless of the temperature during irradiation, the level of fat did not have a significant effect on the D_{10} value. Salmonellae behaved like *E. coli* O157:H7 in low-fat beef, but temperature did not have a significant effect when the pathogen was irradiated in high-fat ground beef. Significantly higher D_{10} values were calculated for *C. jejuni* irradiated in frozen than in refrigerated low-fat beef. *C. jejuni* was more resistant to irradiation in low-fat beef than in high-fat beef when treatment was at −17 to −15°C. Regardless of the fat level and temperature during inactivation, these pathogens were highly sensitive to gamma irradiation. An applied dose of 2.5 kGy would be sufficient to kill $10^{8.1}$ *E. coli* O157:H7, $10^{3.1}$ salmonellae, and $10^{10.6}$ *C. jejuni*, resulting in a high probability of complete inactivation of populations much higher than those occasionally present in ground beef patties.

Gamma irradiation has been used as a method of preserving foods in several countries, including Belgium, France, Japan, and the Netherlands (12). The process involves exposing the food to a specific dose of ionizing irradiation from, for example, ^{60}Co, a radioisotope of cobalt (21). Irradiation is known to initiate a chain of events leading to the impairment of structural or metabolic functions, such as fragmentation of DNA and the eventual death of microbial cells (4, 15), thus improving the microbiological quality of foods by reducing the number of spoilage and pathogenic microorganisms. Unlike thermal inactivation, irradiation at low doses does not significantly alter the sensory quality of foods (17). In 1985, the U.S. Food and Drug Administration approved the use of irradiation (1.0 kGy) to control *Trichinella spiralis* in pork (7). The use of 3.0 kGy for poultry, largely to eliminate salmonellae, was authorized in 1990 (8). Current regulations, however, do not permit irradiation treatment to preserve beef.

Due to recent outbreaks of foodborne illness associated with the ingestion of undercooked ground beef containing *Escherichia coli* O157:H7, there has been renewed interest in the use of ionizing irradiation to ensure the microbiological safety of foods. Studies have shown that irradiation can be an effective means of controlling human pathogens such as salmonellae (26), *Campylobacter jejuni* (13), *E. coli* O157:H7 (24), *Listeria monocytogenes* (11) and, at much higher doses, *Clostridium botulinum* (2) in poultry. The efficacy of irradiation treatment in eliminating potential pathogens in vacuum-packaged fresh beef cuts (16) and ground beef (27) has also been reported.

Several factors influence the resistance of microorganisms to inactivation by irradiation. Examples include the chemical composition and the physical state of the suspending medium (14), the temperature during irradiation, water activity (6), and the physiological state of the cells (19). Anellis et al. (1) reported that the D_{10} value of *Streptococcus faecium* increased from 0.09 to 0.38 kGy when the temperature during irradiation treatment was reduced from 5 to −196°C. Proteinaceous substances can also provide a protective effect against irradiation (5), and the presence of α,β-unsaturated carbonyl compounds in meat can sensitize bacterial cells to irradiation (18). Bacterial cells lyophilized in ground beef are reported to be less resistant to irradiation than cells lyophilized in a culture medium (14).

To date, irradiation inactivation studies with pathogenic bacteria have been largely done with small, laboratory-scale irradiators. Realizing that microbial response to irradiation can be influenced by intrinsic and extrinsic factors, we determined the independent and interacting effects of fat content in raw ground beef and of temperature on rates of inactivation of *E. coli* O157:H7, salmonellae, and *C. jejuni*. To the extent possible, the investigation was done under commercial beef processing and irradiation treatment conditions, in order to obtain information with the greatest potential for practical application to the meat industry.

MATERIALS AND METHODS

Determination of growth curves for test pathogens. Bacteria in the stationary phase of growth were used as inocula for ground beef. Hence, studies were initially done to determine growth curves for all strains (serovars) of test pathogens.

E. coli O157:H7 strains used were CA1 (raw ground beef isolate), E0019 (calf feces isolate), 505B (beef isolate), 932 (human feces isolate), and 204P (pork isolate). Each strain was cultured individually in tryptic soy broth, pH 7.3 (Difco,

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Detroit, Mich.), at 37°C. Cultures were transferred by loop at 24-h intervals, twice in 10 ml of tryptic soy broth in screw-cap test tubes (16 by 150 mm) and a third time in 50 ml of tryptic soy broth in a 250-ml Erlenmeyer flask. Samples (1.0 ml) were withdrawn from cultures at 4-h intervals over a 32-h incubation period at 37°C. Serial dilutions (1:10) in sterile 0.1% peptone (pH 7.0) were prepared, and appropriate dilutions (0.1 ml) were surface spread in duplicate on tryptic soy agar (Difco). Colonies were counted after 24 to 48 h of incubation at 37°C.

Salmonella serovars used were USDA SB-1 (*Salmonella dublin*, raw chicken isolate), obtained from Stan Bailey, USDA-ARS Russell Research Center, Athens, Ga.; CDC-2550-71 (*S. dublin*, cow isolate), obtained from the Centers for Disease Control and Prevention, Atlanta, Ga.; D1439 (*Salmonella enteritidis*, human feces isolate), obtained from James Dickson, Iowa State University, Ames; and ST (*Salmonella typhimurium*, cow isolate) and S11 (*S. typhimurium*, human feces isolate), from our laboratory stock culture collection. Two serovars each of *S. dublin* and *S. typhimurium* were examined because of their high incidence among bovine isolates. Each serovar was individually cultured by the procedure described above for *E. coli* O157:H7.

The strains of *C. jejuni* used were D484 (beef isolate) and EDL2 (cow feces isolate), obtained from the Centers for Disease Control and Prevention, and CR01, A74C, and SP92 (chicken isolates), obtained from Norman Stern, USDA-ARS Russell Research Center. Each strain was individually cultured in brucella broth (BB), pH 7.0 (Difco), supplemented with ferrous sulfate (F) (0.5 g liter^{-1}), sodium bisulfite (B) (0.2 g liter^{-1}), and pyruvic acid (P) (0.5 g liter^{-1}) (BB-FBP). Three consecutive 24-h transfers using loop inocula were made in 10 ml of BB-FBP in screw-cap test tubes (16 by 150 mm). A fourth 24-h transfer of 0.1 ml was made in 100 ml of BB-FBP in a 250-ml Erlenmeyer flask. All tubes and flasks were incubated at 42°C under a microaerophilic atmosphere (5% oxygen, 10% CO_2, and 85% nitrogen). Samples (1.0 ml) were withdrawn from cultures at 4-h intervals over a 32-h incubation period. Serial dilutions (1:10) in 0.1 M potassium phosphate-buffered saline (PBS), pH 7.2, were prepared and surface plated (0.1 ml) in duplicate on brucella agar (Difco) supplemented with FBP. Plates were incubated at 42°C under a microaerophilic atmosphere, and colonies were counted after 30 to 48 h.

Preparation of inocula for ground beef. Strains (serovars) of each test pathogen were cultured according to the procedure described for the growth curve analyses. Cultures were grown for 29 to 31 h to obtain cells in the stationary phase of growth. Cells from *E. coli* O157:H7 and *Salmonella* cultures were collected by centrifugation (5,000 × *g*, 20 min) at 21°C, whereas cells from *C. jejuni* cultures were harvested at 5°C. Pellets were resuspended in sterile 0.1% peptone (*E. coli* O157:H7 and salmonellae) or 0.1 M potassium PBS solution, pH 7.2 (*C. jejuni*). Suspensions of cells of each test pathogen were combined in a mixture (80 ml) containing approximately equal populations of each strain (serovar).

Ground beef. Raw ground (2.38-mm grind) beef, both low fat (8.2 to 13.9%) and high fat (26.8 to 27.1%), was obtained from a commercial beef processor and stored at −18°C at the Center for Food Safety and Quality Enhancement until used. Storage time did not exceed 5 weeks. Prior to inoculation with a five-strain mixture of each test pathogen, ground beef was thawed at 1 to 3°C over a 2-day period.

Fat and moisture contents (percent) of ground beef were measured using a CEM Fat and Moisture Analyzer (CEM Corp., Matthews, N.C.). Protein content was estimated from the nitrogen content, determined by the Kjeldahl method, by using a conversion factor of 6.25 (29).

Inoculation and preparation of beef patties. Five-strain (serovar) mixtures of each pathogen were tested in separate experiments. Cell suspensions (40 ml) were inoculated into 3,100 g of low- or high-fat ground beef (2 to 4°C). After thorough hand mixing (hands were protected with latex gloves) of the inoculated ground beef, patties (100 g) (9.5 by 1.2 cm) were prepared with a home-style hamburger press (M. E. Heuck Co., Cincinnati, Ohio). Individual patties were placed in polyethylene stomacher bags, heat sealed, and labeled. Individual packages were placed in a freezer (−16°C) or refrigerator (4°C) within 40 min after inoculation of the test pathogen.

Handling of inoculated beef before irradiation. After 20 to 22 h at freezing or refrigeration temperature, patties were packed in styrofoam containers (Liafoam, Baltimore, Md.) and placed in insulated coolers (Rubbermaid, Gott Corp., Winfield, Kan.). Separate coolers were used for frozen and refrigerated patties. To maintain the temperature of the patties during transport, Polar packs (−18°C) (Midlands Chemical Co., Omaha, Neb.) were placed between styrofoam containers. The coolers were sealed, transported to Vindicator, Inc., Mulberry, Fla., and placed in a freezer (−17 to −15°C) or refrigerator (3 to 5°C). The time elapsed between removal of patties from frozen or refrigerated storage at the Center for Food Safety and Quality Enhancement and arrival at Vindicator, Inc. was 5 to 6 h.

Irradiation treatment. Eight ground beef patties (two low-fat frozen, two low-fat refrigerated, two high-fat frozen, and two high-fat refrigerated) were placed in a cardboard box (21.5 by 21.5 by 4.0 cm). Patties from each of the four treatment combinations (two fat levels and two temperatures) were placed on the bottom of the box. A sheet of cardboard (21.2 by 21.2 cm) was placed on top of the patties, and an additional four patties were placed on top in such a way that when a low-fat frozen patty was at the bottom, a high-fat refrigerated patty was on the top. Three boxes fitted with lids were prepared. Each box constituted one replicate. Gammachrome YR dosimeters (Harwell Laboratory, Atomic Energy Authority, United Kingdom) were placed in a central position on the top external side of the lids of boxes representing replicates 1 and 2 and on the bottom external side of a third box (replicate 3) to determine the actual dose absorbed by the patties. The three stacked boxes were centered on top of a 10-cm-thick styrofoam block placed on a turntable (2.3 rpm) approximately 220 cm from the ^{60}Co irradiation source. The patties were exposed to gamma irradiation at the desired doses of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 kGy. Twenty-four unirradiated ground beef patties (three replicates of eight [two low-fat frozen, two low-fat refrigerated, two high-fat frozen, and two high-fat refrigerated]), subjected to the same storage, transport, and handling conditions as the irradiated patties, served as the control (0 irradiation dose). Actual doses applied are listed in Table 1.

Although, laboratory-scale ^{60}Co sources may provide more uniform application of treatment, they are not suitable for treating commercial-size lots of ground beef. All irradiation treatments were therefore done at a facility (Vindicator, Inc.) with a commercial-size ^{60}Co Gamma Beam 650 irradiator (Nordion International Inc., Kanata, Ontario, Canada). The dosimetry system used and the equipment for measuring the dose absorbed by beef patties were calibrated according to national standards established by the National Institute of Standards and Technology. Determination of the density of the boxes of patties was a part of this standard procedure.

Handling of inoculated beef after irradiation. Immediately after gamma irradiation treatment, individual packages of beef patties were labeled with the dose applied and were returned

TABLE 1. Desired and actual (absorbed) gamma irradiation doses for ground beef patties inoculated with test pathogens[a]

Pathogen	Actual dose for ground-beef patties after application of desired dose (kGy):					
	0.5	1.0	1.5	2.0	2.5	3.0
E. coli O157:H7	0.401 (0.007)	0.605 (0.025)	0.975 (0.119)	1.307 (0.089)	1.971 (0.081)	2.165 (0.276)
Salmonellae	0.400 (0.008)	0.663 (0.043)	0.983 (0.089)	1.327 (0.139)	1.799 (0.174)	2.201 (0.223)
C. jejuni	0.412 (0.007)	0.709 (0.028)	1.099 (0.042)	1.569 (0.062)	2.082 (0.053)	2.410 (0.076)

[a] Actual doses are the average of three replicates. Values in parentheses indicate ± standard deviation.

to frozen (−17 to −14°C) or refrigerated (2 to 5°C) storage at the Vindicator facility. After all treatments were administered, the patties were packed in styrofoam boxes and coolers in a manner identical to that described above. The coolers were transported back to the Center for Food Safety and Quality Enhancement within 6 h and were held frozen or refrigerated for 12 to 14 h. Frozen (−17 to −14°C) patties were thawed at 21 to 23°C (1 to 2 h) in preparation for microbiological analysis. Refrigerated (4°C) patties were subjected to microbiological analysis without any adjustment in temperature.

Microbiological analyses. Viable populations of cells in 29- to 31-h cultures of each strain (serovar) of test pathogen, as well as populations in the cell suspensions of five-strain (serovar) mixtures, were determined. Each ground beef patty (100 g) inoculated with *E. coli* O157:H7 or salmonellae was combined with sterile 0.1% peptone water (200 ml) at 21 to 23°C; 0.1 M potassium PBS (pH 7.2) was used as a diluent for enumerating viable cells of *C. jejuni* in each patty. Uninoculated (control) samples were also analyzed for the presence of each test pathogen. Beef and diluent were pummelled at medium speed in a Stomacher 400 Laboratory Blender (Teckman, Cincinnati, Ohio) for 1 min. Serial dilutions (1:10, 0.1 ml) were prepared and surface plated in duplicate on an appropriate enumeration medium. Populations of mesophilic aerobic bacteria in uninoculated ground beef were also determined.

***E. coli* O157:H7.** Appropriate serial dilutions (0.1 ml) of pummelled beef and peptone diluent were surface plated on sorbitol MacConkey agar (pH 7.1) (Unipath-Oxoid U.S., Columbia, Md.) supplemented with 4-methylumbelliferyl-β-D-glucuronide (MUG) (0.2 g liter^{-1}). The MUG reagent was added to heat-sterilized (121°C, 15 min), molten (47 to 50°C) sorbitol MacConkey agar. Colonies typical of *E. coli* O157:H7 (sorbitol-negative, MUG-negative) were counted after incubation at 37°C for 20 to 22 h. Randomly selected colonies were confirmed as *E. coli* O157:H7 by microscopic examination and by the API-20E miniaturized diagnostic kits (Analytab Division, Sherwood Medical, Plainview, N.Y.), the *E. coli* O157 latex agglutination assay (Unipath-Oxoid U.S.), and the Bacto *E. coli* antiserum H7 assay (Difco).

Salmonellae. Diluted, pummelled samples (0.1 ml) were surface plated on bismuth sulfite agar (pH 7.6) (Difco). Plates were incubated at 37°C for 48 h, and colonies typical of *Salmonella* spp. were counted. Random presumptive colonies were confirmed by microscopic examination and by appropriate biochemical tests (9).

C. jejuni. Diluted samples (0.1 ml) were surface plated on BB-FBP agar supplemented with 50 ml of defibrinated horse blood (Lampire Biologicals, Pipersville, Pa.), 0.33 μg of cefoperazone (Cefobid, Pfizer, Inc., New York, N.Y.) ml^{-1} and 200 μg of cycloheximide (Sigma, St. Louis, Mo.) ml^{-1}. Sterile defibrinated horse blood and antibiotics were added to 950 ml of heat-sterilized (121°C, 15 min), cooled (50°C) basal medium before the molten agar was dispensed into plates. Colonies which formed on plates incubated at 42°C under a microaerophilic atmosphere between 24 and 36 h were counted and recorded as presumptive *C. jejuni*. Randomly selected colonies were confirmed by microscopic examination and by appropriate biochemical tests (22).

Mesophilic aerobic microorganisms. Uninoculated ground beef (100 g) was combined with 200 ml of 0.1% peptone, pummelled at medium speed with a stomacher for 1 min, and surface plated (0.1-ml amounts) in duplicate on plate count agar (Difco). Plates were incubated at 30°C for 48 h before colonies were counted.

Statistical analysis. Each treatment combination, i.e., pathogen, fat level, irradiation temperature, and irradiation dose, was done in triplicate. Two patties per treatment combination were analyzed, and microbiological analyses were done in duplicate. The number of survivors of each pathogen after gamma irradiation treatment, expressed as $\log_{10}$ CFU/g of beef, was plotted against the irradiation dose. A regression line was fitted to sets of data by using the regression procedure of the SAS statistical package (20). For each pathogen, six regression lines were generated for each patty, replicate, and treatment combination. In those instances where inactivation curves were characterized by tailing (salmonellae and *C. jejuni*), regression lines were also fitted to data points that did not contribute to tailing. Regression coefficients, slopes, and 95% confidence limits were determined for all regression lines.

The irradiation resistance of each pathogen subjected to each treatment combination was assessed by calculating the D_{10} values obtained by taking the negative reciprocal of the slope for each regression line. This procedure yielded six D_{10} values for each pathogen. D_{10} values were then compared by using a general linear model (20), and differences between mean D_{10} values were determined using Duncan's multiple range test. An analysis of variance was also conducted to determine if D_{10} values were affected by the omission of datum points responsible for the tailing or shouldering of the inactivation curves.

RESULTS AND DISCUSSION

Chemical composition and microbiological quality of raw ground beef. The fat, protein, and moisture contents of ground beef used in experiments involving each test pathogen are

TABLE 2. Fat, protein, and moisture contents of ground beef before inoculation with test pathogens

Pathogen	Level of fat	Component (%)		
		Fat	Protein	Moisture
E. coli O157:H7	Low	13.9	18.0	67.3
	High	27.1	15.2	56.3
Salmonellae	Low	12.5	18.5	68.0
	High	27.5	15.2	55.2
C. jejuni	Low	8.2	19.3	70.3
	High	26.8	16.0	56.0

TABLE 3. Mean populations of pathogens in inocula, aerobic microorganisms in ground beef, and pathogens in inoculated ground beef

Pathogen	Population (log_{10} CFU) in:				
	Inoculum[a] (per ml)	Ground beef[b] (per g)			
		Aerobic microorganisms		Pathogen	
		Low fat	High fat	Low fat	High fat
E. coli O157:H7	9.44	4.85	5.43	7.18	7.13
Salmonellae	9.08	4.53	5.05	6.94	6.91
C. jejuni	9.16	3.52	4.30	6.45	6.49

[a] Five-strain mixture.
[b] Populations of aerobic mesophilic microorganisms in ground beef were determined before inoculation with test pathogens. Populations of pathogens were determined within 10 min after inoculating 3,100 g of ground beef with 40 ml of inoculum.

listed in Table 2. The amount of fat ranged from 8.2 to 13.9% in low-fat beef and from 26.8 to 27.5% in high-fat beef. Although the effectiveness of ionizing doses of gamma irradiation to inactivate pathogenic bacteria could be influenced by the levels of protein and moisture, only the influence of two substantially different levels of fat, in low- and high-fat products, was investigated. Urbain (28), however, suggested that in aqueous environments the lethal effect of irradiation increases because more free radicals are produced. Proteins and carbohydrates tend to have a protective effect as they compete with bacteria for interaction with free radicals produced during hydrolysis of water. Further studies would have to be conducted to determine their influence on the irradiation inactivation of microorganisms.

Populations of aerobic, mesophilic microorganisms in uninoculated ground beef are listed in Table 3. Aerobic colony counts ranged from $10^{3.52}$ to $10^{5.43}$ CFU/g. Low-fat ground beef generally contained lower populations of aerobic mesophiles than did high-fat ground beef. Regardless of the fat content, however, the ground beef used in this study had lower aerobic, mesophilic counts than ground beef sold in retail stores (10). Freshly prepared commercial ground beef has been reported to contain aerobic mesophiles between $10^{4.51}$ and $10^{6.48}$ CFU/g. None of the test pathogens was detected in uninoculated ground beef.

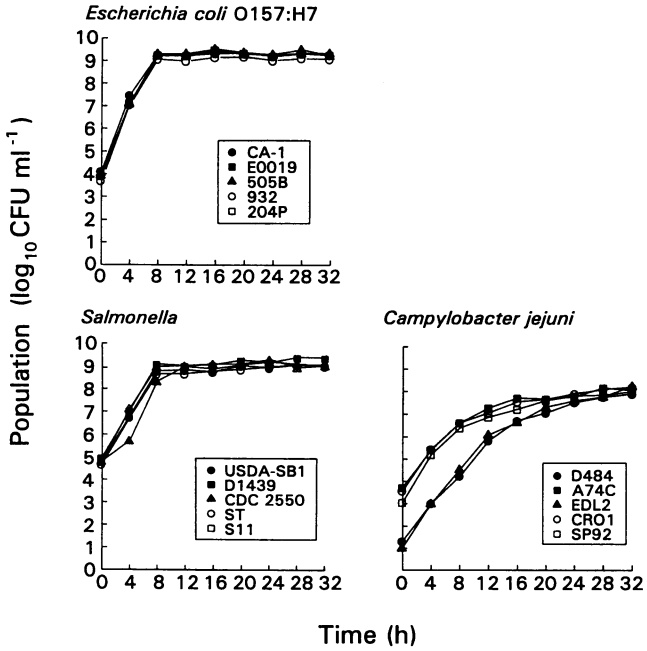

FIG. 1. Growth curves for 5 strains (serovars) of *E. coli* O157:H7, salmonellae, and *C. jejuni*.

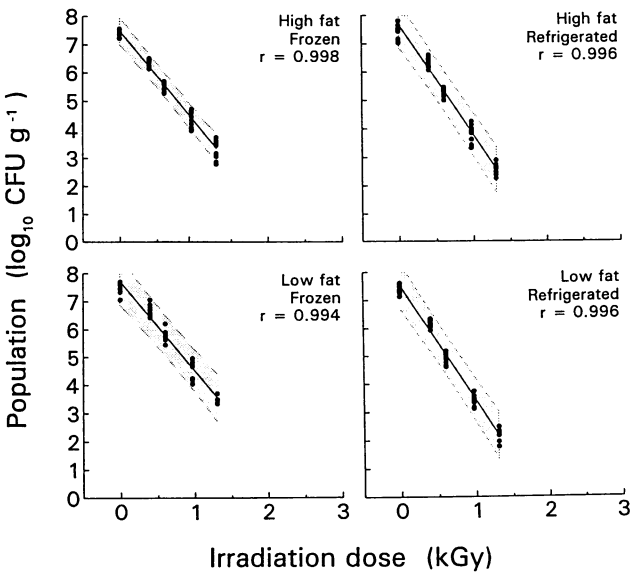

FIG. 2. Irradiation inactivation curves for *E. coli* O157:H7 inoculated in high-fat frozen, high-fat refrigerated, low-fat frozen, and low-fat refrigerated ground beef. Shaded areas indicate 95% confidence limits.

Growth curve analyses. The age and physiological state of bacterial cells have been shown to influence their response to

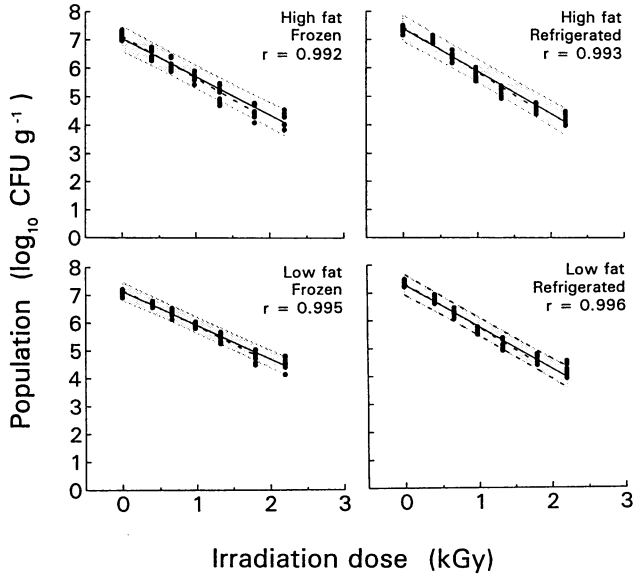

FIG. 3. Irradiation inactivation curves for salmonellae inoculated in high-fat frozen, high-fat refrigerated, low-fat frozen, and low-fat refrigerated ground beef. Shaded areas indicate 95% confidence limits.

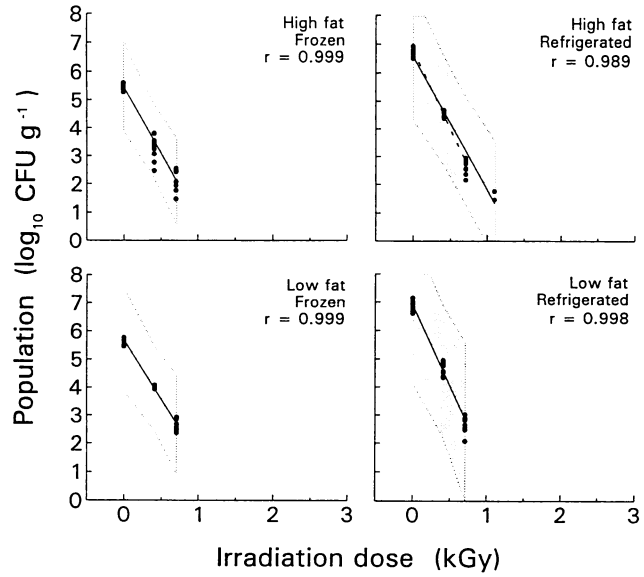

FIG. 4. Irradiation inactivation curves for *C. jejuni* inoculated in high-fat frozen, high-fat refrigerated, low-fat frozen, and low-fat refrigerated ground beef. Shaded areas indicate 95% confidence limits.

ionizing radiation. Stapleton (19) reported that the irradiation resistance of *E. coli* is highest during the lag phase of growth, decreases during the logarithmic phase, and increases again during the stationary phase of growth. Although bacterial cells in the logarithmic growth phase are generally more sensitive to stress than are stationary-phase cells, there are some exceptions. Lambert and Maxcy (13) reported that the irradiation resistance of *C. jejuni* cells was not affected by age. In any case, it was desirable to know the growth phase of cells used to inoculate ground beef. Growth curves for *E. coli* O157:H7, salmonellae, and *C. jejuni* are shown in Fig. 1. After 29 to 31 h, all cultures were in a stationary phase of growth. *E. coli* O157:H7 and salmonellae reached the stationary phase within 8 h, whereas 16 to 20 h was required for *C. jejuni*.

Populations of pathogens in the inocula and in ground beef immediately after inoculation are listed in Table 3. All inocula contained more than 10^9 CFU/ml. Slight decreases in populations of viable cells would be expected to occur during holding times between inoculation and irradiation (43 to 45 h) and between irradiation and analysis (24 to 25 h). To minimize any effect of transport or handling conditions, control patties (inoculated but not irradiated) were subjected to the same transport and handling conditions as patties treated with gamma irradiation.

Gamma irradiation inactivation curves. Irradiation inactivation curves were obtained for *E. coli* O157:H7 (Fig. 2), salmonellae, (Fig. 3), and *C. jejuni* (Fig. 4) subjected to all combinations of test parameters, i.e., low- and high-fat beef irradiated at −17 to −15°C and 3 to 5°C. Shaded areas in these figures indicate 95% confidence limits. Regression coefficients for all treatments were high (≥0.989). Regardless of test parameters, a reduction in population with increasing irradiation doses was observed. Tailing of inactivation curves was observed for salmonellae subjected to all combinations of test parameters and for *C. jejuni* in high-fat refrigerated ground beef, as indicated by the dashed lines in Fig. 3 and 4, respectively. Tailing of curves may have been due to differences in the irradiation resistance of test cells. A mutant strain of *S. typhimurium* LT2 exhibiting resistance to irradiation has been reported by Davis and Sinskey (3). Slight differences in the physiological age of cells or the distribution of cells in ground beef may also have contributed to tailing of inactivation curves.

D_{10} values (kGy) for the three pathogens in low- and high-fat frozen and refrigerated ground beef are listed in Table 4. Values were calculated for inactivation curves with and without tailing. Statistical analysis revealed that there was no significant difference ($P \leq 0.05$) in the D_{10} values obtained for the two types of curves. D_{10} values for the pathogens ranged from 0.241 to 0.307 kGy for *E. coli* O157:H7, from 0.621 to 0.800 kGy for salmonellae, and from 0.175 to 0.235 kGy for *C. jejuni*.

Our study indicates that D_{10} values for pathogens in frozen ground beef were generally higher than those calculated for refrigerated beef. Regardless of the fat level, significantly ($P < 0.05$) higher D_{10} values were observed for *E. coli* O157:H7 when irradiated at −17 to −15°C than when irradiated at 3 to 5°C. A similar effect of temperature was reported by Thayer et al. (26). D_{10} values for *E. coli* O157:H7 in mechanically deboned chicken irradiated at 5 and −5°C were 0.26 kGy and 0.42 kGy, respectively. The protective effect of low temperature may be attributed to the suppression of indirect debilitating effects of reactive intermediates, primarily ·OH radicals resulting from water hydrolysis. Freezing immobilizes water molecules, and as a consequence, the diffusion of free radicals is restricted (4).

At a given irradiation temperature, the level of fat did not have a significant effect on D_{10} values for *E. coli* O157:H7. Thayer and Boyd (24) also observed that large variations in the fat contents and the protein contents of mechanically deboned chicken meat and finely ground lean beef did not alter the

TABLE 4. D_{10} values of pathogenic bacteria in low- and high-fat ground beef[a]

Pathogen	D_{10} values (kGy) of bacteria in:			
	Low-fat beef		High-fat beef	
	Frozen	Refrigerated	Frozen	Refrigerated
E. coli O157:H7	0.307 a (0.015)	0.241 b (0.012)	0.305 a (0.023)	0.251 b (0.028)
Salmonellae				
With tailing	0.800 a (0.054)	0.624 c (0.089)	0.745 ab (0.057)	0.661 bc (0.031)
Without tailing	0.756 a (0.057)	0.621 b (0.027)	0.675 ab (0.060)	0.618 b (0.028)
C. jejuni				
With tailing	0.235 a (0.017)	0.175 c (0.005)	0.207 b (0.016)	0.199 bc (0.017)
Without tailing	0.235 a (0.017)	0.175 c (0.005)	0.207 b (0.016)	0.178 c (0.017)

[a] Mean values in the same row that are not followed by the same letter are significantly different ($P \leq 0.05$). Values in parentheses indicate ± standard deviation.

TABLE 5. Populations of pathogens that would theoretically be killed in ground beef by treatment with gamma irradiation[a]

Pathogen	Population (log_{10} CFU/g) killed by:				
	0.5 kGy	1.0 kGy	1.5 kGy	2.0 kGy	2.5 kGy
E. coli O157:H7	1.64	3.26	4.89	6.51	8.14
Salmonellae	0.62	1.25	1.88	2.50	3.13
C. jejuni	2.13	4.26	6.38	8.51	10.64

[a] Calculated on the basis of the highest D_{10} value for each pathogen (Table 4). Population killed (theoretical value) = dose ÷ D_{10} value.

sensitivity of *E. coli* O157:H7 to gamma irradiation. The D_{10} value of *E. coli* O157:H7 in ground beef at 5°C was reported to be 0.27 kGy.

Salmonellae behaved like *E. coli* O157:H7 in low-fat beef, but temperature did not have a significant effect on the D_{10} value when the pathogen was in high-fat beef. D_{10} values for salmonellae were 2.4- to 2.6-fold higher than D_{10} values for *E. coli* O157:H7. D_{10} values for *Salmonella* spp. have been reported to range from 0.38 to 0.77 kGy at 2°C in mechanically deboned chicken (25), whereas a D_{10} value of 0.57 kGy has been observed for the pathogen in ground beef treated at 18 to 20°C (23). At any given temperature, during irradiation, the level of fat did not significantly influence D_{10} values for salmonellae.

Significantly higher D_{10} values were calculated for *C. jejuni* in frozen than in refrigerated high-fat beef. The D_{10} values for *C. jejuni* suspended in high-fat ground beef, however, were lower than those suspended in low-fat beef. A D_{10} value of 0.19 kGy at 0 to 5°C was reported for *C. jejuni* in ground turkey (13).

The order of sensitivity of test pathogens to gamma irradiation was *C. jejuni* > *E. coli* O157:H7 > salmonellae. This order was not influenced by the fat level or by the temperature of ground beef during irradiation treatment. These differences in irradiation resistance may be attributed to such factors as cell size and the structural arrangement of the DNA within the cell. According to Diehl (4), different species or strains of the same species may require different doses to achieve the same degree of inactivation. Furthermore, *C. jejuni* is particularly sensitive to changes in environmental stress, which may include ionizing irradiation.

On the basis of the highest D_{10} values for each pathogen (Table 4), populations which would theoretically be killed in ground beef subjected to gamma irradiation doses of 0.5, 1.0, 1.5, 2.0, and 2.5 kGy were calculated (Table 5). Depending upon populations of pathogens anticipated to be present in ground beef and the desired probability of completely inactivating these populations, the necessary gamma irradiation dose can be theoretically selected. An applied dose of 2.5 kGy would be sufficient to kill $10^{8.1}$ *E. coli* O157:H7, $10^{3.1}$ salmonellae, and $10^{10.6}$ *C. jejuni*. Since such populations are considerably greater than those occasionally found in ground beef, the application of 2.5 kGy would, with a high probability, result in complete inactivation of these pathogens.

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