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 ≤ 0.05) on raw and cooked ground turkey meat. High populations (~10^8 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 (~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 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 (Automicrobial 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 1/10 volume of sterile Butterfield’s phosphate (0.25 M KH₂PO₄, adjusted to pH 7.2 with NaOH).

**Radiation source, techniques, and dosimetry.** The Lockhead 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 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 ± 1°C (3). The FWT-60 dosimeters were read at 600 nm with an FWT-92 reader. In some studies 5-mm-diameter alanine dosimeters (Bruker Instruments, Römnstetten, Germany) were used, and the free-radical signal was measured using a Bruker SMS 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 ± 1°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 ±2% of the target dose.

Microbiological analysis: Plate count. Samples were assayed for CFU by standard pour-plating 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 ± 0.05°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 (λ_{excitation} = 365 nm, λ_{emission} = 460 nm) (FID) (12). Riboflavin was determined in the same 2% trichloroacetic acid (TCA) extracts used for the FID determination of thiamine and assayed fluorometrically, λ_{excitation} 450 nm, λ_{emission} = 530 nm (11).

Reducant 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 dithiothreitol. 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/trichlymyetamine (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 phenylthiocarbamyl 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 × two substrates × 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 thermometer). 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 cm³/645 cm²/24 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* (~7.6 × 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 × seven doses × 4 days × 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 × 10^3 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 (~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 × five doses × two temperatures × 7 days × 3 replications) = 420. Analyses = total plate count, isolate identification, enrichment culture, meat pH, moisture, and aw.

**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 ~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. Irradiation 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) × (2 raw or cooked meat) × (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) × (three replicates) × (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 × 10^3 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 ~3.6 × 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 (>10^6.86) at 4°C on either the raw or the cooked turkey stored at 2°C. Thus, 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.

![FIGURE 1. Multiplication and/or survival of ~3 × 10^6 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.](image)

![FIGURE 2. Multiplication and/or survival of ~3 × 10^6 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.](image)
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 (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...
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 (≤4°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 10⁶ 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.](https://meridian.allenpress.com/jfp/article-pdf/61/8/979/1661337/0362-028x-61_8_979.pdf)
TABLE 1. Pooled analyses of extracts of raw and cooked turkey meat

<table>
<thead>
<tr>
<th>Peak</th>
<th>Nonhydrolyzed (nmol/ml)</th>
<th>Hydrolyzed (nmol/ml)</th>
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<tr>
<td>Unknown (0.12)</td>
<td>195 ± 90</td>
<td>398 ± 67</td>
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<tr>
<td>Unknown (0.14)</td>
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<tr>
<td>Asp</td>
<td>118 ± 40</td>
<td>198 ± 63</td>
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<tr>
<td>Glu</td>
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<td>Asn</td>
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<tr>
<td>Ser</td>
<td>216 ± 57</td>
<td>363 ± 19</td>
</tr>
<tr>
<td>Gln + Gly</td>
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<td>859 ± 67</td>
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<tr>
<td>His</td>
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<tr>
<td>His + β-Ala</td>
<td>1,361 ± 216</td>
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<tr>
<td>Arg + GABA</td>
<td>2,082 ± 395</td>
<td>2,643 ± 244</td>
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<td>Thr</td>
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<tr>
<td>Ala + Anserine</td>
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<tr>
<td>Pro + 1MHis</td>
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<tr>
<td>Unknown (0.85)</td>
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</tr>
<tr>
<td>Lys</td>
<td>69 ± 14</td>
<td>91 ± 2</td>
</tr>
</tbody>
</table>

*a Numbers in parentheses, retention times relative to norleucine.

b GABA, γ-aminobutyric acid.

c 1MHis, 1-methylhistidine.

than on raw turkey, aqueous extracts of raw and cooked turkey were prepared and filter sterilized. Surprisingly, when these extracts were used as substrates for cultivation of *L. monocytogenes*, very little growth occurred on the extracts from the cooked meat. The pH values of the two extracts were 6.43 ± 0.05 and 6.58 ± 0.03 for raw and cooked meat, respectively. Because *L. monocytogenes* requires the vitamins biotin, thiamine, and riboflavin, and the amino acids arginine, cysteine, isoleucine, leucine, methionine, and valine for growth (30), the extracts were analyzed for each of these growth factors. Premaratne et al. (30) discovered that glucose may not be replaced in minimal medium for propagation of *L. monocytogenes* by any of several other sugars. Both extracts contained 30 to 40 μg of thiamine and 36 to 69 μg of riboflavin per liter of extract, or 10 times these amounts per gram of turkey. An analysis for biotin was not performed. Aqueous extracts from raw and cooked turkey contained 214 ± 20 mg and 112 ± 5 mg/liter of glucose, respectively.

Analysis of free amino acids extracted from samples showed four major components with retention times relative to norleucine (rtt) of 0.40 (Gln), 0.53, 0.58, and 0.85 (Table 1). Upon acid hydrolysis, several changes in the pattern occurred. The large peak at 0.58 rtt was diminished and thus represents a mixture of Ala and anserine (β-Ala-1-methylhistidine). Following hydrolysis, residual Ala could be detected, and the hydrolysis products of anserine, β-Ala (which coelutes with His, 0.45 rtt) and 1-methylhistidine (0.59 rtt) were observed. Authentic anserine and its hydrolysis products confirmed these identifications. The largest chromatographic peak (0.53 rtt) was significantly larger after hydrolysis. Its retention time corresponded to a mixture of carnosine (β-Ala-histidine), Arg, and γ-amino butyric acid (GABA), that could not be resolved by the column. Authentic GABA was mixed with carnosine and the hydrolysiss standard amino acid mixture, and these two coeluted with Arg. The increase in this peak following hydrolysis masked the loss of about 250 nmol of carnosine per ml of extract. This conclusion was reached because the increase in the β-Ala + His peak exceeded the amount of β-Ala expected, relative to the 1-methylhistidine released by the hydrolysis of anserine (800 nmol/ml). These amounts would be 10 times greater in the tissue; however, as has been noted (27), anserine is the dominant β-alanyl peptide in avian breast muscle. The GABA in the samples could arise in two ways, either from the decarboxylation of Glu by the muscle tissue or by bacterial degradation of Arg or its metabolites. The observed increases in the 0.53-rtt peak could be from the hydrolysis of peptides. In addition, Gln and Asn were converted on hydrolysis to their respective dicarboxylic acids. The peak at 0.85 rtt was abolished, as were two minor peaks at 0.12 and 0.14 rtt; these changes are accompanied by an increase in Gly and a new peak at 0.13 rtt. These changes could be related to Gly conjugates of benzylic acids, which on hydrolysis would yield Gly and the 0.13-rtt peak. Thus, the dominant peaks observed in these extracts are small physiological peptides and GABA, which are known to occur in tissue extracts; only a small percentage of the material represents free amino acids. It was discovered recently that *L. monocytogenes* has an active transport system for dipeptides and tripeptides that can supply amino acids essential for its growth (41).

The hydrolyzed and unhydrolyzed samples were quantitated and the results reported in Table 1. All four samples were somewhat similar with regard to the quantities and types of extracted amino compounds, although one of each of the extracts prepared from both the raw and cooked meat was relatively low in the 0.53-rtt peak; following hydrolysis, the values were comparable to the other two extracts. This indicates that the parent compound of these peaks is present at a relatively constant amount. The extracts from both raw and cooked turkey contained all of the essential amino acids for the growth of *L. monocytogenes*. Our results did not provide substantive evidence of differences in critical nutrients between extracts of raw versus cooked turkey meat.

The titration of the raw and cooked turkey meat with DCPIP required 9.05 and 7.00 ml of titrant, respectively, indicating that the amount of reducing equivalents was higher in the raw than in the cooked meat. Thus, bacterial survival should have been higher in the raw than in the cooked turkey when it was irradiated; this is the opposite of what was observed.

The sensitivity of *Salmonella typhimurium* to radiation is inversely proportional to the water content, not the a_w, of the substrate (37). The loss in moisture due to cooking in experiment 4 was not large (7.61 ± 1.00%), and we did not observe a difference in radiation resistance in that experiment. Water activity is known to influence the ability of bacteria to multiply; however, typical measurements for raw and cooked turkey meat were 0.997 and 0.992, respectively. This is a minor difference and would not be likely to influence the ability of *Listeria* to multiply. There was a small pH change when the meat was cooked, shifting from approximately 6.8 to 7.1 after cooking. The sum of the
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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