

Research Note

Effect of Irradiation Temperature on Inactivation of *Escherichia coli* O157:H7 and *Staphylococcus aureus*[†]

DONALD W. THAYER* AND GLENN BOYD

Food Safety Research Unit, USDA, ARS, NAA, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA

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ABSTRACT

The resistance of *Escherichia coli* O157:H7 and *Staphylococcus aureus* in ground beef to gamma radiation was significantly ($P < 0.05$) higher at subfreezing temperatures than above freezing. Ground beef was inoculated (ca. 2×10^8 CFU/g) with five isolates of either *E. coli* O157:H7 or *S. aureus* and subdivided into 25-g samples, vacuum packaged in barrier pouches, and tempered to 20, 12, 4, 0, -4, -12, -20, -30, -40, or -76°C before gamma irradiation. The studies were repeated twice. The D_{10} -values for both of these pathogens increased significantly at subfreezing temperatures, reaching maxima at approximately -20°C. The D_{10} -values for *E. coli* O157:H7 at 4 and -20°C were 0.39 ± 0.04 and 0.98 ± 0.23 kGy, respectively. The D_{10} -values for *S. aureus* at 0 and -20°C were 0.51 ± 0.02 and 0.88 ± 0.05 kGy, respectively.

The recent approval for the use of ionizing irradiation to inactivate foodborne pathogens in red meats makes it important to understand the effect of processing temperature. Processors may choose to use relatively low doses of radiation and deep-frozen products to avoid changes in the sensorial properties. Unfortunately, some processors might not appreciate the combined effects of irradiation dose and temperature on the survival of pathogens. Thayer and Boyd (7) reported that the D_{10} -values of *E. coli* O157:H7 in mechanically deboned chicken meat at 5 and -5°C were 0.28 ± 0.02 and 0.44 ± 0.03 kGy, respectively; also, a similar increased survival of *Staphylococcus aureus* at -20°C was reported (6).

These studies were initiated to provide D_{10} -values for a gram-negative and a gram-positive pathogen, *Escherichia coli* O157:H7 and *S. aureus*, at several irradiation processing temperatures from 20 to -76°C. The latter temperature was chosen in case some processors used dry ice to keep meat frozen during processing when portions of the product could actually reach that temperature, allowing for greater pathogen survival. The authors are unaware of other detailed studies that provide D_{10} -values for these pathogens over this temperature range. This study will test the hypothesis that the previously observed increased survival of *E. coli* O157:H7 and *S. aureus* when irradiated in hard-frozen meat will be reflected in increased D_{10} -values.

MATERIALS AND METHODS

Cultures. *E. coli* O157:H7 isolates ATCC 35150, ATCC 43889, ATCC 43894, 93-437, and ENT C9490 were maintained

and cloned on tryptic soy agar (Difco Laboratories, Detroit, Mich.) and incubated at 37°C. Isolate 93-437 was obtained from the Oregon Public Health Laboratory, Portland, Ore. ENT C9490 was obtained from the Centers for Disease Control and Prevention, Atlanta, Ga.

S. aureus isolate B124 was obtained from L. Nakamura, U.S. Department of Agriculture, Peoria, Ill. The following isolates were obtained from the American Type Culture Collection, Manassas, Va.: ATCC 25923, ATCC 13565, ATCC 14458, and ATCC 27154. 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., Hazelwood, Mo.) (1, 4).

Substrate. Commercial lean ground beef (64.8% moisture, 12.3% fat, 0.89% ash, and 19.7% protein) was obtained from a local grocery store, then mixed and vacuum packaged in 100-g portions in no. 400 polyethylene Stomacher bags. Each bag was then vacuum sealed within a barrier pouch. The packages of meat were rapidly frozen and sterilized by gamma irradiation to an absorbed dose of 42 kGy at -76°C and stored at -70°C until needed.

Meat inoculation. Each isolate of *E. coli* or *S. aureus* was cultured independently in tryptic soy broth (Difco) at 37°C. Equal amounts of 18-h cultures of each isolate, *E. coli* O157:H7 or *S. aureus*, were mixed and harvested by centrifugation and resuspended in 1/10 volume of Butterfield's phosphate. Sterile ground beef was mixed with the inoculum in the ratio of 10 ml inoculum to 100 g of meat for an approximate initial population of 10^8 CFU/g. Inoculated ground beef was subdivided into 5.00 ± 0.05 -g samples and vacuum sealed within no. 400 Stomacher bags. These pouches were either stored at -70°C or chilled to irradiation temperature using appropriate means, i.e., ice, dry ice, or refrigerated water bath immediately before irradiation.

Irradiation. Samples were irradiated with a temperature-controlled, ¹³⁷Cs, self-contained, gamma-radiation source (Lockheed Georgia, Marietta, Ga.) (8) with a dose rate of 0.10 kGy min⁻¹. The dose rate was established using alanine transfer do-

* Author for correspondence. Tel: 215-233-6582; Fax: 215-233-6559; E-mail: dthayer@arserrc.gov.

† Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over other of a similar nature not mentioned.

simeters from the National Institutes of Standards and Technology, Gaithersburg, Md. Corrections for source decay were made monthly. Routine dosimetry was performed using 5-mm-diameter alanine dosimeters (Bruker Instruments, Rjeomstettem, Germany), and the free-radical signal was measured using a Bruker EMS 104 EPR analyzer (2). The target doses were 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 kGy; the actual doses for duplicate samples irradiated at 4°C in the study of *E. coli* O157:H7 were: 0.39 and 0.40, 0.79 and 0.82, 1.17 and 1.24, 1.57 and 1.65, 1.88 and 2.04, and 2.31 and 2.37 kGy, respectively. This dose uniformity was achieved at all temperatures and in each study.

Microbiological analysis. Samples were assayed for CFU by standard pour-plate procedures using tryptic soy agar with serial dilutions in sterile Butterfield's phosphate. Petri plates were incubated at 37°C for 24 h 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, N.J.).

Statistical analysis. D_{10} -values were determined as described previously (8). Statistical calculations were performed with the general linear models procedure of the SAS statistical package (3, 5). Regression techniques were used to fit second-order response-surface models to the data in order to predict the effects of irradiation dose and temperature on the number of survivors of *E. coli* O157:H7 or *S. aureus*. Each study was repeated twice.

RESULTS AND DISCUSSION

Reduced irradiation temperatures resulted in greater survival of *E. coli* O157:H7. The analysis of variance for the inactivation of *E. coli* O157:H7 on ground beef by ionizing radiation produced the following results: \log survivor CFU/g = $-0.027 - 0.016 \times \text{temperature (}^\circ\text{C)} - 3.117 \times \text{kGy} - 0.030 \times \text{temperature} \times \text{kGy} - 0.0003 \times \text{temperature}^2 + 0.235 \text{ kGy}^2$ ($R^2 = 0.91$). This equation predicts that a dose of 1.2 kGy would inactivate 4.59, 3.43, 2.51, and 1.83 log CFU/g at 20, 0, -20, and -40°C, respectively; the actual values were 4.44 ± 0.52 , 3.33 ± 0.98 , 2.33 ± 0.09 , and 1.74 ± 0.09 log CFU/g. The effects of temperature, radiation dose, interaction between temperature and dose, and (temperature)² terms on the survival of *E. coli* O157:H7 were significant ($P < 0.05$). Many of the D_{10} -values for the inactivation of *E. coli* O157:H7 at a given temperature, such as at 0°C, differed significantly ($P < 0.05$) from values obtained at other temperatures (Table 1).

Reduced irradiation temperature resulted in greater survival of *S. aureus* in ground beef (Table 1). The analysis of variance for the inactivation of *S. aureus* on ground beef by ionizing radiation produced the following results: \log survivor = $-0.045 - 0.020 \times \text{temperature (}^\circ\text{C)} - 2.280 \times \text{kGy} - 0.017 \times \text{temperature} \times \text{kGy} - 0.0003 \times \text{temperature}^2 + 0.130 \times \text{kGy}^2$ ($R^2 = 0.90$). The effects of temperature, radiation dose, interaction between temperature and dose, and (temperature)² terms were significant ($P < 0.01$). This equation predicts that a radiation dose of 1.2 kGy will inactivate 3.52, 2.59, 1.91, and 1.46 log CFU/g at 20, 0, -20, and -40°C, respectively. The actual values were 4.93 ± 0.17 , 3.76 ± 0.20 , 7.20 ± 0.03 , and 6.98 ± 0.29 log. The effects of temperature, radiation dose, interaction between temperature and dose, and (temperature)²

TABLE 1. D_{10} -values \pm SD in kGy for the inactivation of *E. coli* O157:H7 or *S. aureus* in ground beef by ionizing radiation^a

	20°C	12°C	4°C	0°C	-4°C	-12°C	-20°C	-30°C	-40°C	-76°C
<i>E. coli</i>	0.25 \pm 0.02	0.29 \pm 0.04	0.39 \pm 0.04	0.37 \pm 0.05	0.36 \pm 0.04	0.61 \pm 0.06	0.98 \pm 0.23	1.07 \pm 0.23	0.66 \pm 0.05	1.11 \pm 0.28
<i>S. aureus</i>	0.42 \pm 0.02	0.43 \pm 0.02	0.51 \pm 0.02	0.38 \pm 0.02	0.48 \pm 0.05	0.74 \pm 0.10	0.88 \pm 0.05	0.87 \pm 0.09	0.85 \pm 0.24	0.82 \pm 0.12

^a Data represent the pooled regression analysis of two inactivation studies at each temperature, with 9 degrees of freedom. The D_{10} -values are the reciprocal of the slope of the regression, and the standard deviation was calculated from the standard error for the regression.

terms on the survival of *S. aureus* were significant ($P < 0.05$). The D_{10} -values for *S. aureus* between 20 and -76°C are presented in Table 1. Many of the D_{10} -values for the inactivation of *S. aureus* at a given temperature, such as at 0°C , differed significantly ($P < 0.05$) from values obtained at other temperatures (Table 1).

Over the narrow temperature range of 0 to -20°C , the inactivation of *E. coli* O157:H7 and of *S. aureus* in ground beef followed Arrhenius kinetics. The regression of the log(base 10) of the D_{10} -values for *E. coli* O157:H7 from 0 to -20°C is described by the following linear regression equation with an R^2 value of 0.83: ($\text{Log } D = -4.48 + 1,114T$, where $T = 1/[273.16 + ^{\circ}\text{C}]$, and the D_{10} -value is reported in kGy). The regression of the log(base 10) of the D_{10} -value for *S. aureus* from 0 to -20°C is described by the following linear regression equation with an R^2 value of 0.78: ($\text{Log } D = -4.83 + 1,214T$, where $T = 1/[273.16 + ^{\circ}\text{C}]$, and the D_{10} -value is reported in kGy).

The increased survival of both *E. coli* O157:H7 and *S. aureus* at reduced temperatures could produce significant inactivation errors if inappropriate D_{10} -values are used or if there are significant variations of the actual temperature within the product. Thayer and Boyd (8) found that the inactivation of *Listeria monocytogenes* by gamma radiation between 5 and -20°C followed Arrhenius kinetics, with the D_{10} -value increasing from 0.44 to 1.21 kGy. In this study, the D_{10} -values for the inactivation of *E. coli* O157:H7 at -12 and -20°C were significantly different; how-

ever, the D_{10} -values for -20 , -30 , -40 , and -76°C did not differ. We conclude that predictions of the inactivation of foodborne pathogens in frozen products should be based on ionizing-radiation D_{10} -values at the lowest temperature to which the food will be exposed during irradiation.

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