Journal of Food Protection, Vol. 74, No. 4, 2011, Pages 627–630
doi:10.4315/0362-028X.JFP-10-421

Research Note

Inactivation of Avirulent *Yersinia pestis* in Beef Bologna by Gamma Irradiation†

CHRISTOPHER H. SOMMERS* AND BRENDAN A. NIEMIRA

U.S. Department of Agriculture, Agriculture Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA

MS 10-421: Received 30 September 2010/Accepted 18 December 2010

ABSTRACT

Yersinia pestis, a psychrotrophic pathogen capable of growth at refrigeration temperatures, can cause pharyngeal and gastrointestinal plague in humans that consume contaminated foods. Because *Y. pestis* is listed as a select agent for food safety and defense, evaluation of food safety intervention technologies for inactivation of this pathogen is needed. Ionizing (gamma) radiation is a safe and effective intervention technology that can inactivate pathogens in raw and processed meats, produce, and seafood. In this study, we investigated the effect of temperature on the ability of ionizing radiation to inactivate avirulent *Y. pestis* in beef bologna. The mean (±standard error of the mean) radiation D_{10}-values (the radiation dose needed to inactivate 1 log unit or 90% of the population of a microorganism) for avirulent *Y. pestis* suspended in beef bologna samples were 0.20 (±0.01), 0.22 (±0.01), 0.25 (±0.02), 0.31 (±0.01), 0.35 (±0.01), and 0.37 (±0.01) kGy at temperatures of 5, 0, −5, −10, −15, and −20°C, respectively. When incorporated into a three-dimensional mesh, the predictive model followed a parabolic fit ($R^2 = 0.84$), where the log reduction $= -0.264 - (0.039 \times \text{temp}) - (3.833 \times \text{dose}) - (0.0013 \times \text{temp}^2) - (0.728 \times \text{dose}^2)$. These results indicate that ionizing radiation would be an effective technology for control of *Y. pestis* in ready-to-eat fine emulsion sausage products.

Yersinia pestis, the causative agent of plague, is typically transmitted to humans via rodents that carry infected fleas. Although rare, pharyngeal plague and gastrointestinal plague can be contracted through consumption of raw or cooked meat products infected with *Y. pestis (1, 2, 4, 9).* In a recent foodborne outbreak of *Y. pestis* infection, 17 of the 83 individuals who contacted gastrointestinal plague from the consumption of contaminated camel meat died from the disease *(8).* One thing that makes this pathogen so dangerous is its ability to grow at refrigeration temperatures as low as 4°C in liquid egg products, raw beef, and frankfurters, even in the presence of antimicrobials such as sodium diacetate and potassium lactate, which are commonly used in ready-to-eat meats *(7, 12, 19).* Because *Y. pestis* is listed as a select agent for both food safety and food defense *(13, 18),* evaluation of the efficacy of nonthermal food processing intervention technologies for inactivation of *Y. pestis* in food products is needed.

The use of ionizing radiation to inactivate foodborne pathogens in meat and poultry has been approved by the U.S. Food and Drug Administration (FDA) *(23),* and in the United States these foods are now irradiated commercially for pathogen reduction. A petition to allow the irradiation of multi-ingredient foods was filed with the FDA by the National Food Processors Association (now the Grocery Manufacturers Association) in 1999; this new rule would allow irradiation of ready-to-eat meat products such as bologna *(6).* Following an attack on the nation's food supply, ionizing radiation may be a useful technology, an ''insurance policy,'' that could be used to protect the nation's food supply.

The FDA currently lists 4.5 kGy as the maximum allowable dose for irradiation of refrigerated red meat and 7 kGy as the maximum allowed dose for frozen product *(23).* The difference in the upper radiation dose limits for refrigerated versus frozen meats is based on the differences in radiation resistance of pathogens in the refrigerated versus frozen state. Approximately 75% of the damage done to macromolecules such as DNA is indirect through radicals, primarily hydroxyl radicals, produced by the radiolysis of water *(13, 16).* The increased radiation resistance of microorganisms at subfreezing temperatures has been attributed to the lower water activity of meat at subfreezing temperatures and to decreased hydroxyl radical mobility in the frozen state *(3, 20).* Therefore, when predictive equations are developed for inactivation of pathogens on foods that can be preserved by freezing, irradiation is conducted at both refrigeration and subfreezing temperatures to develop those equations. These predictive equations are particularly useful for the radiation processing industry, the food processing industry, governmental regulatory agencies, and international standards organizations.

* Author for correspondence. Tel: 215-836-3754; Fax: 215-233-6445; E-mail: christopher.sommers@ars.usda.gov.

† Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Although models describing the effect of temperature and cooking for the inactivation of foodborne pathogens are abundant, similar models that describe the effect of temperature on radiation inactivation of foodborne pathogens are relatively rare. Temperature during irradiation is a critical factor that affects bacterial inactivation; however, predictive models are relatively rare because most research facilities are unable to control temperature during the irradiation process and because the work is time-consuming. Models currently available include inactivation of *Listeria monocytogenes, Escherichia coli* O157:H7, *Staphylococcus aureus,* and *Yersinia* spp. in raw meat *(14, 15, 21, 22).*

In this study, the radiation resistance of *Y. pestis* suspended in beef bologna was investigated, and a predictive model was developed to describe the radiation dose needed to produce appropriate log reductions in pathogen populations at refrigeration and subfreezing temperatures. This predictive model is the first to describe the effect of temperature on the radiation resistance of a foodborne pathogen, in this case *Y. pestis,* in a ready-to-eat meat product.

MATERIALS AND METHODS

Beef bologna manufacture. Ground beef (15% fat) was emulsified in a cutter-mixer (model HCM40, Hobart, Troy, OH). Cure ingredients and additives (wt/wt, per kilogram of meat) were 3% sodium chloride, 3% dextrose, 0.5% sodium tripolyphosphate, 0.05% sodium erythorbate, 0.02% sodium nitrite, and 20% deionized water. The emulsion was stuffed into 10-cm fibrous collagen casings (Dewied Int., Santa Fe, NM) to produce bologna. The sausages were then cooked (model KL-50 Smokehouse, Koch Inc., Kansas City, MO) to an internal product temperature of 73°C. The dry bulb setting was 90°C and the wet bulb setting was 63°C for a relative humidity of approximately 47%. After the desired internal temperature was reached, the sausages were immediately chilled in a sterile brine bath and the casings were removed in an aseptic manner. The sausages were then sectioned and vacuum packaged to 0.26 mm Hg with a vacuum packager (model A300, Multivac, Inc., Kansas City, MO), overpacked in gas- and moisture-impermeable foil bags (Mil-B-131-H, Bell Fibre Products Corp., Columbus, GA), and stored at −20°C. The bologna was then irradiated (25 kGy at −20°C) to inactivate background microflora *(11).* The bologna pH was 6.7 before and after irradiation.

***Y. pestis*.** Four avirulent *Y. pestis* strains (KUMA, Yokohama, KIM5, and CO99) were obtained from Dr. Susan Straley (Department of Microbiology and Immunology, University of Kentucky, Lexington) and Dr. Robert Brubaker (Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing) through Dr. George Paoli (U.S. Department of Agriculture, Eastern Regional Research Center, Wyndmoor, PA). The virulence state of *Yersinia* spp. does not affect their radiation resistance *(17).* The isolates were maintained at 0 to 4°C on brain heart infusion (BHI) agar (Difco, BD, Sparks, MD) until ready for use.

Propagation and inoculation. The four *Y. pestis* strains were cultured independently in 25 ml of BHI medium (20°C) in 50-ml sterile tubes at 30°C (150 rpm) for 24 h. The bacteria were then sedimented by centrifugation (1,700 × *g*) and resuspended as a cocktail in a 10-fold reduced volume of Butterfield phosphate buffer (BPB; Applied Research Institute, Newtown, CT). The four-strain cocktail was then diluted 1:10 by volume into 45 g of sterile beef bologna and mixed in a stomacher mixer (Seward, London, UK) for 2 min. The inoculated bologna was then aliquoted (5 g) into no. 400 polyethylene stomacher bags and vacuum packed (26 mm Hg; model A400, Multivac). This procedure resulted in a *Y. pestis* population of approximately 10^8 CFU/g of bologna.

The vacuum-packaged samples, including nonirradiated controls, were placed in an isotemp bath (−20, −15, −10, or −5°C) for approximately 60 min before irradiation. Samples were then transferred to the temperature controlled irradiator chamber for irradiation.

Irradiation. A Lockheed Georgia Company (Marietta, GA) self-contained ^{137}Cs irradiator, with a dose rate of 0.086 kGy/min, was used for all exposures *(17).* The radiation source consisted of 23 individually sealed source pencils in an annular array. The cylindrical sample chamber (22.9 by 63.5 cm) was located central to the array when placed in the operating position. Inoculated samples were placed vertically and centrally in the sample chamber with a 4-mm-thick polypropylene bucket to ensure a good dose uniformity ratio (<1.1:1.0), which is the maximum radiation absorbed divided by the minimum absorbed as determined by appropriate dose mapping experiments. The temperature during irradiation, monitored by thermocouple, was maintained by introduction of the gas phase from a liquid nitrogen source directly into the top of the sample chamber. The absorbed dose was verified with tempered 5-mm alanine pellets that were then evaluated with an EMS 104 EPR analyzer (Bruker, Billerica, MA).

Sample dilution and plating. After irradiation, the samples were assayed for CFU by surface plating. Approximately 45 ml of BPB was added to the sample bags containing the 5 g of bologna. The samples were then mixed by stomaching for 2 min, and 1:10 serial dilutions were made in BPB. The diluted samples (0.1 ml) were then surface plated onto BHI agar plates. The plates (three per dilution) were incubated at 30°C for 3 days and then scored.

D_{10}-values. The mean plate counts of the treated samples (N) were divided by the average control plate counts (N_0) to produce a survivor ratio (N/N_0). The $\log(N/N_0)$ was then used to determine the D_{10}-values and other statistical measures. D_{10}-values were the reciprocal of the slope of the linear regression as determined by a least squares analysis *(5).*

Statistical analysis. Each experiment was conducted independently three times. Statistical analysis functions of MS Excel (Microsoft Corp., Redmond, WA) were used for routine calculations, descriptive statistics, and analysis of variance (ANOVA). The predictive model for determination of log survivor ratios was developed with Sigma Plot version 8.0 (SPSS Science, Chicago, IL). A parabolic model of the form $f = y_0 + ax + by + cx^2 + dy^2$ was used to describe the response curve surface, consistent with published methodologies used to model the interaction of temperature and radiation response of *Yersinia* spp. *(15, 16).*

RESULTS AND DISCUSSION

Ionizing radiation is a safe and effective method for inactivation of foodborne pathogens in a variety of foods, including raw and ready-to eat meat products. In addition to inactivating foodborne pathogens, irradiation can decrease the virulence of surviving bacteria through induction of virulence plasmid loss and mutation of chromosomal virulence factors *(13, 15).* In this study, avirulent *Y. pestis*

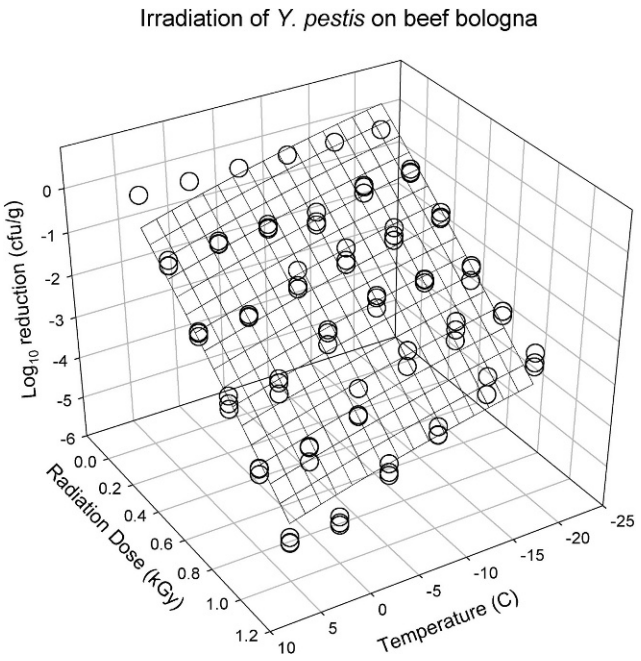

FIGURE 1. *A three-dimensional mesh plot of log reduction data for* Y. pestis *as a function of radiation dose and product temperature. The predictive equation followed a parabolic fit* ($R^2 = 0.84$), *where log reduction* $= -0.264 - (0.039 \times \text{temp}) - (3.833 \times \text{dose}) - (0.0013 \times \text{temp}^2) - (0.728 \times \text{dose}^2)$. *Open circles represent log reduction values for* Y. pestis *at specific temperatures and radiation doses.*

was inoculated into beef bologna, which was subsequently refrigerated or frozen and irradiated at specific temperatures to determine the effect of temperature on the ability of gamma radiation to inactivate the pathogen in a fine emulsion sausage product.

The mean ($\pm$standard error of the mean [SEM]) radiation D_{10}-values of avirulent *Y. pestis* suspended in beef bologna were 0.20 ($\pm$0.01), 0.22 ($\pm$0.01), 0.25 ($\pm$0.02), 0.31 ($\pm$0.01), 0.35 ($\pm$0.01), and 0.37 ($\pm$0.01) kGy at 5, 0, -5, -10, -15, and -20°C, respectively. The predictive model followed a parabolic fit ($R^2 = 0.84$) where the log reduction $= -0.264 - (0.039 \times \text{temp}) - (3.833 \times \text{dose}) - (0.0013 \times \text{temp}^2) - (0.728 \times \text{dose}^2)$ (Fig. 1 and Table 1). These results indicate that relatively low gamma radiation doses of 1.0 to 2.0 kGy would be needed to provide effective protection (a 5-log reduction) against this pathogen for refrigerated and frozen ready-to-eat meats such as beef bologna.

Temperature (or more accurately the physical state of water) can have a profound effect on the radiation resistance of a microorganism. In one study, the D_{10}-values for *E. coli* O157:H7 suspended in beef were 0.41 kGy at 5°C and 0.62 kGy at -15°C *(10)*. In another study, the D_{10}-values for *L. monocytogenes* were 0.45 kGy in refrigerated (5°C) ground beef and 1.21 kGy in beef frozen to -20°C *(21)*. The D_{10}-values for *E. coli* O157:H7 were 0.39 and 0.98 kGy in ground beef irradiated at 4 and -20°C, respectively, and the D_{10}-values for *S. aureus* were 0.48 and 0.87 kGy in ground beef irradiated at 5 and -20°C, respectively *(22)*. In our previous research, the mean ($\pm$SEM) radiation resistance of *Y. pestis* suspended in raw ground pork was 0.16 ($\pm$0.02), 0.27 ($\pm$0.02), 0.36 ($\pm$0.02), and 0.54 ($\pm$0.03) kGy at 10, 0, -10, and -20°C, respectively *(15)*.

TABLE 1. *Radiation* D_{10}*-values of* Y. pestis *suspended in beef bologna samples at refrigeration and subfreezing temperatures*[a]

Temp (°C)	D_{10}-value (kGy) Mean	SEM	R^2
5	0.20 A	0.01	0.99
0	0.22 A	0.01	0.99
−5	0.25 A	0.01	0.98
−10	0.31 AB	0.02	0.95
−15	0.35 B	0.01	0.98
−20	0.37 B	0.01	0.98

[a] Each experiment was conducted independently three times ($n = 3$). SEM, standard error of the mean. Values with the same letter are not significantly different, as determined by an ANOVA ($P < 0.05$).

These results provide the necessary information for governmental agencies and the food and radiation processing industries to control *Y. pestis* in ready-to-eat meat products if the need were to arise.

ACKNOWLEDGMENT

We thank Robert Richardson for the technical assistance in this study.

REFERENCES

1. Albaji, A., S. Kharabsheh, S. Al-Azab, M. Al-Kayad, Z. Amr, M. Abu-Baker, and M. Chu. 2005. A 12-case outbreak of pharyngeal plague following consumption of camel meat, in north-eastern Jordan. *Ann. Trop. Med. Parasitol.* 99:789–793.
2. Bin Saeed, A., N. Al-Hamdan, and R. Fontaine. 2005. Plague from eating raw camel liver. *Emerg. Infect. Dis.* 11:1456–1457.
3. Bruns, M. W., and R. B. Maxcy. 1979. Effect of irradiation temperature and drying on survival of highly radiation resistant bacteria in complex menstrua. *J. Food Sci.* 44:1743–1746.
4. Christie, A. B., T. H. Chen, and S. S. Elberg. 1980. Plague in camels and goats; their role in human epidemics. *J. Infect. Dis.* 141:724–726.
5. Diehl, J. F. 1995. Safety of irradiated food, 2nd ed. Marcel Dekker, New York.
6. Government Accounting Office. 2010. Food irradiation: FDA could improve its documentation and communication of key decisions on food irradiation petitions. GAO-10-309R. Federal oversight of food irradiation. Available at: http://www.gao.gov/new.items/d10309r.pdf. Accessed 26 May 2010.
7. Gurtler, J., R. Rivera, H. Zhang, and C. H. Sommers. 2010. Behavior of avirulent *Yersinia pestis* in liquid whole egg as affected by storage temperature, antimicrobials and thermal pasteurization. *J. Food Saf.* 30:537–557.
8. Leslie, T., C. A. Whitehouse, S. Yingst, C. Baldwin, F. Kakar, J. Mofleh, A. S. Hami, L. Mustafa, F. Omar, E. Ayazi, C. Rossi, B. Noormal, N. Ziar, and R. Kakar. 2010. Outbreak of gastroenteritis caused by *Yersinia pestis* in Afghanistan. *Epidemiol. Infect.* Available at: http://journals.cambridge.org/action/displayAbstract;jsessionid=F4660B80D0254DE3F36413054AE80FA8.tomcat1?fromPage=online&aid=7847218. Accessed 30 August 2010.
9. Lindsay, J. A. 1997. Chronic sequelae of foodborne disease. *Emerg. Infect. Dis.* 3:443–452.
10. López-González, V., P. S. Murano, R. E. Brennan, and E. A. Murano. 1999. Influence of various commercial packaging conditions on survival of *Escherichia coli* O157:H7 to irradiation by electron beam versus gamma rays. *J. Food Prot.* 62:10–15.
11. Sommers, C. H., and S. Bhaduri. 2001. Loss of crystal violet binding activity in stationary phase *Yersinia enterocolitica* following gamma irradiation. *Food Microbiol.* 18:367–374.

12. Sommers, C. H., and P. Cooke. 2009. Inactivation of avirulent *Yersinia pestis* in Butterfield's phosphate buffer and frankfurters by UVC (254 nm) and gamma radiation. *J. Food Prot.* 72:755–759.
13. Sommers, C. H., A. P. Handel, and B. A. Niemira. 2002. Radiation resistance of *Listeria monocytogenes* in the presence or absence of sodium erythorbate. *J. Food Sci.* 67:2266–2270.
14. Sommers, C. H., N. Kesser, X. Fan, F. M. Wallace, J. S. Novak, A. P. Handel, and B. A. Niemira. 2004. Irradiation of ready-to-eat meats: eliminating *Listeria monocytogenes* while maintaining product quality, p. 77–89. *In* V. Komolprasert and K. Morehouse (ed.), Irradiation of food and packaging. American Chemical Society, Washington, DC.
15. Sommers, C. H., and B. A. Niemira. 2007. Effect of temperature on the radiation resistance of *Yersinia pestis* suspended in raw ground pork. *J. Food Saf.* 27:317–325.
16. Sommers, C. H., B. A. Niemira, M. Tunick, and G. Boyd. 2002. Effect of temperature on the radiation resistance of virulent *Yersinia enterocolitica*. *Meat Sci.* 61:323–328.
17. Sommers, C. H., and J. Novak. 2002. Radiation resistance of plasmid-containing versus plasmid-less *Yersinia enterocolitica*. *J. Food Prot.* 65:556–559.
18. Takhistov, P., and C. M. Bryant. 2006. Protecting the food supply. *Food Technol.* 60:34–43.
19. Tamplin, M., and S. Bhaduri. Behavior of *Yersinia pestis* strains KIM5 and CDC A1122 in raw ground beef. Unpublished data.
20. Taub, I. A., J. W. Halliday, and M. D. Sevilla. 1979. Chemical reactions in proteins irradiated at subfreezing temperatures. *Adv. Chem. Serol.* 180:109–140.
21. Thayer, D. W., and G. Boyd. 1995. Radiation sensitivity of *Listeria monocytogenes* on beef as affected by temperature. *J. Food Sci.* 60: 237–240.
22. Thayer, D. W., and G. Boyd. 2001. Effect of irradiation temperature on inactivation of *E. coli* O157:H7 and *Staphylococcus aureus*. *J. Food Prot.* 64:1624–1626.
23. U.S. Food and Drug Administration. 2005. Irradiation in the production, processing, and handling of food. 21 CFR Part 179. Docket 1999F-4372. *Fed. Regist.* 70(157):48057–48073.