

Variations in Radiation Sensitivity of Foodborne Pathogens Associated with the Suspending Meat

D.W. THAYER, G. BOYD, J.B. FOX, JR., L. LAKRITZ, and J.W. HAMPSON

ABSTRACT

Longissimus dorsi from beef, pork, and lamb and turkey breast and leg meats were inoculated with *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus*, and the gamma radiation resistance of the pathogens were determined under identical conditions. At 5°C the respective radiation D-values of *E. coli* O157:H7 and *L. monocytogenes* did not vary with the suspending meat. The D-value for a mixture of *Salmonella* spp. was significantly lower on pork than on beef, lamb, turkey breast, and turkey leg meats. The D-value for *S. aureus* was significantly lower on lamb and mechanically deboned chicken meat than on the other meats. All values were, nevertheless, within expected ranges.

Key Words: pathogens, bacteria, radiation sensitivity

INTRODUCTION

SINCE BOTH THE COMPOSITION and the radiation chemistry of the major chemical components of beef, pork, lamb, and poultry meats are well known, it should be possible to predict changes that may occur when those food products are irradiated (Diehl, 1990; Josephson, 1983; Merritt and Taub, 1983; Taub, 1981). Diehl and Scherz (1975) and Taub et al. (1976) concluded that it is possible to assess wholesomeness with a reasonable degree of certainty by extrapolating chemical data from one food and applying it to another that is generically related. The concept of "chemiclearance" may be important in decisions about approval for irradiation of red meats and possibly other meats not included in existing regulations by the U.S. Food & Drug Administration and for which extensive toxicological data are scarce. The primary purpose for irradiating meats is to control or eliminate foodborne pathogens. Yet, there are many published examples of significantly different radiation D-values for a given pathogen on different meats under irradiation conditions that were apparently very similar (Urbain, 1986). Primary factors such as temperature during irradiation and presence or absence of oxygen can significantly alter the estimates of radiation sensitivity. Some secondary factors which may also have an influence are growth media and incubation conditions for propagation and estimation of pathogen populations following irradiation. In order to accept the concept of chemiclearance for toxicological data, regulatory agencies must have confidence that the efficacy of the process to control or eliminate foodborne pathogens is reasonably predictable between generically related products as well as between poultry and red meats.

Our objective was to test the "null hypothesis" that the radiation resistance of *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus* on different meats does not differ when irradiation and culture conditions are identical. A second parallel study tested the "null hypothesis" that chemical changes in meats irradiated under identical conditions are either the same or predictable from chemical composition or physical structure.

MATERIALS & METHODS

Experimental design

The radiation resistance of two gram-positive and two gram-negative foodborne pathogens were studied simultaneously on beef, lamb, pork, turkey breast meat, and turkey leg meat. To avoid possible effects of culture variation, all five meats were inoculated from the same culture at the same time and irradiated under identical conditions at the same time. Because tissues are known to have animal-to-animal variations, three independent lots of each meat were obtained at different times, and the entire study was replicated.

Cultures

Three isolates of *Escherichia coli* O157:H7 35150, 43889, and 43894; *Salmonella dublin* 15480, *S. enteritidis* 13076, *S. newport* 6962, *S. senftenberg* 8400, *S. typhimurium* 14028; *Staphylococcus aureus* 25923, and 13565; and *Listeria monocytogenes*, 15313, 43256, 49594, and 7644 were obtained from the American Type Culture Collection, Rockville, MD. *E. coli* 93-937 was associated with a restaurant outbreak and was obtained from the Oregon Public Health Laboratory (Portland, OR), and ENT C9490 was associated with the major outbreak in Oregon during 1992 and was obtained from the Communicable Disease Center (Atlanta, GA). *S. aureus* B124 was obtained from the Eastern Regional Research Center culture collection. All cultures were maintained and cloned on Tryptic Soy Agar (TSA, Difco, Detroit, MI). Culture identity was confirmed by gram stains and from reactions on GNI or GPI cards, as appropriate, of the Vitek AMS Automicrobic System (bioMérieux Vitek, Inc., USA, Hazelwood, MO) (Aldridge et al., 1977; Knight et al., 1990). Each isolate was cultured independently in 100 mL of tryptic soy broth (TSB, Difco, Detroit, MI) at 35°C with agitation at 150 rpm on a rotary shaker for 18 hr at 35°C. Equal amounts of the culture of each isolate of a pathogen were mixed, and the culture was harvested by centrifugation. A tenfold inoculum was prepared by resuspending the cells in 1/10 volume of Butterfield's phosphate (0.25M KH₂PO₄ adjusted to pH 7.2 with NaOH).

Substrates and packaging conditions

All meats were purchased locally the day after slaughter. Three lots of each meat were obtained at different times. Pork was purchased from Leidy of Souderton, PA; beef (steer) from Carl Venezia of Conshohocken, PA; lamb, and turkey from C. Fehl's of Springhouse, PA. The Longissimus dorsi of the mammals and the breast and all of the leg muscles of the turkey were used. A single muscle was selected from the red meats to avoid introducing variances due to tissue. Turkey breast meat is relatively homogeneous, and virtually no information is available on the effects of radiation on foodborne pathogens on either breast or leg meats. Turkey also provides a comparison to data already available for the survival of these pathogens on chicken. All muscles were carefully trimmed of fat, cubed, and frozen in dry ice. The meat was then pulverized in a Hobart silent cutter to yield a homogeneous material. Mechanically deboned chicken meat (MDCM) consisting of ≈ 90% rib and 10% back meat was obtained in 18-kg lots from a commercial manufacturer of poultry frankfurters, Tyson Foods, New Holland, PA. The meat substrates were subdivided into 100 ± 0.05g amounts and then spread thinly and vacuum sealed in Stomacher 400 (Tekmar Co., Cincinnati, OH) polyethylene bags. These bags were then vacuum sealed in high barrier pouches fabricated with 0.025 mm polycaprolactam (nylon 6) as the outside layer, 0.0090 mm aluminum foil as the middle layer, and 0.051 mm polyethylene terephthalate as the inner layer (American National Can Company, Des Moines, IA) to provide better protection during handling and to prevent oxygen transmission to the samples. The meat was frozen at -50°C and sterilized by gamma irradiation to a dose of 42 kGy at -30°C. Prior research (Thayer et al., 1987; Thayer, 1990;

Table 1—Effect of suspending meat on gamma radiation D-values at 5°C for foodborne pathogens *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* spp., and *S. aureus*

Pathogen	Beef	Lamb	Pork	Turkey breast	Turkey leg	MDCM
	D(kGy) ± SE					
<i>E. coli</i> O157:H7	0.30 ± 0.02 ^a	0.32 ± 0.02 ^a	0.30 ± 0.01 ^a	0.30 ± 0.01 ^a	0.29 ± 0.04 ^a	ND
<i>L. monocytogenes</i>	0.45 ± 0.03 ^b	0.47 ± 0.04 ^b	0.48 ± 0.02 ^b	0.50 ± 0.03 ^b	0.47 ± 0.03 ^b	ND
<i>Salmonella</i> spp.	0.70 ± 0.04 ^c	0.67 ± 0.04 ^c	0.51 ± 0.03 ^d	0.71 ± 0.04 ^c	0.71 ± 0.04 ^c	ND
<i>S. aureus</i>	0.46 ± 0.02 ^e	0.40 ± 0.03 ^f	0.43 ± 0.02 ^e	0.45 ± 0.03 ^e	0.46 ± 0.05 ^e	0.41 ± 0.03 ^f

a,b,c,d,e,f Different letters in the same row indicate significant differences ($P < 0.05$); MDCM = mechanically deboned chicken meat; SE = standard error; ND = not determined; *Salmonella* spp. = *S. dublin*, *S. enteritidis*, *S. newport*, *S. senftenberg*, and *S. typhimurium*.

Thayer and Boyd, 1991) demonstrated that such treatments did not significantly alter the wholesomeness and nutritional characteristics or the response of *Salmonella typhimurium* on the meat to gamma radiation. Both sterile and non-sterile meat were stored at -50°C until use.

Radiation source and irradiation techniques

The self-contained gamma-radiation source of ^{137}Cs had a strength of approximately 134,000 Ci (4.95 PBq) and a dose rate of 0.108 kGy min^{-1} . The dose rate was established using National Physical Laboratory (Middlesex, United Kingdom) dosimeters. Variations in doses absorbed by experimental samples were minimized by placement within a uniform area of the radiation field. Samples were maintained at $5 \pm 0.5^{\circ}\text{C}$ during irradiation by injecting the gas phase from liquid nitrogen into the irradiation chamber. Sample temperature was monitored continuously during irradiation.

Inoculation of meat for determination of D_{10} values

Sterile meat was thawed rapidly in about 5 min by submerging the package in a 50°C water bath, inoculated with sufficient cells for a final population of $\approx 10^9$ stationary-phase cells/g, and mixed in sterile Number 400 polyethylene Stomacher bags by stomaching for 90 sec using a Stomacher 400 (Tekmar Co., Cincinnati, OH). Aliquots of $5.0 \pm 0.05\text{g}$ of inoculated meat were transferred aseptically to radiation-sterilized oxygen permeable poultry bags (E-300, Cryovac Division, W. R. Grace & Co., Duncan, SC). These bags complied with U.S. regulations. Inoculated meat was spread uniformly over an area of about $10 \times 10\text{ cm}$ within the bags and heat sealed in vacuo.

Determination of D_{10} values

Inoculated meat samples received radiation doses of 0 to 2.0 kGy in increments of 0.235 kGy at $5 \pm 0.5^{\circ}\text{C}$. The studies of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* were repeated three times. The study of *S. aureus* was repeated twice.

Microbiological analysis

Samples were assayed for colony-forming units (CFU) by standard pour-plate procedures using TSA with serial dilutions in sterile Butterfield's phosphate. Petri plates containing *E. coli* O157:H7, *Salmonella* spp. or *S. aureus* were incubated for 24 hr at 35°C before counting. Petri plates containing *L. monocytogenes* were incubated for 48 hr at 35°C before counting. CFU were counted on three petri plates having 30 to 300 colonies with a New Brunswick Scientific Biotran II® automated colony counter (New Brunswick Scientific Co., Inc., Edison, NJ).

Proximate analysis

Water and fat were determined by CEM methods (AOAC, 1990) with one modification: After determining moisture content, the samples for fat determination were blended with 100 mL methylene chloride, the slurry was quantitatively transferred to filter paper in a Buchner funnel, and the methylene chloride removed by suction. Protein and ash were determined using these CEM microwave furnaces; MAS-300 furnace for ash (CEM, 1990; Zheng and Dotson, 1994) and Kjel-FAST furnace for Kjeldahl digestion (CEM, 1987). After sulfuric acid/ H_2O_2 digestion in the CEM oven, the alkalization and distillation of the ammonia was completed in a Kjeltac apparatus (AOAC, 1990).

Statistical analysis

Cultural responses were expressed as the logarithm of the CFU/g. For each experiment, the average (N) CFU value of 3 plate counts for each replicate sample was determined and divided by the average of the 3 zero-dose values (N_0) to give a survivor value (N/N_0). The \log_{10} survivor values ($\log_{10}(N/N_0)$) were then used for subsequent calculations. The D-values (dose in kGy resulting in a 90% reduction of viable CFU) were the reciprocals of the slopes of the linear regressions of the log survivor values as determined by least squares analysis. The zero-dose values were excluded from the calculation of the regression to avoid shoulder effects as described by Thayer et al. (1990). Statistical calculations were performed with the general linear models procedure of the SAS statistical package (Freund et al., 1986; SAS Institute, Inc. 1987). The regressions were tested for differences by analysis of covariance.

RESULTS

Radiation D_{10} values

The D-values for the four pathogens on beef, lamb, pork, turkey breast, and turkey leg were compared (Table 1). The D-value for *S. aureus* was greater than expected, based on our previous studies with MDCM (Thayer and Boyd, 1992). Therefore an additional set of three samples of MDCM was inoculated with the mixture of *S. aureus* isolates and the D-value for each replicate was determined.

The D-values for *E. coli* O157:H7 and *L. monocytogenes* were not significantly ($P < 0.05$) different on any of the 5 substrates tested (Table 1). The D-values for *Salmonella* on beef, lamb, turkey breast, and turkey leg meats were not different (Fig. 1). The D-value for *Salmonella* was lower ($P < 0.05$) on pork meat than on any of the other meats (Table 1, Fig. 1). The D-value for *S. aureus* on lamb or chicken meat was lower ($P < 0.05$) than when these microorganisms were on the other meats. The D-value on turkey leg meat was different from that on chicken meat (Table 1, Fig. 2).

Proximate analysis

The proximate analyses of the beef, lamb, MDCM, pork, turkey breast, and turkey leg meats were also compared (Table 2). The major differences between these products were in low and high fat contents in turkey breast meat and MDCM, respectively (Table 2).

DISCUSSION

WE KNOW OF NO PREVIOUS REPORTED STUDY in which radiation survival of foodborne pathogens was determined on 5 meat substrates under identical conditions. The "null hypothesis" that the suspending meat would not alter the radiation D-values for either *E. coli* O157:H7 or *L. monocytogenes* when the culture and irradiation conditions were identical was accepted. The null hypothesis was not accepted for the radiation survival of either *Salmonella* or *S. aureus*. We eliminated culture bias by inoculating meat samples from each of the four species at the same time using a single inoculum preparation. Animal bias was tested by performing three independent studies with different lots of meat from different animals for each of the studies. Thus, the differences in responses of *Salmonella* and *S. aureus* to

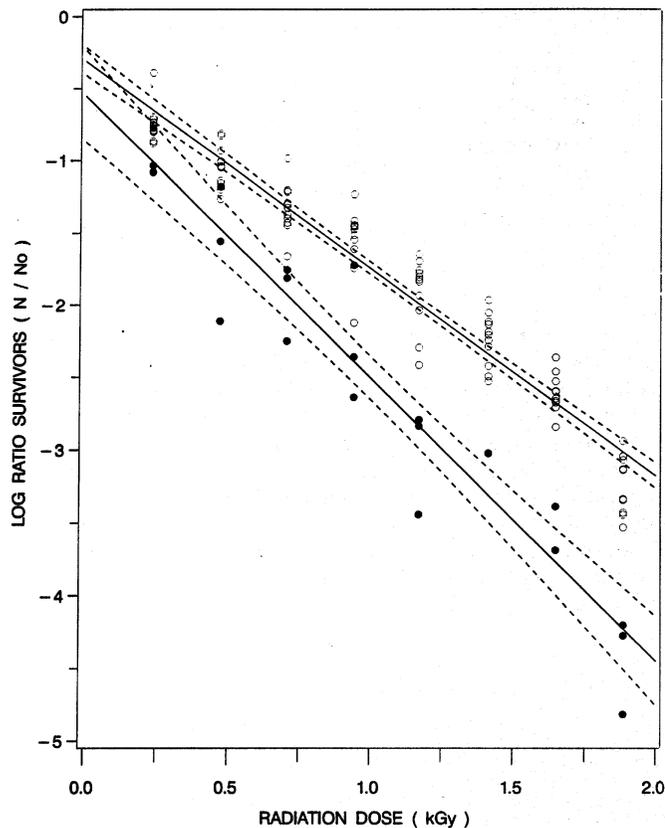


Fig. 1—Effect of gamma radiation on the survival of a mixture of *S. dublin*, *S. enteritidis*, *S. newport*, *S. senftenberg*, and *S. typhimurium* suspended on the *L. dorsi* of beef, pork, and lamb, turkey breast meat, and turkey leg meat. The linear regressions for the survival curves of the *Salmonella* on beef, lamb, turkey breast, and turkey leg meats are identical and are plotted as a single regression (○); the linear regression for the survival of *Salmonella* on pork (●) is plotted separately. The regression curves are shown with their 95% confidence limits as dashed lines.

gamma radiation must be associated with chemical and/or physical properties of the meats. With exception of the MDCM, all meats were chopped using identical techniques, and the same muscle tissues were used for comparison of the red meats. So at the macro scale the physical state of each of the meats should have been very similar. The purpose of our companion study, mentioned earlier, was to examine these meats for any changes in chemical properties when irradiated.

Thayer and Boyd (1993) found a D-value of 0.28 kGy for *E. coli* O157:H7 irradiated in vacuo on finely ground lean beef at 5°C and a value of 0.27 kGy on either lean ground beef or MDCM. No effect of atmosphere during irradiation was found, nor was there an apparent effect of fat since the beef contained 2.6% and the MDCM contained 21.3% fat. Grant and Patterson (1991) obtained a D-value of 0.34 kGy for a nonpathogenic strain of *E. coli* irradiated in the presence of air on fresh pork fillets. Patterson (1988a) found D-values of 0.27 and 0.39 kGy for nonpathogenic *E. coli* irradiated at 10°C in vacuo or in air. The results of our present study were very similar to those reported previously, but they expand data for *E. coli* O157:H7 to include pork, lamb, and turkey.

Huhtanen et al. (1989) found a mean D-value of 0.43 kGy at 2–4°C for several isolates of *L. monocytogenes* on chicken meat. Patterson (1988b) found D-values of 0.417–0.553 kGy depending on strain and plating medium for *L. monocytogenes* on chicken mince. Beuchat et al. (1993) found D-values of 0.51 to 0.61 kGy for *L. monocytogenes* on ground beef irradiated at a commercial radiation source and did not find effects for either fat content or temperature on the results. In our study we found

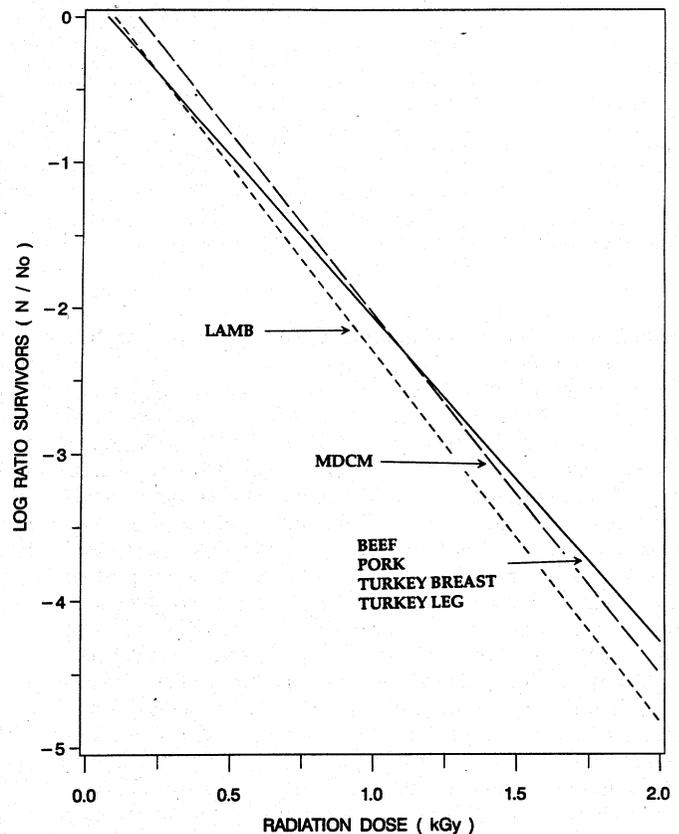


Fig. 2—Effect of gamma radiation on the survival of *S. aureus* on the *L. dorsi* of beef, pork, and lamb, turkey breast meat, turkey leg meat, and mechanically deboned chicken (MDCM). The survival curves for *S. aureus* on beef, pork, turkey breast, and turkey leg meats are identical and are presented as a single regression line. The survival curves for *S. aureus* on lamb, and MDCM are significantly different.

D-values of 0.45–0.50 kGy for *L. monocytogenes* irradiated at 5°C on beef, pork, and lamb *L. dorsi* muscle, turkey breast meat, and turkey leg meat. Individual values were not significantly different ($P > 0.05$) and are similar to those reported for chicken meat and hamburger. We could not find published radiation D-values of *L. monocytogenes* on pork, lamb, and turkey meats.

Many investigators examined effects of gamma radiation on *Salmonella* on various foods. Tarkowski et al. (1984) found D-values from 0.55 kGy for *S. typhimurium* to 0.78 kGy for *S. stanley* on raw ground beef irradiated at 18–20°C. Grant and Patterson (1991) determined the gamma radiation D-values for two strains of *S. typhimurium* irradiated on pork in air at 10°C to be 0.86 and 0.40 kGy. Thayer et al. (1990) determined the D-values for six species of *Salmonella* irradiated on MDCM at 4°C that in air ranged from 0.42 for *S. arizona* to 0.77 kGy for *S. enteritidis*. Patterson (1988a) found a D-value of 0.50 kGy for *S. typhimurium* irradiated in air on chicken mince at 10°C. No published radiation D-value determinations for *Salmonella* on turkey or lamb could be found. The radiation D-values we found for a mixture of five serovars were within the range of values reported previously on all of the meats and are identical with the exception of the values for pork. We could not account for the reduction in D-value on the mixture of *Salmonella* when irradiated on pork compared to D-values on the other meats. Values from proximate analysis of these products were very similar (Table 2). For example, the mean fat content of the pork samples was 3.07%, and the mean for all meats was 3.23%. Maxcy and Tiwari (1973) found D-values for *S. enteritidis* were 0.70 and 0.49 kGy in low- and high-fat beef, respectively. Beuchat et al. (1993), however, did not find a significant effect of

Table 2—Protein, fat, moisture, ash, and pH of meats

Meat	Protein % ± SD ^a	Fat % ± SD	Moisture % ± SD	Ash % ± SD	Total % ± SD	pH ± SD
Beef L. dorsi	19.4 ± 1.63	5.20 ± 1.34	70.8 ± 0.91	0.84 ± 0.07	96.3 ± 1.60	5.67 ± 0.10
Lamb L. dorsi	20.0 ± 1.83	3.85 ± 0.71	72.7 ± 1.12	0.96 ± 0.04	97.5 ± 1.51	5.53 ± 0.06
Pork L. dorsi	20.7 ± 2.01	3.08 ± 1.69	72.0 ± 0.30	1.08 ± 0.02	96.9 ± 0.75	5.39 ± 0.28
Turkey breast	23.4 ± 0.88	1.38 ± 0.73	72.6 ± 1.06	1.02 ± 0.08	98.5 ± 1.12	5.76 ± 0.13
Turkey leg	20.1 ± 0.96	2.65 ± 0.62	74.6 ± 0.65	0.97 ± 0.03	98.3 ± 0.83	6.03 ± 0.34
MDCM ^a	16.4 ± 5.59	21.9 ± 0.14	64.2 ± 0.14	0.97 ± 0.04	103.4 ± 5.34	6.71 ± 0.14

^a SD = standard deviation; MDCM = mechanically deboned chicken meat.

fat content in beef on the radiation D-value for a mixture of five serovars of *Salmonella*.

The similarity of total fat content of the meat samples tends to eliminate fat level as a factor. If we examine the fatty acid composition of beef, pork, lamb, and turkey, the only major difference between these meats was that turkey had lower ratios of stearic and high ratios of linoleic acids than the other meats (Anderson, 1990, 1992; Posati, 1979). But the D-value for *Salmonella* was the same on both beef and turkey. We postulated that the difference may be due to a radiolytic product formed either during irradiation of the inoculated meat or possibly during the initial sterilization of the meat. The amounts of such radiolytic products, however, would be extremely small (ppb level, Merritt and Taub, 1983). Maxwell and Rady (1989) found only negligible changes in fatty acid profiles for neutral lipids of chicken muscle and for fatty acyl residues of skin lipids following irradiation in air at doses up to 10 kGy at 2–5°C. Thayer (1990) found no significant changes in fatty acids of radiation-sterilized chicken meat. If even relatively minor amounts of fatty acids such as linolenic acid were produced as radiolytic products we might have found some effect on *L. monocytogenes*, which is inhibitory at 100 µg/mL (Wang and Johnson, 1992). The pathogen cells were, however, not cultivated on the irradiated meats. Unless the reaction took place during irradiation any radiolytic products would be expected to be at non-toxic levels after dilution of the cultures for counting.

Thayer and Boyd (1992) obtained a D-value of 0.36 kGy for stationary phase cells of *S. aureus* ATCC 13565 irradiated at 0°C *in vacuo* on MDCM. Patterson (1988a) found D-values of 0.37 to 0.42 for a *S. aureus* isolate when irradiated in chicken mince under various atmospheres. Differences between D-values under different atmospheres were not considered significant. D-values of 0.58 and 0.30 kGy were found by Maxcy and Tiwari (1973) for the radiation resistance of *S. aureus* in low- and high-fat beef, respectively. However, Beuchat et al. (1993) did not find such an effect of fat content on radiation sensitivity of *S. aureus*. We found D-values from 0.40 kGy in lamb meat to 0.46 kGy in beef, pork, turkey breast, and turkey leg meats. Those values seemed higher than those obtained previously (Thayer and Boyd, 1992) with MDCM. We thus included a study with three replicate samples of MDCM and found a D-value of 0.41 kGy. As mentioned previously, results with lamb and MDCM were significantly different from those with beef. Nevertheless, the range of values was not great and was in reasonable agreement with the few published values available. Some reports indicate gamma radiation produces greater lethality for microorganisms at reduced pH. However, no significant difference in pH values of the meats were noted either before or after irradiation (Table 2). Differences in reductant values between skeletal muscle and liver, but not between skeletal muscles, were associated with differences in rates of radiolysis of thiamin by Fox et al. (1993).

We did not test for presence of antibiotics or other antimicrobial substances in the meats used to suspend the organisms. Possibly such substances could be present and interact with the test bacteria. However, this was considered unlikely since the meats were obtained from USDA inspected facilities. The samples were diluted immediately following irradiation and were not at any time incubated on the meat itself.

Extrapolations based on the higher radiation D-values for either *Salmonella* or *Staphylococcus* would provide greater protection for consumers. Because we could not explain changes in radiation D-values for *Salmonella* and *S. aureus* on the different meats, it seems prudent to not assume D-values for a pathogen would be the same on all meats. However, irradiation of non-frozen meats, based on current regulations for poultry, to a minimum dose of 1.5 kGy with a maximum dose of 3.0 kGy would greatly decrease any danger of *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp. and *Staphylococcus aureus* reaching the consumer.

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