

Fate of Gamma-Irradiated *Listeria monocytogenes* during Refrigerated Storage on Raw or Cooked Turkey Breast Meat†

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ABSTRACT

The radiation resistance and ability of *Listeria monocytogenes* ATCC 7644, 15313, 43256, and 49594 to multiply on irradiated, air-packed, refrigerated raw or cooked turkey breast meat nuggets (ca. 25 g) and ground turkey breast meat was investigated. Gamma-radiation D values for *L. monocytogenes* were significantly different on raw and cooked nuggets, 0.56 ± 0.03 kGy and 0.69 ± 0.03 kGy, respectively; but they were not significantly different ($P \leq 0.05$) on raw and cooked ground turkey meat. High populations ($\sim 10^9$ CFU/g) of *L. monocytogenes* declined during 14 days of storage at 4°C in both irradiated and nonirradiated samples of raw but not of cooked ground turkey breast meat. A moderate inoculum ($\sim 10^3$ CFU/g) did not survive a radiation dose of 3 kGy. The population increased in cooked but not in raw samples of irradiated ground turkey meat stored at either 2 or 7°C for 21 days. The D value changed significantly from 0.70 ± 0.04 to 0.60 ± 0.02 kGy when the product was cooked to an internal temperature of 80°C before irradiation. Growth on either raw or cooked turkey meat did not alter the radiation resistance of *L. monocytogenes*. Analyses were performed for pH, a_w , moisture, and reducing potential of raw and cooked turkey meat and for pH, amino acid profile, thiamine, and riboflavin contents of aqueous extracts of raw and cooked turkey meats without identifying the factor or factors involved in differences in the survival and multiplication of *L. monocytogenes* on raw and cooked meat.

Listeria monocytogenes was found on a number of foods, including raw, undercooked, and cooked poultry and poultry products; in some cases listeriosis in humans was linked to its presence on these foods (10, 19, 21, 22, 32, 34, 42). These products can become vectors of disease because *L. monocytogenes* can multiply on them during refrigerated storage (5, 8, 14, 15, 17, 18, 25, 31, 35, 43). Even in well-run processing plants, cooked poultry products may become contaminated before they are packaged; at least one such case has been documented (42). Because ionizing irradiation can be applied after packaging, it might provide an excellent means to eliminate *L. monocytogenes* from prepackaged ready-to-eat poultry and poultry products.

The radiation resistance of *L. monocytogenes* on both raw and cooked chicken and turkey has been measured under a number of conditions (7, 20, 26, 28, 37, 40). Temperature and the medium in which the bacteria are suspended are two of the factors affecting the radiation resistance of *Listeria* (9, 28, 36).

These studies test four hypotheses: (i) that the radiation resistance of *L. monocytogenes* on raw or cooked turkey meat is the same, (ii) that the recovery and multiplication of high numbers of *L. monocytogenes* on aerobically packed and irradiated raw or cooked ground turkey meat during 14

days of refrigerated (4°C) storage would not differ, (iii) that the recovery and multiplication of low numbers of *L. monocytogenes* on aerobically packed irradiated raw or cooked ground turkey meat during prolonged storage at 2°C or 7°C would not differ, and (iv) that the order of cooking and irradiation would not alter the survival of *L. monocytogenes*.

MATERIALS AND METHODS

Bacterial cultures. Four isolates of *L. monocytogenes* (ATCC 7644, 15313, 43256, and 49594) were obtained from the American Type Culture Collection, Rockville, Md. These cultures were maintained and colonies isolated by streaking on tryptic soy agar (TSA, Difco Laboratories, Detroit, Mich.) at 37°C. Culture identity was confirmed by Gram stains and from reactions on gram-positive identification (GPI) cards using the Vitek AMS (Automicrobic System, bioMérieux Vitek, Inc., USA, Hazelwood, Mo.) (1). Each isolate was cultured independently in 100 ml of tryptic soy broth (TSB, Difco) in a baffled 500-ml Erlenmeyer culture flask at 37°C with agitation at 150 rpm on a rotary shaker for 18 h. Equal amounts of the cultures of each isolate were mixed together, and the mixed culture was sedimented by centrifugation. Tenfold inocula were prepared by resuspending the cells in $\frac{1}{10}$ volume of sterile Butterfield's phosphate (0.25 M KH_2PO_4 , adjusted to pH 7.2 with NaOH).

Radiation source, techniques, and dosimetry. The Lockheed Georgia Company self-contained, gamma-radiation source strength at the time of this study was ca. 119,384 Ci (4.42 Pbq) with a dose rate of $0.106 \text{ kGy/min}^{-1}$. The dose rate was established using National Physical Laboratory (Middlesex, UK) dosimeters. Corrections for decay of the source are made monthly. Routine dosimetry was performed using barrier pouches containing five Far West FWT-60 radiochromic film dosimeters that were equilibrated

to 51% relative humidity (RH) (Far West Technology, Inc., Goleta, Calif.). The pouches containing the dosimeters were taped to individual samples. A standard curve was developed with the pouches of dosimeters surrounded by 5 mm of polystyrene, and the temperature was maintained at $5 \pm 1^\circ\text{C}$ (3). The FWT-60 dosimeters were read at 600 nm with an FWT-92 reader. In some studies 5-mm-diameter alanine dosimeters (Brucker Instruments, Rjeomstettem, Germany) were used, and the free-radical signal was measured using a Bruker EMS 104 EPR analyzer (4). Variations in sample dose absorption were minimized by placing small samples within a uniform area of the radiation field, by irradiating the samples within a polypropylene container (4 mm wall) to absorb Compton electrons, and by using the same geometry for sample irradiation during each study. Samples were maintained at $5 \pm 1^\circ\text{C}$ during irradiation by thermostatically controlled injection of the gas phase from liquid nitrogen into the top of the irradiation chamber. Sample temperature was monitored continuously during irradiation with thermocouples taped to two samples in the chamber. Based on measurements of dosimeter responses in several experiments, the actual dose was within $\pm 2\%$ of the target dose.

Microbiological analysis: Plate count. Samples were assayed for CFU by standard pour-plate procedures using TSA with serial dilutions in sterile Butterfield's phosphate. The samples (25 g) were diluted 10-fold and homogenized with a Stomacher lab blender (model 400, Tekmar Co., Cincinnati, Ohio) for 90 s and pour plated in triplicate at appropriate dilutions. All samples were incubated at 37°C for 48 h. CFU were counted at a dilution giving 30 to 300 CFU per plate with a New Brunswick Scientific Biotran II automated colony counter.

Enrichment culture. In experiment 3 the initial sample dilutions were made in UVM *Listeria* enrichment broth (Difco). The enrichment broth was incubated overnight at 37°C and checked for the presence of viable *L. monocytogenes* by plating on TSA. Culture identity was confirmed as described above.

Chemistry. Moisture was determined by CEM methods (2). Water activity (a_w) was measured on duplicate 5.0-g samples using the AquaLab model CX-2T water activity instrument (Decagon Devices, Inc., Pullman, Wash.). The temperature was maintained at $25 \pm 0.05^\circ\text{C}$. Initial measurements were made with the instrument calibrated at 75.5% RH.

Thiamine was determined as previously described by flow injection determination, conversion to thiochrome, and fluorometric measurement ($\lambda_{\text{excitation}} = 365 \text{ nm}$, $\lambda_{\text{emission}} = 460 \text{ nm}$) (FID) (12). Riboflavin was determined in the same 2% trichloroacetic acid (TCA) extracts used for the FID determination of thiamine and assayed fluorometrically, $\lambda_{\text{excitation}} = 450 \text{ nm}$, $\lambda_{\text{emission}} = 530 \text{ nm}$ (11).

Reductant levels in the raw and cooked turkey meat were determined by titration with 2,6-dichlorophenol-indophenol (DCPIP) (12). In general, DCPIP is readily reduced by mild reductants, including sulfhydryl groups (2, 24). Meat samples (1.0 g) were blended under nitrogen with 20 ml of Tris/SDS/EDTA buffer (10.4 g Tris, 5 g SDS [sodium dodecyl sulfate] and 1.2 g EDTA adjusted to pH 8.0 with 0.1 N NaOH and diluted to 1.0 liter). Standards and samples were titrated at 70 to 80°C under flowing nitrogen to prevent reoxidation of the 2 mM DCPIP. Solutions of DCPIP were standardized with ascorbic acid, cysteine, and dithio-

threitol. Ascorbic acid titrated to a rosy-pink end point; the sulfhydryl compounds to a greyish-blue end point.

The pH of the meat was determined using an Orion Research Digital Ionalyzer/501 meter by pressing a combination spear-tipped pH electrode (Orion 86300, Orion Research Inc., Beverly, Mass.) into the meat or solution, as appropriate.

Free amino acids were separated with a Pico*Tag Ultrafiltration device (Millipore Corporation, Milford, Mass.); samples were frozen until used. Aliquots (20 μl) were removed and placed in analysis tubes that had been pyrolyzed at 500°C . The samples were evaporated in a Waters Pico*Tag Work Station and then dried from a mixture of methanol/water/triethylamine (2:2:1). For hydrolysis, a 20- μl aliquot of each sample was dried in a pyrolyzed tube and reacted with 6 N HCl containing 1.0% phenol in the gas phase. The amino acids were quantitated as their phenylthiocarbonyl derivatives using the Waters Pico*Tag high-pressure liquid chromatography (HPLC) system for hydrolysates.

Glucose concentrations of clarified extracts of raw or cooked meat, as described below, were determined using the Sigma Diagnostics glucose procedure no. 510 and kit (Sigma Chemical Co., St. Louis, Mo.) according to directions of the manufacturer for deproteinized blood, plasma, or serum.

Determination of gamma-radiation D_{10} values and statistical analysis. For each experiment, the average (N) CFU/plate of three plates, at a dilution giving 30 to 300 CFU/plate, was determined and divided by the average of the three zero-dose values (N_0) to give a survivor value (N/N_0). The log of this value was then used in the calculations of the D_{10} value. There are a number of methods in use for the determination of radiation D_{10} values. We define the D_{10} value as the gamma radiation dose resulting in a 90% reduction of viable *L. monocytogenes*; and we determine that value by least-squares analysis of the regression of a minimum of five survival values, excluding the zero-dose value, versus radiation dose (39). Each study was repeated independently at least twice. Statistical calculations were performed with the general linear models procedure of the SAS statistical package (13, 33). Regressions were tested for differences by analysis of covariance.

Experiment 1: Objective. To test the hypothesis that the radiation resistance of *L. monocytogenes* is the same on raw or cooked turkey meat nuggets.

Experimental design. Substrate = raw or cooked turkey breast meat nuggets. Sample size = ~ 25 g. Irradiation dose = 0, 0.25, 0.50, 0.75, 1.0, 1.25, 1.50, 1.75, and 2.0 kGy. Study replications = 2. Samples (nine doses \times two substrates \times two replications) = 36.

Substrate. Boneless and skinless turkey breast meat was purchased from a local market. Approximately 60% of the meat was cooked in a microwave oven, with frequent turning, to an internal temperature of 85°C (measured with a calibrated thermocouple). Both raw and cooked meat, separately, were cut into approximately 25-g pieces, "nuggets" averaging 12.5 mm in thickness. The nuggets were vacuum packaged in stomacher 400 polyethylene bags. These bags were then vacuum sealed in Freshstuff oxygen barrier pouches (oxygen transmission 0.6 to 0.8 $\text{cm}^3/645 \text{ cm}^2/24 \text{ h}$ at 3.2°C and 90% RH) (American National Can

Company, Des Moines, Iowa). The packaged meat was rapidly frozen on dry ice and sterilized with a gamma-radiation dose of 42 kGy at -30°C . The sterile meats were stored at -50°C until used.

Inoculation. Each nugget was inoculated with 0.1 ml of the cocktail of the four isolates of *L. monocytogenes* ($\sim 7.6 \times 10^6$ CFU per g) and vacuum packaged within a sterile no. 400 stomacher bag.

Experiment 2: Objective. To test the hypothesis that *L. monocytogenes* would not recover and multiply on aerobically packed and irradiated raw or cooked ground turkey meat during 14 days of storage at 4°C .

Experimental design. Substrate = raw or cooked ground turkey meat. Sample size = ~ 25 g. Irradiation dose = 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 kGy. Storage temperature = 4°C . Sampling times = 1, 3, (7 or 8), and 14 days. Study replications = 2. Samples (two substrates \times seven doses \times 4 days \times two replications) = 112. Analyses = total plate count, isolate identification, enrichment culture.

Substrate. Ground turkey was purchased locally, packed in 200-g amounts in stomacher bags, overbagged and vacuum sealed in Freshstuff barrier pouches, and radiation sterilized, as described above. Approximately two thirds of the turkey was cooked to an internal temperature of 85°C using a microwave oven prior to sterilization. Four 200-g bags of sterile meat (raw or cooked) were required for each replicate of the study.

Inoculation. Sterile ground turkey (raw or cooked) was inoculated with enough of the mixture of strains of *L. monocytogenes* in TSB (20 ml/200 g of meat) for a population of approximately 3×10^9 CFU/g after stomaching for 90 s. Following mixing, 25-g aliquots of the inoculated meat were placed within sterile poultry pouches (Cryovac E300) that were then vacuum (-0.95 bar) sealed. These pouches were chosen for their oxygen permeability and suitability for irradiation of poultry.

Experiment 3: Objective. To determine the fate of *L. monocytogenes* when present in more realistic numbers ($\sim 10^3$ CFU/g) on raw or cooked ground turkey following irradiation within an oxygen permeable package during storage at 2 and 7°C . The null hypotheses were that neither the cooking of the turkey nor storage temperature would alter the recovery and multiplication of *L. monocytogenes*.

Experimental design. Substrate = raw or cooked ground turkey meat. Sample size = 25 g. Irradiation dose = 0, 1.0, 2.0, 3.0, and 4.0 kGy. Storage temperature = 2 or 7°C . Sampling times = 0, 1, 3, 7, 10, 14, 17, and 21 days. Study replications = three. Samples (two meat treatments \times five doses \times two temperatures \times 7 days \times 3 replications) = 420. Analyses = total plate count, isolate identification, enrichment culture, meat pH, moisture, and a_w .

Substrate. Commercial ground turkey was purchased locally, packaged, and irradiation sterilized (either with or without cooking), as described above.

Inoculation. Sufficient inoculum was added to the meat for a population of $\sim 10^3$ CFU/g after being mixed with the turkey. Inoculation, preparation of 25-g aliquots, and packaging was as described for experiment 2.

Assay. In addition to pour plate analyses for surviving *L. monocytogenes*, enrichment cultures were performed at days 0 and 21.

Experiment 4: Objective. To test the hypothesis that the radiation resistance of *L. monocytogenes* is the same on, or grown on, raw or cooked ground turkey breast meat.

Experimental design. Substrate = sterile raw or cooked ground turkey meat. Sample size = 5.0 g. Radiation dose = 0, 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, and 2.4 kGy at 5°C . Cultures were grown in TSB and inoculated onto, or grown on, the meat. Study replications = two. Samples (nine radiation doses) \times (2 raw or cooked meat) \times (2 grown on meat) = 72 samples. Samples were taken at 0, 6, and 18 h to allow rough estimates of growth rate and to determine the effects of culture growth on the pH of the meat. Analyses = CFU and pH. Packaging: 5.0 g per sample spread over an area of approximately 10×10 cm within a sterile E300 poultry pouch and vacuum sealed.

Substrate. Commercial ground turkey was purchased locally, packaged in 100-g amounts, frozen, and irradiation sterilized, as described above. Just before use, approximately 150 g of sterile turkey were thawed and placed inside a 15-cm-diameter sterile plastic petri dish and cooked in a microwave oven to an internal temperature of 85°C using the defrost cycle. The turkey lost approximately 7.6% of its total weight when cooked.

Inoculation. Sterile ground turkey (100 g) in a stomacher bag was inoculated with 1 ml of cells from an equal mixture of each of the *L. monocytogenes* isolates in TSB by kneading the meat in the bag by hand. After mixing, the population of *L. monocytogenes* in the meat was approximately 2×10^3 CFU/g. After incubation for 24 h at 37°C , 5-g portions of the meat were placed in sterile Cryovac E300 pouches and vacuum sealed at -0.95 bar.

Experiment 5: Objective. To test the hypothesis that the order of cooking or irradiation does not affect the radiation D value or survival of *L. monocytogenes* on turkey.

Experimental design. Substrate = inoculated raw or cooked ground turkey. Sample size = 25 g. Cooking was done with a microwave oven to an internal temperature of 72 to 75°C in accordance with the Code of Federal Regulations 9:381.150, which specifies a minimum internal temperature of 160°F (72°C) for poultry rolls and certain other products (6). This minimum temperature would cause the least injury to the cells. Treatments = raw irradiated, cooked irradiated, and irradiated cooked. Radiation dose = 0, 0.30, 0.60, 0.90, 1.20, 1.50, 1.80, 2.10, and 2.40 kGy. Experimental replicates = three. Samples = (three treatments) \times (three replicates) \times (nine radiation doses) = 81.

Substrate. Commercial ground turkey was purchased locally, packaged, and irradiation sterilized as described in experiment 2.

Inoculation. Sterile ground turkey (raw or cooked) was inoculated with enough (20 ml/200 g of meat) of the mixture of strains of *L. monocytogenes* in TSB for a final population of approximately 3×10^9 CFU/g after stomaching for 90 s. Following mixing, 25-g aliquots of the inoculated raw turkey meat were placed within sterile poultry bags (Cryovac E300), which were then vacuum (-0.95 bar) sealed. Alternatively, the 25-g aliquots of inoculated turkey were placed inside a 5- by 0.5-cm plastic petri plate and cooked in a microwave oven to an internal temperature of 72 to 75°C , either before or after irradiation. All samples were vacuum sealed within sterile poultry bags during irradiation.

This entire experiment was repeated with a cooking temperature of 80°C . This temperature was chosen to allow sufficient *L. monocytogenes* to survive to be able to evaluate the com-

bined effect of the two treatments. This subexperiment was repeated twice; other conditions were the same as described above.

RESULTS AND DISCUSSION

Experiment 1. The D values for the inactivation of *L. monocytogenes* on raw and cooked turkey nuggets were 0.56 ± 0.03 kGy and 0.69 ± 0.03 kGy, respectively. These D values were judged significantly ($P < 0.05$) different by analysis of covariances. Starting with an initial population of $10^{6.86}$ CFU/g, significantly more *L. monocytogenes* survived when irradiated on cooked than on raw turkey nuggets. Thus, the null hypothesis that there was no significant difference between the survival of *L. monocytogenes* on raw or cooked turkey nuggets was rejected. The D value on cooked turkey meat was significantly ($P < 0.05$) higher than we previously found on raw turkey (0.50 ± 0.03 kGy) (39). The obvious conclusion is that cooking the meat resulted in the production of some substance(s) that provided protection to the cells of *L. monocytogenes* from gamma radiation, perhaps by competition for free radicals. This question is addressed in a subsequent experiment.

Experiment 2. A high inoculum was used for both raw and cooked ground turkey meat to ensure survival of *L. monocytogenes* at all radiation doses. As in experiment 1, the D value for the inactivation of *L. monocytogenes* was higher when the cells were irradiated on cooked (0.63 ± 0.06 kGy) turkey than when the cells were irradiated on raw turkey (0.55 ± 0.03 kGy). Analysis of covariances indicated, however, that these values were not significantly different ($P > 0.05$), although the trend of a higher D value and survival on cooked meat was similar to that obtained with the turkey nuggets reported above. The computed D value for inactivation of *L. monocytogenes* on both raw and cooked turkey at day zero was 0.59 ± 0.03 kGy. Analysis of variances for the 14-day study indicated significant effects for cooked versus raw turkey, radiation dose, days, and for interactions between radiation dose and cooking treatment and radiation dose and days. These effects are apparent in Figures 1 and 2. At a storage temperature of 4°C, *L. monocytogenes* tended to decrease in number on raw turkey following a radiation dose of ≥ 1.0 kGy but either increased in number following a low radiation dose or did not decline in number on the cooked turkey. These results perhaps mean that the cooked meat, in addition to protecting the cells from radiation, provided a better medium for multiplication and/or recovery of injured cells of *L. monocytogenes*. A pure ground meat product, not a product mixed with spices and possibly antioxidants as would be used in preparation of commercial nuggets, was used to separate the effects of radiation, cooking, and storage from those that may be due to spice mixtures.

Experiment 3. *L. monocytogenes* populations, initially $\sim 3.6 \times 10^3$ CFU/g, multiplied on both raw and cooked ground meat during storage at 2°C; however, the increase in population was greater on cooked meat (Figs. 3 and 4). The population of *L. monocytogenes* declined during storage at 2°C on raw turkey irradiated with either 1 or 2 kGy. CFU of *L. monocytogenes* were not detectable by pour plating (>10

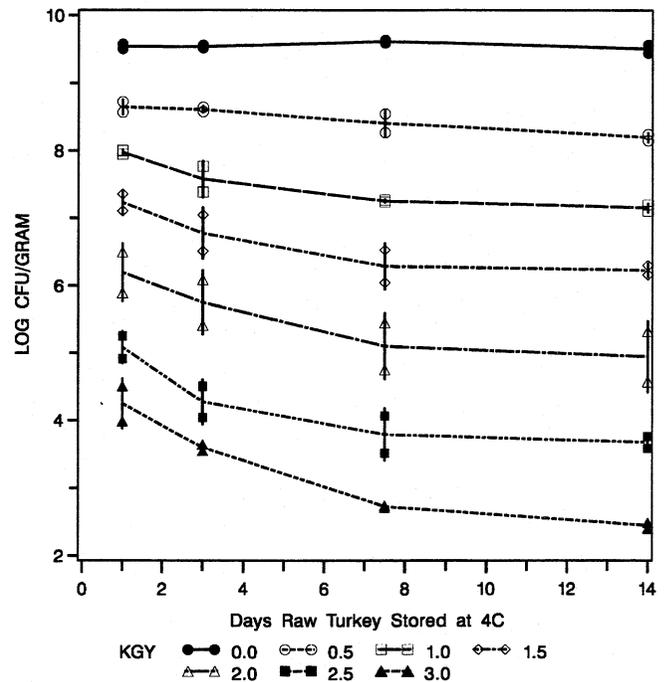


FIGURE 1. Multiplication and/or survival of $\sim 3 \times 10^9$ CFU/g of *L. monocytogenes* inoculated onto radiation-sterilized raw ground turkey meat stored in oxygen-permeable packaging at 4°C for 14 days after gamma-radiation doses of 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 kGy. Bars represent one standard deviation.

CFU/g) on irradiated (3 or 4 kGy) raw turkey nuggets stored at 2°C. Though undetectable by pour plating, one of three samples that received a dose of 3 kGy and that was stored at 2°C for 21 days contained viable *L. monocytogenes*. No

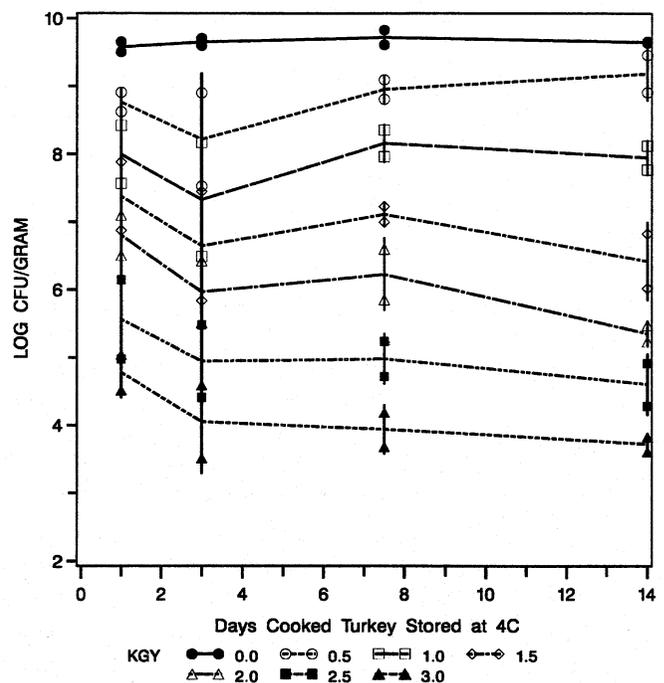


FIGURE 2. Multiplication and/or survival of $\sim 3 \times 10^9$ CFU/g of *L. monocytogenes* inoculated onto radiation-sterilized cooked (internal temperature 85°C) ground turkey meat stored in oxygen-permeable packaging at 4°C for 14 days after gamma-radiation doses of 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 kGy. Bars represent one standard deviation.

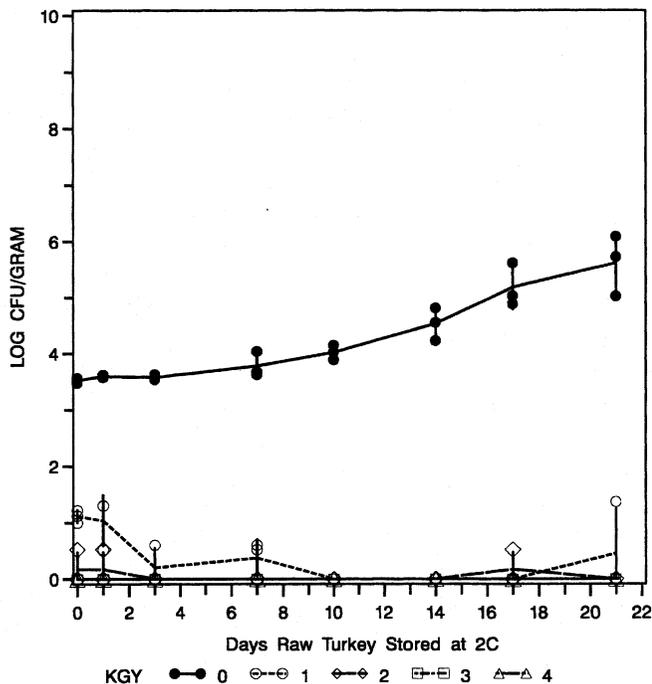


FIGURE 3. Multiplication, survival, and inactivation of $\sim 3.6 \times 10^3$ CFU/g of *L. monocytogenes* inoculated onto radiation-sterilized raw ground turkey meat stored in oxygen-permeable packaging at 2°C for 21 days after gamma-radiation doses of 0, 1, 2, and 4 kGy. Bars represent one standard deviation.

viable *L. monocytogenes* were detected by enrichment culture following a 3-kGy treatment at day zero. None of the irradiated (4 kGy) raw samples that was stored at 2°C contained viable *L. monocytogenes*. None of the irradiated

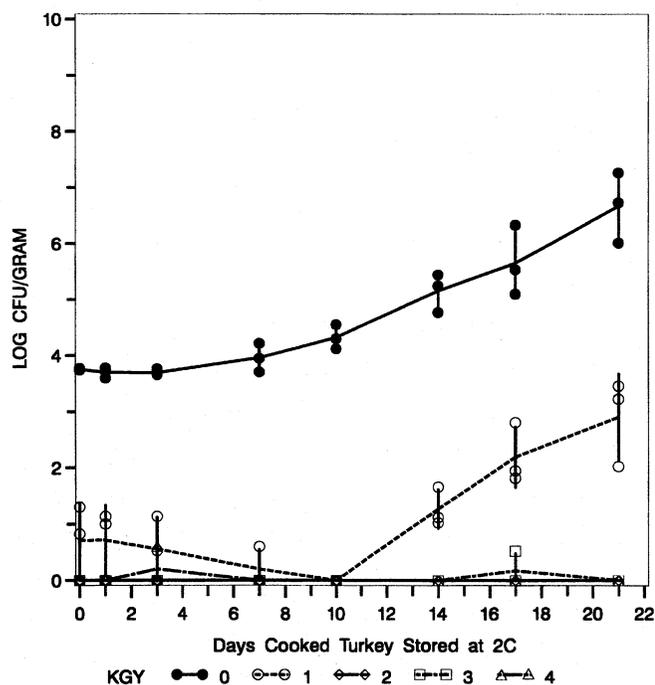


FIGURE 4. Multiplication, survival, and inactivation of $\sim 3.6 \times 10^3$ CFU/g of *L. monocytogenes* inoculated onto radiation-sterilized cooked (internal temperature 85°C) ground turkey meat stored in oxygen-permeable packaging at 2°C for 21 days after gamma-radiation doses of 0, 1, 2, 3, and 4 kGy. Bars represent one standard deviation.

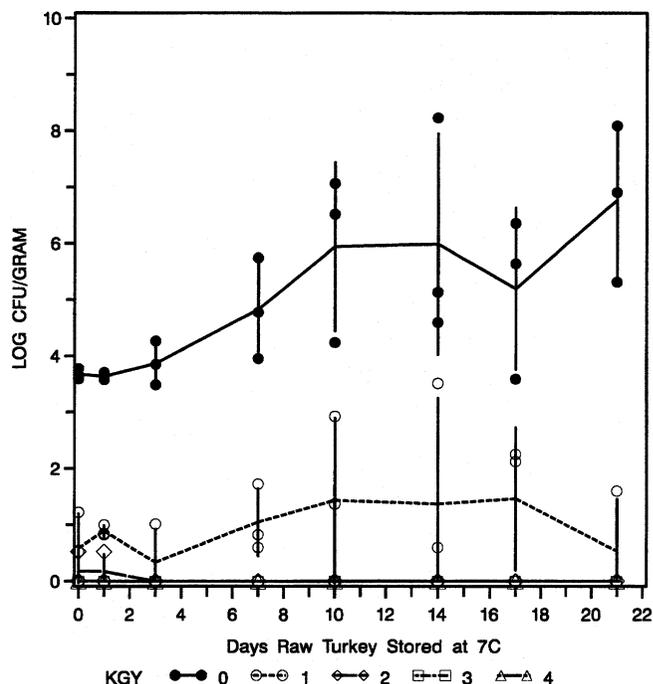


FIGURE 5. Multiplication, survival, and inactivation of $\sim 3.6 \times 10^3$ CFU/g of *L. monocytogenes* inoculated onto radiation-sterilized raw ground turkey meat stored in oxygen-permeable packaging at 7°C for 21 days after gamma-radiation doses of 0, 1, 2, and 4 kGy. Bars represent one standard deviation.

(3 or 4 kGy) samples stored at 7°C contained viable *L. monocytogenes* (Figs. 5 and 6). Though viable *L. monocytogenes* were detected at day zero following a 2-kGy treatment, these small populations declined during storage; by

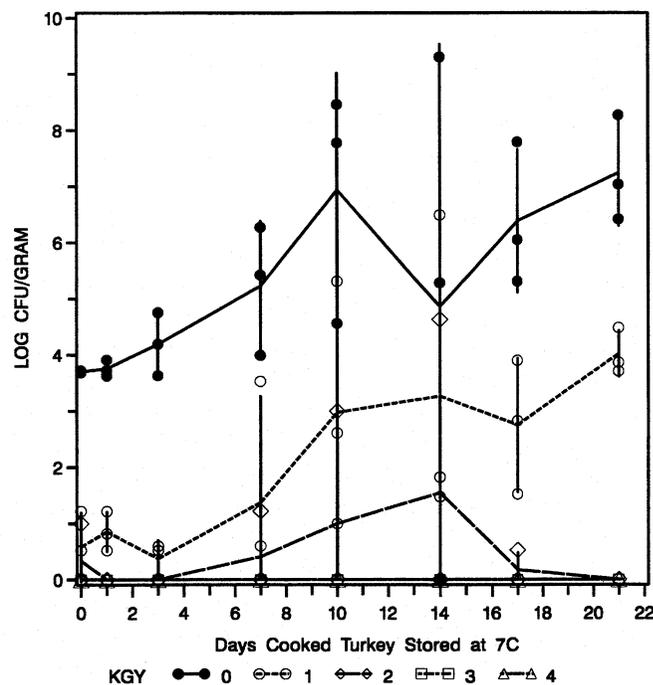


FIGURE 6. Multiplication, survival, and inactivation of $\sim 3.6 \times 10^3$ CFU/g of *L. monocytogenes* inoculated onto radiation-sterilized cooked (internal temperature 85°C) raw ground turkey meat stored in oxygen-permeable packaging at 7°C for 21 days after gamma-radiation doses of 0, 1, 2, 3, and 4 kGy. Bars represent one standard deviation.

day 21 viable *L. monocytogenes* were detected in only one replicate in samples stored at either 2 or 7°C.

Significantly more multiplication of *L. monocytogenes* on irradiated (0, 1, and 2 kGy) turkey occurred at 7°C than at 2°C, and again the increase was greater on cooked meat (Figs. 5 and 6). Patterson et al. (29) observed significantly longer lag phases (18 versus 1 day) for *L. monocytogenes* grown at 6°C on cooked poultry meat compared to that on raw poultry following a dose of 2.5 kGy. We did not observe such an extension of the lag phase for irradiated *L. monocytogenes* growing on either raw or cooked meat.

Our results and those of others tend to indicate that a few *L. monocytogenes* cells might survive a 3-kGy treatment but would be unlikely to multiply or to represent a significant hazard, providing that the meat is well refrigerated ($\leq 4^\circ\text{C}$). We observed that though a few *L. monocytogenes* did survive a 3-kGy treatment, their number decreased during storage and that viable *L. monocytogenes* were undetectable after 10 days storage with the exception of one of six samples at 21 days. Apparently, the radiation was lethal to *L. monocytogenes* cells. Kamat and Nair (20) concluded that a dose of 3 kGy was necessary to eliminate 10^3 CFU of cells of *L. monocytogenes* from air-packed, frozen chicken meat. These authors (20) incubated unirradiated chicken meat samples at 2 to 4°C for 15 days and observed multiplication of four strains of *L. monocytogenes* on unirradiated meat but could not detect survivors on irradiated (3 kGy) samples; however, enrichment cultures were apparently not performed. Lewis and Corry (23) found surviving *Listeria* on 30 of 32 irradiated (2.5 kGy) chickens by enrichment culture.

Experiment 4. Analysis of covariances confirmed that the radiation D value (0.57 ± 0.04 kGy) for inactivation of *L. monocytogenes* cells grown on ground cooked turkey meat was not significantly ($P > 0.05$) different than D values obtained when the cells were grown in TSB and inoculated onto either ground raw (0.60 ± 0.04 kGy) or cooked ground turkey meat (0.55 ± 0.04 kGy) or when the cells were grown on raw turkey meat (0.62 ± 0.05 kGy). The D value calculated from all of the data is 0.56 ± 0.02 kGy. In this experiment we did not confirm our earlier observation that the D value was greater on cooked than on raw turkey meat, but that experiment was conducted on nuggets, not ground meat. It is possible that the effect is due to surface changes in the meat. Examination of the plate counts obtained at 0, 6, and 18 h in each case tended to indicate that there was a slightly greater growth rate on the cooked meat during the first 6 h, but that difference was not apparent at 18 h. The pH values of the raw meats were initially 6.78 and 6.75 and were 6.59 and 6.62 after 18 h growth of *L. monocytogenes*. The pH values of the cooked meats were initially 6.80 and 6.84 and were 6.69 and 6.71 after 18 h incubation. We conclude that the differences observed in experiments 2 and 3 between the multiplication and survival of *L. monocytogenes* on raw and cooked turkey meat may not be due entirely to increased D values.

Experiment 5. The D value for *L. monocytogenes* in ground raw turkey was 0.71 ± 0.03 kGy; in turkey that was

heated to a temperature of 72 to 75°C and then irradiated it was 0.70 ± 0.04 kGy; and in turkey that was irradiated and then heated it was 0.70 ± 0.03 kGy. These values do not differ significantly ($P > 0.05$). Analysis of variances revealed significant effects ($P < 0.01$) for cooking and irradiation but not for the interaction between cooking and irradiation. This study was repeated with an increase in the cooking temperature from the minimum allowed processing temperature of 72°C to 80°C. Either heating to 80°C or irradiating to a dose of 2 kGy would inactivate approximately 2 logs of *L. monocytogenes* (Fig. 7). The following D values were obtained in turkey meat: when raw, 0.73 ± 0.04 kGy; when irradiated and cooked, 0.70 ± 0.04 ; and when cooked and irradiated, 0.60 ± 0.02 kGy. The values obtained with the raw and the irradiated and cooked meats did not differ significantly ($P > 0.05$). However, the D values obtained with the latter two products differed significantly ($P < 0.05$) from that obtained with cooked and irradiated meat. Grant and Patterson (16) observed that irradiation (0.8 kGy) of *L. monocytogenes* increased its sensitivity to heat and resulted in a marked reduction in its thermal D values. Our results did not confirm their observation, but the current study was not designed to allow the computation of thermal D values. We conclude that synergistic effects may depend upon the extent of the first treatment, and that processors may obtain significantly enhanced inactivation of *L. monocytogenes* if the cooking step produces an internal temperature of approximately 80°C. It is assumed that the irradiation treatment will be applied after cooking and packaging.

Experiment 6. In an effort to identify some of the possible factors influencing the increased growth of *L. monocytogenes* and its radiation survival on cooked rather

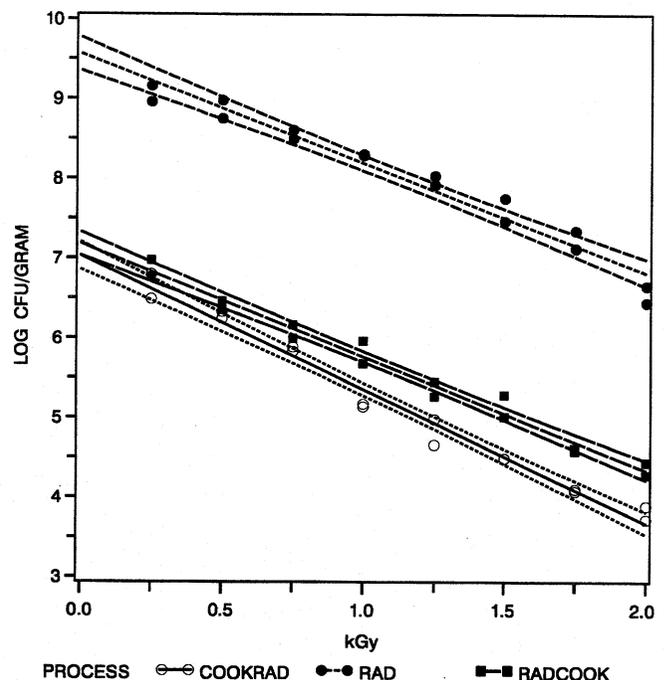


FIGURE 7. Effect of radiation and the order of cooking to an internal temperature of 80°C or irradiation on the survival of *L. monocytogenes*. The 95% confidence limits are indicated for each regression.

effects of a decreased amount of water, perhaps providing slightly greater protection from radiation in some of the experiments, and increased pH may have acted together to provide better conditions for survival and multiplication of *Listeria* on the cooked meat. Alternative hypotheses are that the raw turkey contained a natural inhibitor for multiplication of *L. monocytogenes*, which was inactivated by cooking the meat, or that cooking released nutrients from the meat. No evidence of such an inhibitory substance for the multiplication of *L. monocytogenes* was found when raw turkey meat was overlaid with agar. The conflicting results between some of the experiments do not lend overwhelming support for either conclusion, but the observation that there was greater survival and multiplication of *L. monocytogenes* on cooked rather than on raw meat was consistent throughout the entire study. The interaction of heat injury with radiation injury may help to reduce this effect.

We conclude that survival of irradiated *L. monocytogenes* may be greater on cooked meats than on raw meats and its multiplication rate may also be greater during refrigerated storage on cooked meats.

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REFERENCES

- Aldridge, C., P. W. Jones, S. F. Gibson, J. W. Lanham, M. C. Meyer, R. Vannest, and R. A. Charles. 1977. Automated microbiological detection/identification system. *J. Clin. Microbiol.* 6:406-413.
- AOAC. 1990. Official methods of analysis, 15th ed. Association of Official Analytical Chemists. Washington, D.C.
- ASTM. 1996. E1275-93 standard practice for use of a radiochromic film dosimetry system. 1996 Annual Book of ASTM Standards 12.02:735-739.
- ASTM. 1996. E1607-94 standard practice for use of the alanine-EPR dosimetry system. 1996 Annual Book of ASTM Standards 12.02:838-843.
- Broome, C. V., B. Gellin, and B. Schwartz. 1990. Epidemiology of listeriosis in the United States, chapter 10. *In* A. J. Miller, J. L. Smith, and G. A. Somkuti (ed.), *Foodborne listeriosis*. Elsevier, Amsterdam, The Netherlands.
- CFR (Code of Federal Regulations). 1995. § 381.150 Cooking temperature requirements for poultry rolls and certain other poultry products, 9 CFR chapter III (1-1-95 edition), p. 478.
- Dion, P., R. Charbonneau, and C. Thibault. 1994. Effect of ionizing dose rate on the radioresistance of some food pathogenic bacteria. *Can. J. Microbiol.* 40:369-374.
- Duffy, L. L., P. B. Vanderlinde, and F. H. Grau. 1994. Growth of *Listeria monocytogenes* on vacuum-packed cooked meats: effects of pH, a_w , nitrite and ascorbate. *Int. J. Food Microbiol.* 23:377-390.
- Farag, M. D. E. H., K. Shamsuzzaman, and J. Borsa. 1990. Radiation sensitivity of *Listeria monocytogenes* in phosphate buffer, trypticase soy broth, and poultry feed. *J. Food Prot.* 53:648-651.
- Farber, J. M., G. W. Sanders, and M. A. Johnston. 1989. A survey of various foods for the presence of *Listeria* species. *J. Food Prot.* 52:456-458.
- Fox, J. B., Jr., S. A. Ackerman, and D. W. Thayer. 1992. Fluorometric determination of thiamin vitamers in chicken. *J. Assoc. Off. Anal. Chem.* 75:346-354.
- Fox, J. B., Jr., L. Lakritz, and D. W. Thayer. 1993. Effect of reductant level in skeletal muscle and liver on the rate of loss of thiamin due to γ -radiation. *Int. J. Radiat. Biol.* 64:305-309.
- Freund, R. J., R. C. Littell, and P. C. Spector. 1986. SAS system for linear models. SAS Institute Inc., Cary, N.C.
- Gilbert, R. J., K. L. Miller, and D. Roberts. 1989. *Listeria monocytogenes* and chilled foods. *Lancet* i(8634):383-384.
- Glass, K. A., and M. P. Doyle. 1989. Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. *Appl. Environ. Microbiol.* 55:1565-1569.
- Grant, I. R., and M. F. Patterson. 1995. Combined effect of gamma radiation and heating on the destruction of *Listeria monocytogenes* and *Salmonella typhimurium* in cook-chill roast beef and gravy. *Int. J. Food Microbiol.* 27:117-128.
- Hudson, J. A., and S. J. Mott. 1993. Growth of *Listeria monocytogenes*, *Aeromonas hydrophila* and *Yersinia enterocolitica* on cooked beef under refrigeration and mild temperature abuse. *Food Microbiol.* 10:429-437.
- Ingham, S. C., and C. L. Tautorus. 1991. Survival of *Salmonella typhimurium*, *Listeria monocytogenes* and indicator bacteria on cooked uncured turkey loaf stored under vacuum at 3°C. *J. Food Safety* 11:285-292.
- Kaczmarek, E. B., and D. M. Jones. 1989. Listeriosis and ready-cooked chicken. *Lancet* i(8637):549.
- Kamat, A. S., and M. P. Nair. 1995. Gamma irradiation as a means to eliminate *Listeria monocytogenes* from frozen chicken meat. *J. Sci. Food Agric.* 69:415-422.
- Kerr, K. G., S. F. Dealler, and R. W. Lacey. 1988. Materno-fetal listeriosis from cook-chill and refrigerated food. *Lancet* ii(8620):1133.
- Kerr, K. G., N. A. Rotowa, P. M. Hawkey, and R. W. Lacey. 1990. Incidence of *Listeria* spp. in pre-cooked, chilled chicken products as determined by culture and enzyme-linked immunoassay (ELISA). *J. Food Prot.* 53:606-607, 629.
- Lewis, S. J., and J. E. L. Corry. 1991. Survey of the incidence of *Listeria monocytogenes* and other *Listeria* spp. in experimentally irradiated and in matched unirradiated raw chickens. *Int. J. Food Microbiol.* 12:257-262.
- Mahler, H. R. 1955. Diaphorases, p. 707-711. *In* S. P. Colowick and N. O. Kaplan (eds.), *Methods in enzymology*, vol. II. Academic Press, New York.
- Marshall, D. L., L. S. Andrews, J. H. Wells, and A. J. Farr. 1992. Influence of modified atmosphere packaging on the competitive growth of *Listeria monocytogenes* and *Pseudomonas fluorescens* on precooked chicken. *Food Microbiol.* 9:303-309.
- Mead, G. C., W. R. Hudson, and R. Ariffin. 1990. Survival and growth of *Listeria monocytogenes* on irradiated poultry carcasses. *Lancet* 335(8696):1036.
- Olsman, W. J., and P. Slump. 1981. Methods of determination of connective tissue free muscle protein in meat products, p. 195-240. *In* R. Lawrie (ed.), *Developments in meat science*, vol. II. Applied Science Publishers, London.
- Patterson, M. 1989. Sensitivity of *Listeria monocytogenes* to irradiation on poultry meat and in phosphate-buffered saline. *Lett. Appl. Microbiol.* 8:181-184.
- Patterson, M. F., A. P. Damoglou, and R. K. Buick. 1993. Effects of irradiation dose and storage temperature on the growth of *Listeria monocytogenes* on poultry meat. *Food Microbiol.* 10:197-203.
- Premaratne, R. J., W. Lin, and E. A. Johnson. 1991. Development of an improved chemically defined minimal medium for *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 57:3046-3048.
- Pruett, W. P., Jr., P. P. Graham, J. A. Marcy, and N. G. Marriott. 1993. Fate of *Listeria monocytogenes* and *Salmonella* spp. in thermally processed color-modified turkey meat. *J. Muscle Foods* 4:1-12.
- Rijpens, N. P., G. Jannes, and L. M. F. Herman. 1997. Incidence of *Listeria* spp. and *Listeria monocytogenes* in ready-to-eat chicken and turkey products determined by polymerase chain reaction and line probe assay hybridization. *J. Food Prot.* 60:548-550.
- SAS Institute Inc. 1987. SAS/STAT® guide for personal computers, version 6 edition. SAS Institute Inc., Cary, N.C.
- Schwartz, B., C. V. Broome, G. R. Brown, A. W. Hightower, C. A. Ciesielski, S. Gaventa, B. G. Gellin, and L. Mascola. 1988. Association of sporadic listeriosis with consumption of uncooked hot dogs and undercooked chicken. *Lancet* ii(8614):779-782.
- Shineman, T. L., and M. A. Harrison. 1994. Growth of *Listeria monocytogenes* on different muscle tissues. *J. Food Prot.* 57:1057-1062.
- Thayer, D. W., and G. Boyd. 1995. Radiation sensitivity of *Listeria monocytogenes* on beef as affected by temperature. *J. Food Sci.* 60:237-240.

37. Thayer, D. W., G. Boyd, J. B. Fox, Jr., L. Lakritz, and J. W. Hampson. 1995. Variations in radiation sensitivity of foodborne pathogens associated with the suspending meat. *J. Food Sci.* 60:63-67.
38. Thayer, D. W., G. Boyd, J. B. Fox, Jr., and L. Lakritz. 1995. Effects of NaCl, sucrose, and water content on the survival of *Salmonella typhimurium* on irradiated pork and chicken. *J. Food Prot.* 58:490-496.
39. Thayer, D. W., G. Boyd, W. S. Muller, C. A. Lipson, W. C. Hayne, and S. H. Baer. 1990. Radiation resistance of *Salmonella*. *J. Ind. Microbiol.* 5:373-390.
40. Varabiouff, Y., G. E. Mitchell, and S. M. Nottingham. 1992. Effects of irradiation on bacterial load and *Listeria monocytogenes* in raw chicken. *J. Food Prot.* 55:389-391.
41. Verheul, A., A. Hagting, M.-R. Amezaga, I. R. Booth, F. M. Rombouts, and T. Abee. 1995. A di- and tripeptide transport system can supply *Listeria monocytogenes* Scott A with amino acids essential for growth. *Appl. Environ. Microbiol.* 61:226-233.
42. Wenger, J. D., B. Swaminathan, P. S. Hayes, S. S. Green, M. Pratt, R. W. Pinner, A. Schuchat, and C. V. Broome. 1990. *Listeria monocytogenes* contamination of turkey franks: evaluation of a production facility. *J. Food Prot.* 53:1015-1019.
43. Wimpfheimer, L., N. S. Altman, and J. H. Hotchkiss. 1990. Growth of *Listeria monocytogenes* Scott A, serotype 4 and competitive spoilage organisms in raw chicken packaged under modified atmospheres and in air. *Int. J. Food Microbiol.* 11:205-214.