C18

SURVIVAL AND INJURY OF *LISTERIA MONOCYTOGENES*, *LISTERIA INNOCUA* AND *LISTERIA IVANOVII* IN GROUND PORK FOLLOWING ELECTRON BEAM IRRADIATION

RODRIGO TARTÉ R.[1,2], ELSA A. MURANO[1,3], and DENNIS G. OLSON[1,2]
Departments of [1]Food Science and Human Nutrition, [2]Animal Science, and [3]Microbiology, Immunology and Preventive Medicine
Iowa State University, Ames, Iowa 50011, USA

Key Words: Irradiation, pork, *Listeria*

BACKGROUND

Food irradiation has been researched for many decades (*11*), and it can eliminate bacterial pathogens, including *Listeria monocytogenes* (*6, 15, 18, 20*). In the USA, legal approval of ionizing radiation to treat foods of animal origin is limited to raw, packaged poultry at 1.5 to 3.0 kGy for the elimination of pathogens (*21*) and pork carcasses and fresh cuts at 0.3 to 1.0 kGy for the destruction of *Trichinella spiralis* (*4*). Currently, the Food and Drug Administration is reviewing a petition to allow the irradiation of fresh or frozen raw meat, at doses of 1.5 to 4.5 kGy, to reduce microbial pathogens and parasites and extend product shelf life (*5*).

OBJECTIVES

Objectives were (i) to determine the effect of electron beam irradiation on the survival of five strains of *Listeria* in fresh ground pork, and (ii) to evaluate radiation-induced sublethal injury of the five strains by recovering them on both permissive and selective plating media.

MATERIALS AND METHODS

Cultures. Strains studied were *Listeria monocytogenes* NADC 2045 (clinical isolate Scott A, serotype 4b), *L. monocytogenes* NADC 2783 (a hamburger isolate, serotype 4b), *L. monocytogenes* ATCC 15313 (which is nonhemolytic and, hence, nonpathogenic), *L. innocua* NADC 2841, and *L. ivanovii* NADC 3518.

Test sample preparation. Radiation-sterilized ground lean pork was inoculated with approx. 2 x 10^6 cells/g of an early stationary phase culture of *Listeria*. This meat was then packed (in 25-g portions) into sterile 60 x 15 mm polystyrene petri dishes; lids were placed and held with Parafilm laboratory film wrapped around the periphery of the plates. Samples were kept at 4°C overnight (15-16 h) prior to irradiation.

Irradiation and Dosimetry. Irradiation was done at the Iowa State University Linear Accelerator Facility, which is equipped with an MeV CIRCE III Linear Electron Accelerator (MeV Industrie S.A., Jouy-en-Josas, France). Samples were irradiated in duplicate at six target average dose levels (0, 0.25, 0.50, 0.75, 1.00 and 1.25 kGy) in electron beam mode. Absorbed radiation doses were determined by the use of dosimeter alanine pellets placed on the top and bottom surfaces of one of the duplicate petri dishes. Immediately following irradiation, absorbed doses were determined by electron paramagnetic resonance on a Bruker EMS 104 EPR Analyzer.

Determination of survivors. Following irradiation, serially diluted sample homogenates were surface plated in duplicate onto plates of tryptic soy agar supplemented with 0.6% yeast extract (TSAYE) for the enumeration of uninjured and sublethally injured cells and of modified Oxford medium (MOX) for the enumeration of uninjured cells. Plates were incubated aerobically at 37°C for 48 h (TSAYE) or 72 h (MOX). After incubation, colonies on plates were counted and recorded as colony count per g of sample.

Calculation of D_{10} values. D_{10} values were determined separately for the TSAYE and MOX plate counts by plotting the number of survivors (as $\log_{10}$ colony count/g) as a function of irradiation dose (in kGy) to obtain a regression curve, where the D_{10} value is the reciprocal of the absolute value of the slope of the curve. The entire experiment was replicated three times.

Determination of Sublethal Injury. For each replication, linear regression equations were used to determine the number of cells recovered on each medium. Percent sublethal injury for each strain at each dose used (0 to 1.25 kGy) was then calculated as:

[(log CFU/g on TSAYE - log CFU/g on MOX)/(log CFU/g on TSAYE)] x 100

Statistical Analysis. D_{10} value data were analyzed as a split-plot design with whole units arranged in a completely randomized fashion (*17*), with strain of *Listeria* as a whole-plot factor and plating medium as a sub-plot factor. Sublethal injury data were analyzed as a 5 x 6 factorial (5 strains vs. 6 doses). The analysis was done using release 6.07 of the Statistical Analysis System (SAS) software program. The ANOVA procedure was used to obtain the analyses of variance and, when these showed significant treatment effects ($P<0.05$), least significant differences (LSD) were calculated to identify significant differences ($P=0.05$) among treatment means (*17*).

RESULTS

There was a linear decrease in cell numbers during irradiation. *Listeria innocua* (D_{10}=0.638 kGy) was significantly more radiation-resistant (LSD at $P=0.05$) than all three strains of *L. monocytogenes* (D_{10}=0.424-0.447 kGy) and *L. ivanovii* (D_{10}=0.378 kGy), as recovered on TSAYE (Fig. 1). D_{10} values for *L. innocua*, *L. ivanovii*, and *L. monocytogenes* ATCC 15313 (Fig. 1) were significantly lower (LSD at $P=0.05$) when cells were recovered on MOX medium. Neither of the two hemolytic strains of *L. monocytogenes* was susceptible to radiation-induced injury (LSD at $P=0.05$) with the doses absorbed, while *L. monocytogenes* ATCC 15313, *L. innocua* and *L. ivanovii* were (Fig. 2). Unirradiated cells of *L. ivanovii* showed significant ($P<0.05$) sublethal injury.

DISCUSSION AND CONCLUSIONS

The D_{10} values determined in this study for *L. monocytogenes* (0.424-0.447 kGy) in irradiated ground pork (Fig. 1) are similar to those reported elsewhere (*1, 2, 7, 9, 12, 13, 16*) and indicate that the irradiation dose range currently allowed for the destruction of *Trichinella spiralis* in pork (0.3-1 kGy) is inadequate for the complete inactivation of *L. monocytogenes*, since it would reduce the numbers by only 0.7 $\log_{10}$ to 2.2 $\log_{10}$. This does not provide an adequate safety margin, since meats contaminated with *L. monocytogenes* could contain higher numbers of the organism than would be inactivated even at 1 kGy (*15*). However, the dose range in a petition to the FDA (*5*) for the irradiation of both beef and pork (1.5-4.5 kGy) would be adequate for the removal of the pathogen from fresh pork, as it would achieve reductions of 3.4 $\log_{10}$ to 10.1 $\log_{10}$, thus providing an acceptable margin of safety.

The nonpathogenic *L. innocua*, which has been shown in some surveys to be more prevalent in meats and meat products than *L. monocytogenes* (*3, 8, 15, 19*), has a greater radiation resistance. It has been shown that *L. innocua* can sometimes outgrow *L. monocytogenes* and make its recovery more difficult (*14*). It is therefore important that any listeriae isolated from irradiated foods be identified to the species level, in order to avoid losses due to unnecessary recalls and/or remedial actions.

Although it is not as prevalent in meats as *L. monocytogenes* and *L. innocua*, *L. ivanovii* is important, since it has been recognized as an occasional human pathogen (*15*). Results indicate that *L. ivanovii* NADC 3518 is no more radioresistant than *L. monocytogenes* (Fig. 1). It is concluded that irradiation doses that would eliminate *L. monocytogenes* would also eliminate *L. ivanovii*.

Most food preservation methods, such as heating, freezing or irradiation, can result in sublethal injury to microorganisms. Injured cells may be unrecoverable with conventional methods that involve the use of selective media (*10*), thus raising the potential for error in reporting mistakenly low numbers of survivors. Under favorable environmental conditions, sublethally injured cells could repair and grow in the food. Previous studies have evaluated the efficacy of selective plating media for the isolation of *L. monocytogenes* following irradiation (*7, 13*). Since results from this study show that D_{10} values were higher on TSAYE than on MOX medium only for the nonhemolytic *L. monocytogenes* ATCC 15313, *L. innocua*, and *L. ivanovii* (i.e., these were susceptible to radiation-induced sublethal injury), but not for the two pathogenic strains of *L. monocytogenes*, it is concluded that MOX medium is appropriate for the isolation of the latter. The results of this study suggest that the use of presently accepted methods for the recovery of *L. monocytogenes* may be acceptable to isolate it from irradiated ground pork. It is recommended that other strains of *L. monocytogenes* be tested before widespread recommendations are made.

REFERENCES

1. El-Shenawy, M. A., A. E. Yousef, and E. H. Marth. 1989. Lebbensm. Wiss. Technol. 22:387-390.
2. Farag, M. D. E.-H. F., K. Shamsuzzaman, and J. Borsa. 1990. J. Food Prot. 53: 648-651.
3. Farber, J. M., G. W. Sanders, and M. A. Hohnston. 1989. J. Food Prot. 52: 456-458.
4. FDA. 1985. 21 CFR Part 179. Fed. Reg. 50:29658-29659.
5. FDA. 1994. Fed. Reg. 59: 43848-43849.
6. Fu, A.-H.. 1994. Ph.D. Dissertation. Iowa State University, Ames, Iowa.
7. Grant, I. R., and M. F. Patterson. 1992. Food Microbiol. 9: 95-103.
8. Grau, F. H. and P. B. Vanderlinde. 1992. J. Food Prot. 55: 4-7.
9. Huhtanen, C. N., R. K. Jenkins, and D. W. Thayer. 1989. J. Food Prot. 52: 610-613.
10. Jay, J. M. 1992. Modern food microbiology, 4th ed. Van Nostrand Reinhold Co., New York.
11. Josephson, E. S. 1983. J. Food Safety 5: 161-190.
12. Monk, J. D., M. R. S Clavero, L. R., M. P. Doyle, and R. E. Brackett. 1994. J. Food Prot. 57: 969-974.
13. Patterson, M. 1989. Lett. Appl. Microbiol. 8: 181-184.
14. Petran, R. L., and K. M. J. Swanson. 1993. J. Food Prot. 56:616-618.
15. Ryser, E. T., and E. H. Marth. 1991. *Listeria*, Listeriosis, and Food Safety. Marcel Dekker, Inc., New York.
16. Shamsuzzaman, K., N. Chuaqui-Offermanns, L. Lucht, T. McDougall, and J. Borsa. 1992. J. Food Prot. 55: 528-533.
17. Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics: a biometrical approach, 2nd ed. McGraw-Hill, New York.
18. Urbain, W. M. 1986. Food Irradiation. Academic Press, Inc., Orlando, FL.
19. Varabioff, Y. 1992. Lett. Appl. Microbiol. 14:167-169.
20. Varabioff, Y., G. E. Mitchell, and S. M. Nottingham. 1992. J. Food Prot. 55: 389-391.
21. U.S. Department of Agriculture. 1992. Irradiation of poultry products. Fed. Reg. 57:43588-43600.

DATA

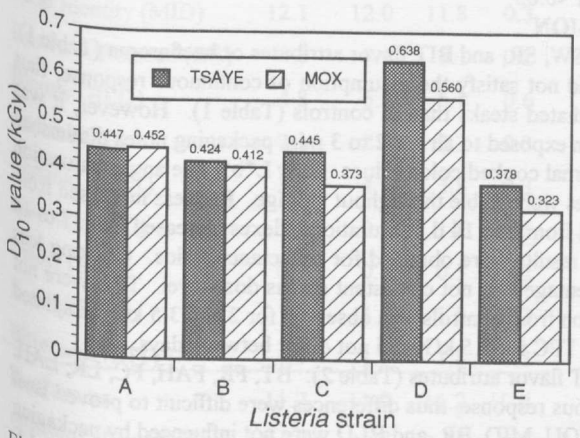

Figure 1. D_{10} values of five strains of *Listeria* in ground pork following electron beam irradiation, as affected by recovery medium (TSAYE or MOX). $LSD_{(0.05)}$ = 0.093 kGy (across strains for one medium) and 0.031 kGy (within strains). A: *L. monocytogenes* NADC 2045 (Scott A), B: *L. monocytogenes* NADC 2783 (hamburger isolate), C: *L. monocytogenes* ATCC 15313, D: *L. innocua* NADC 2841, E: *L. ivanovii* NADC 3518.

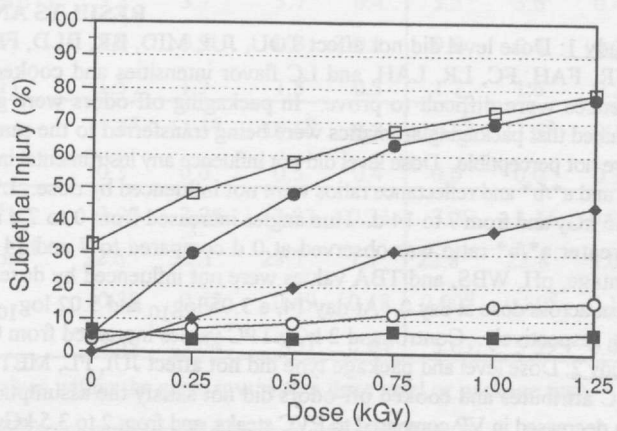

Figure 2. Sublethal injury of five strains of *Listeria* in ground pork following electron beam irradiation. $LSD_{(0.05)}$ = 15.6 kGy. ■ *L. monocytogenes* NADC 2045 (Scott A), ○ *L. monocytogenes* NADC 2783 (hamburger isolate), ● *L. monocytogenes* ATCC 15313, ◆ *L. innocua* NADC 2841, □ *L. ivanovii* NADC 3518.