

# Effects of Gamma Irradiation on the Survival of *Listeria monocytogenes* and on its Growth at Refrigeration Temperature in Poultry and Red Meat

B. GÜRSEL and G. C. GÜRAKAN<sup>1</sup>

Department of Food Engineering, Middle East Technical University, 06531 Ankara, Turkey

**ABSTRACT** Gamma irradiation sensitivity of a strain of *Listeria monocytogenes* was determined in trypticase soy broth supplemented with yeast extract (TSB-YE), in a slurry of chicken breast meat and in raw ground beef. D<sub>10</sub> values in these different media were 0.364, 0.599, and 0.699 kGy, respectively. This organism appeared most sensitive in TSB-YE, more resistant in minced fresh chicken breast meat, and most resistant in fresh minced

beef. It was found that irradiation at 2.5 kGy prior to refrigeration is an efficient way for the preservation of meat products contaminated at 10<sup>3</sup> to 10<sup>4</sup> per gram initial load of *L. monocytogenes* for about 7 d. However, with this initial load, the injured cells might repair themselves and cause a health hazard during storage at 4 C in the presence of air after 7 d.

(Key words: *Listeria monocytogenes*, irradiation, poultry, red meat, refrigeration)

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## INTRODUCTION

Listeriosis is recognized as an important health problem and some major disease outbreaks have been caused by foodborne *Listeria monocytogenes* (Smith and Fratamico, 1995). Although most of the foodborne listeriosis outbreaks have been linked to the consumption of dairy products, recent sporadic cases have been associated with meats as well as other foods (Farber, 1991). In fact, *Listeria* species have been found as common contaminants of poultry carcasses (Johnson *et al.*, 1990). Treatment with ionizing radiation is an excellent way of eliminating *Listeria* species from foods (Farg *et al.*, 1990) and increasing the shelf-life of certain foods. After the approval of irradiation for the control of foodborne pathogens in poultry by the Food and Drug Administration in May 1990, irradiated, uncooked raw poultry has been commercially marketed in the U.S. for the first time (Pszczola, 1993). In poultry meat, D<sub>10</sub> values (the dose required to inactivate a given population to 10% of its initial value) for *L. monocytogenes* were found in the range of 0.42 to 0.55 depending on strain and plating medium (Patterson 1989). However, D<sub>10</sub> values of 0.51 to 1.0 kGy for various strains of *L. monocytogenes* irradiated in raw ground beef have been reported (El-Shenawy *et al.*, 1989). In the literature, conflicting results of the dose required to destroy 10<sup>4</sup> *L. monocytogenes* per gram poultry were reported. Huhta-

nen *et al.* (1989) suggested a dose of 2.0 kGy to eliminate 10<sup>4</sup> cells of *L. monocytogenes* per gram of poultry. Varabioff *et al.* (1992) reported that *L. monocytogenes* was not recovered from air-packaged chickens that had been artificially inoculated with the pathogen before irradiating to 2.5 kGy and storing for 15 d at 4 C. However, in the study of Mead *et al.* (1990), it was shown that poultry carcasses with an initial load of 10<sup>4</sup> *L. monocytogenes* per gram were positive for the pathogen after irradiation to 2.5 kGy and refrigerated storage. The objective of this research was to compare the variations in irradiation sensitivity of a strain of *L. monocytogenes* in two different meat substrates as well as in trypticase soy broth supplemented with yeast extract (TSB-YE) under identical conditions and to determine the ability of this organism to grow aerobically in irradiated and unirradiated raw ground beef and chicken at refrigeration temperature over a period of 15 d.

## MATERIALS AND METHODS

### Organism and Culture Conditions

The strain of Special *Listeria* Culture Collection<sup>2</sup> (SLCC), *L. monocytogenes* SLCC 9488 (4b), obtained from the Institut für Lebensmittel Hygiene des Bundesinstituts für Gesundheitlichen Verbraucherschutz und Veterinar Medizin (BGVV), Berlin, Germany, was used throughout this study. Stock culture was maintained in sterile glycerol at -20 C, and it was propagated in trypticase soy broth containing 0.6% yeast extract (TSB-YE) two to three times at 35 C before use in experiments.

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<sup>1</sup>To whom correspondence should be addressed.

<sup>2</sup>Special *Listeria* Culture Collection, Würzburg, Germany.

## Gamma Irradiation

A Shepherd (Mark 1, Model 22) gamma irradiator<sup>3</sup> located in the Nuclear Research Institute in Animal Health of the Turkish Atomic Energy Commission, Ankara, Turkey, was used in this study. The irradiation source was <sup>137</sup>Cs. The dose rate was 0.026 kGy/min during irradiation. Samples were irradiated with doses of 0.5, 1.0, 1.5, 2.0, and 2.5 kGy at ambient temperature for the determination of D<sub>10</sub> values.

## Preparation of Meat Samples

Meat samples were purchased from a local manufacturer.<sup>4</sup> After chicken was deboned, breast meat was minced and 25-g portions were weighed into sterile stomacher bags.<sup>5</sup> The same procedure was performed for minced fresh meat.

## Sample Preparation for Determination of D<sub>10</sub> Values

The bacterium was grown in TSB-YE for 18 to 20 h at 35 C. The cells were harvested by centrifugation and resuspended in PBS. Inoculum was added to each bag to give approximately 10<sup>8</sup> organisms per gram and mixed well with meat samples. Two replicate samples were irradiated at each dose level, and duplicate plate counts were obtained for each dilution of each sample. Pure bacterial suspensions were also dispensed in 3-mL aliquots into sterile glass vials to determine the sensitivity of the organism in TSB-YE. Survival curves were constructed by plotting the measured colony-forming units per gram or milliliters against irradiation dose on a semi-log graph. The log<sub>10</sub> counts of colony-forming units for each dose in the duplicate samples of two independent trials were averaged and the mean values plotted. Curves were fitted by linear regression. Irradiation sensitivity was expressed as D<sub>10</sub> values.

## Sample Preparation for Storage of Irradiated and Unirradiated Meats at Refrigeration Temperature

Meat samples were irradiated at a dose of 7 kGy to ensure that *Listeria* species that might originally be present in meat were killed. A pure bacterial suspension was serially diluted with 0.1% peptone water to obtain the desired inocula. After the samples were inoculated with sufficient cells to give approximately 10<sup>4</sup> cells per gram, they were irradiated to a dose of 2.5 kGy. It was the dose

required to kill 10<sup>4</sup> organisms per gram according to the D<sub>10</sub> values determined previously. Unirradiated samples were used as controls. All samples were stored at 4 C for 15 d. At each time interval, two chicken and two ground beef samples from each treatment were removed from cold storage and examined for *L. monocytogenes* by direct plating. The counts were averaged.

## Enumeration of Bacteria

The number of survivors in each sample was determined by direct plating onto *Listeria* Selective Agar<sup>6</sup> (LSA) (Oxford formulation) containing antibiotic supplement. Twenty-five-gram samples of inoculated chicken and ground beef were taken and 225 mL of 0.1% sterile peptone was added to samples in stomacher bags. The sample was homogenized for 1 min in a Stomacher 400 Lab blender and serially diluted with 0.1% peptone water; then 0.1-mL portions of an appropriate dilution were surface plated in duplicate on LSA plates. After 40 to 48 h of incubation at 35 C aerobically, colonies typical of *L. monocytogenes* were counted on Petri plates having 30 to 300 colonies. Representative colonies were confirmed as *L. monocytogenes* (Varabioff, 1990).

## Statistical Analysis

Cultural responses expressed as log<sub>10</sub> colony-forming units per gram of meat samples were plotted against irradiation dose. A regression line was fitted to sets of data using the Slidewrite Plus Package.<sup>7</sup> Regression coefficients, slopes, and 95% confidence limits were determined for all regression lines. The irradiation sensitivity of the organism in each medium was assessed by calculating D<sub>10</sub> values defined as the negative reciprocal of the slope. Data from two independent replicate trials were statistically analyzed.

## RESULTS AND DISCUSSION

Survival curves of *L. monocytogenes* SLCC 9488 exposed to gamma irradiation in TSB-YE, chicken homogenate, and ground beef are shown in Figure 1. All the curves were exponential in form; no shoulder region was observed. The D<sub>10</sub> values calculated from linear regression slopes of the samples: TSB-YE, chicken breast meat, and minced meat were 0.364, 0.599, and 0.699 kGy, respectively. The organism was most sensitive in TSB-YE, was more resistant in chicken homogenate, and appeared most resistant in beef homogenate. The higher sensitivity of the organism in TSB-YE is attributable to the higher water content of this medium. Because free radicals are generated from water as a result of irradiation, the radiation sensitivity is higher in aqueous media than in dry form (Farag *et al.*, 1990). The higher sensitivity of the organism in chicken homogenate compared to ground beef could be associated with differences in the chemical and physical composition of

<sup>3</sup>J. L. Shepherd and Associates, Glendale, CA.

<sup>4</sup>Köytür, Ankara, Turkey.

<sup>5</sup>Seward Medical Co., London SE1 1PP, UK.

<sup>6</sup>Oxford formulation, Oxoid, Unipath Ltd., Basingstoke, Hampshire, UK.

<sup>7</sup>Advanced Graphics Software Inc., Carlsbad, CA 92008.

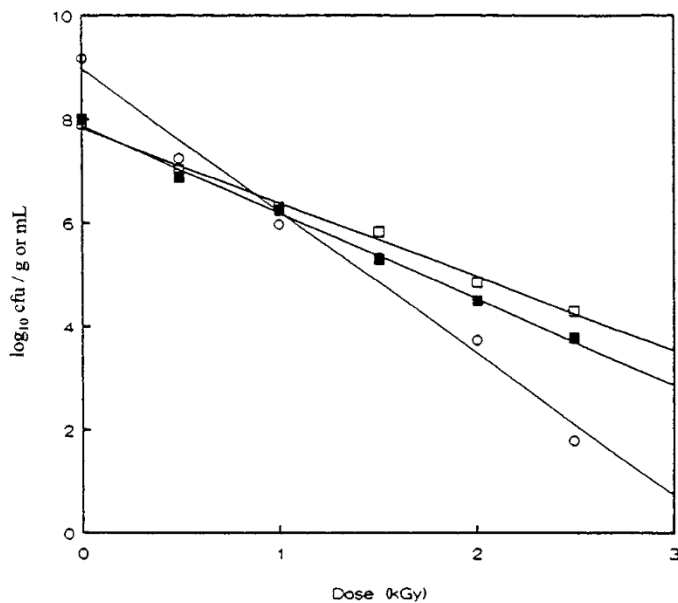


FIGURE 1. Survival curve for *Listeria monocytogenes* SLCC 9488 in TSB-YE (○), minced chicken meat (■), minced beef (□), irradiated with <sup>137</sup>Cs gamma rays.

these two samples. However, this result, obtained at ambient temperature, does not confirm the finding of Thayer *et al.* (1995) in which  $D_{10}$  value of a mixture of *L. monocytogenes* strains did not vary with the suspending meat irradiated at 5 °C. In their study, sample temperature was monitored continuously during irradiation to keep the conditions identical. Thus, we concluded that the variation in  $D_{10}$  values in poultry and red meat we examined could also be due to the uncontrolled temperature we applied during irradiation.

According to these determined  $D_{10}$  values, poultry and meat samples seeded with approximately  $10^3$  to  $10^4$  organisms per gram were irradiated with the dose of 2.5 kGy prior to storage at refrigeration temperature for a 15-d period. This dose was sufficient to eliminate  $10^4$  cells per gram in ground poultry meat and  $10^3$  organisms per gram in ground red meat samples. The ability of *L. monocytogenes* SLCC 9488, with or without exposure to a dose level of 2.5 kGy, to grow on meat samples at 4 °C over a period of 15 d is given in Table 1. There was approximately 2 to 4 log cell reduction of *L. monocytogenes* after 2.5 kGy irradiation. Moreover, it was observed from the survival counts of the control samples that refrigerated storage was effective in suppressing the growth of this organism in both samples during 7 d of storage. However, at 11 d of storage at 4 °C, a slight increase in the count of number of survivors was detected in the beef sample, whereas no significant increase in chicken meat sample was observed. On the other hand, at 15 d of storage, a drastic increase of about 4 log cycles of the organism was observed in both meat samples. This result indicates a very long adaptation period of this organism at refrigeration temperature. Once the cells completed their

TABLE 1. The effect of refrigerated storage on growth of *Listeria monocytogenes* in irradiated and unirradiated minced poultry and red meat<sup>1</sup>

<i>L. monocytogenes</i>								
Time	In chicken meat				In red meat			
	Unirradiated		Irradiated		Unirradiated		Irradiated	
(d)	(cfu/g)							
0	1.2	10 <sup>4</sup>	NC <sup>2</sup>		1.2	10 <sup>3</sup>	NC	
4	2.8	10 <sup>4</sup>	NC		1.5	10 <sup>3</sup>	NC	
7	1.1	10 <sup>4</sup>	NC		4.0	10 <sup>3</sup>	NC	
11	4.2	10 <sup>4</sup>	2.0	10 <sup>4</sup>	3.0	10 <sup>5</sup>	NC	
15	9.2	10 <sup>8</sup>	7.9	10 <sup>7</sup>	1.5	10 <sup>9</sup>	1.5	10 <sup>4</sup>

<sup>1</sup>Values represent the averages of two duplicate samples.

<sup>2</sup>NC = no count or the counts are below the minimum detectability of the methodology (< 10 cells per gram).

long lag period after storage at 4 °C, they started to grow very rapidly.

Irradiation reduced the number of *L. monocytogenes* in both chicken meat and ground beef. No *L. monocytogenes* cells were recovered from irradiated ground meat samples under test conditions until 15 d, whereas about  $2 \times 10^4$  cells were recovered from irradiated chicken samples before 11 but after 7 d. This observation indicates that not all *L. monocytogenes* cells were eliminated by irradiation at 2.5 kGy: some cells survived or the injured cells were able to repair themselves at refrigeration temperature in air-packaged samples. Although Oxford agar medium was reported to be one of the most sensitive *Listeria* isolation agar media for different food products inoculated with low levels of *L. monocytogenes* (Tiwari and Aldenrath, 1990; Dever *et al.*, 1993), the counts of irradiated samples were below the minimum detectability of the methodology (< 10 cells per gram) for up to 11 d. In fact, the recovery of radiation-injured cells in irradiated samples was reported to be hampered by the antibiotics in LSA (Kamat and Nair, 1995).

These results show that an irradiation dose of 2.5 kGy retards the subsequent development of *L. monocytogenes* during storage at 4 °C but did not eliminate it completely from raw chicken or beef when the initial load of the organism was higher than  $10^3$  cells per gram. However, in both meat samples contaminated with  $10^2$  cells per gram, the injured cells were not recovered even after 30 d of refrigeration (results not shown). The faster recovery of injured cells after 11 d in chicken meat than in red meat was associated with the higher number of initial cells ( $10^4$  per gram) in chicken meat.

Varabioff *et al.* (1992), reported that no *L. monocytogenes* was detected in air-permeable stomacher bags during storage at 4 °C for 15 d, whereas this organism was recovered from vacuum-packaged chickens. This result was contradictory to our observation in which the pathogen has appeared in chicken samples irradiated in air-permeable stomacher bags after 11 d and in the fresh meat samples after 15 d. The present

data support the results of Mead *et al.* (1990). In their investigation, 22% of irradiated carcasses initially inoculated with  $10^4$  *L. monocytogenes* (a mixture of four strains) per gram were found to be positive for the pathogen after 21 d of storage. Patterson *et al.* (1993) also reported a duration of 15 d of lag time in raw poultry meat samples irradiated to 2.5 kGy and stored at 6 C. One explanation for these conflicting results could be the different growth rates and specificities of various strains used in different studies. In fact, a faster growth rate was noted for strain SLCC 9488 than for strain ATCC 15313. In conclusion, the storage of irradiated poultry and red meats at 4 C after 7 d should not be recommended.

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