

# Reduction of *Salmonella* spp. and Strains of *Escherichia coli* O157:H7 by Gamma Radiation of Inoculated Sprouts†

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## ABSTRACT

There have been several recent outbreaks of salmonellosis and infections with *Escherichia coli* O157:H7 linked to the consumption of raw sprouts. Use of ionizing radiation was investigated as a means to reduce or to totally inactivate these pathogens, if present, on the sprouts. The radiation *D* value, which is the amount of irradiation in kilograys for a 1-log reduction in cell numbers, for these pathogens was established using a minimum of five doses at  $19 \pm 1^\circ\text{C}$ . Before inoculation, the sprouts were irradiated to 6 kGy to remove the background microflora. The sprouts were inoculated either with *Salmonella* spp. cocktails made with either meat or vegetable isolates or with *E. coli* O157:H7 cocktails made with either meat or vegetable isolates. The radiation *D* values for the *Salmonella* spp. cocktails on sprouts were 0.54 and 0.46 kGy, respectively, for the meat and vegetable isolates. The radiation *D* values for the *E. coli* O157:H7 cocktails on sprouts were 0.34 and 0.30 kGy, respectively, for the meat and vegetable isolates. *Salmonella* was not detected by enrichment culture on sprouts grown from alfalfa seeds naturally contaminated with *Salmonella* after the sprouts were irradiated to a dose of 0.5 kGy or greater. Ionizing radiation is a process that can be used to reduce the population of pathogens on sprouts.

The nutritional content of dry seeds is increased after germination of the seed and growth of the sprout (15). In recent years, this ancient process of sprouting has become popular worldwide to produce a variety of vegetable items. The seedlings or sprouts are grown by commercial producers for sale to retail stores and restaurants or by the consumer in the home. The sprouts, grown hydroponically, are usually eaten raw in salads or on sandwiches but can be cooked, as in oriental-style meals (15).

Seed sprouts taken for analysis can have a high bacterial count, including the possibility of pathogens (16). Some foodborne pathogens isolated from various sprouts are *Salmonella* spp., *Escherichia coli* O157:H7, *Bacillus cereus*, and *Staphylococcus aureus* (16). Other potential foodborne pathogens isolated from sprouts are *Klebsiella pneumoniae* and *Aeromonas hydrophilia* (5, 16).

Since 1988, the number of reported fresh produce, including sproutborne, outbreaks has steadily increased, and the outbreaks have become international (16). The National Advisory Committee on Microbiological Criteria for Foods has issued reports on the microbiological safety evaluation and recommendations on fresh produce and on sprouted seeds. These reports reviewed the history of sprout-related outbreaks, from the first *B. cereus* outbreak in 1973 to the outbreaks related to *E. coli* O157:H7 in 1998. The contamination of fresh produce could occur from any combination of the following: contaminated seeds, contaminated equip-

ment, contaminated water source, or poor hygienic handling (16, 17). With sprouts, the seeds were considered the most likely source of the pathogen. After germinating either artificially contaminated radish seeds or the water used for sprouting with *E. coli* O157:H7, the pathogen was isolated from the sprouts in both procedures (12).

Several unsuccessful methods were tried to decontaminate fresh produce, including raw sprouts, of the pathogenic bacterial population. Park et al. (19) indicated that buffered sodium hypochlorite solution, Bionox, was effective in reducing *Salmonella* by 2 and 3 log/g on vegetables and fruits, respectively, and reported on the chemical analysis of the produce. Further studies are needed to determine the effect of this disinfectant on the organoleptic properties of the produce. To date, there are no chemical or water rinse treatments effective in decontaminating the fresh fruit or vegetable that result in an edible raw product (16). Taormina and Beuchat (22) were able to reduce the population of *E. coli* O157:H7 in the germinated seeds but could not eliminate the pathogen on the sprouts. Since there is no way to guarantee pathogen-free raw sprouts, in 1998 the California Department of Health Services (4) issued an advisory for high-risk individuals on the consumption of raw sprouts after several alfalfa sprout-related outbreaks occurred. In 1999, the U.S. Department of Health and Human Services issued a warning regarding the hazards of eating raw sprouts (26).

It is difficult to clean the surface of fresh produce, since the pathogen may adhere to the surface, and excessive processing may even increase the risk of bacterial growth due to the release of nutrients (16). Currently, there is no way to eliminate the pathogen on fresh sprouts. The purpose of

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this research was to determine whether ionization radiation could be used to decontaminate the sprouts by determining the radiation *D* value, which is the amount of irradiation in kilograys for a 1-log reduction in cell numbers, for *E. coli* O157:H7 strains and *Salmonella* serotypes isolated from vegetable and meat foodborne outbreaks.

## MATERIALS AND METHODS

**Substrate.** The sprouts (radish, alfalfa, and broccoli) were purchased from Windy Hollow Farms, Wagontown, Pa., and arrived within 12 h after harvest. They were shipped at ambient temperature and were refrigerated upon arrival in the laboratory. The sprouts were repackaged into plastic bags (Freshstuff, American National Can Co., Neenah, Wis.; oxygen transmission = 0.6 to 0.8 cm<sup>3</sup> per 254 cm<sup>2</sup> per 24 h at 22.8°C and 50% relative humidity, water vapor transmission = 0.3 to 0.4 g per 254 cm<sup>2</sup> per 24 h at 37.8°C and 90% relative humidity). The bags contained approximately 1 kg of sprouts each and were irradiated to a dose of 6 kGy at 19 ± 1°C to remove background microflora. The irradiated samples were kept refrigerated at 8°C until used.

**Cultures.** *Salmonella enterica* Dublin ATCC 15480, *S. enterica* Enteritidis ATCC 13076, *S. enterica* Newport ATCC 6962, *S. enterica* Senftenberg ATCC 8400, *S. enterica* Typhimurium ATCC 14028, *E. coli* O157:H7 ATCC 35150, *E. coli* O157:H7 ATCC 43889, and *E. coli* O157:H7 ATCC 43894 were obtained from the American Type Culture Collection (Rockville, Md.). *E. coli* O157:H7 93-437 was obtained from the Oregon Public Health Laboratory, Portland, Oreg., and *E. coli* O157:H7 ENT C9490 was obtained from the Centers for Disease Control and Prevention, Atlanta, Ga. The seven vegetable-related isolates were obtained from Dr. William Fett, U.S. Department of Agriculture, Agricultural Research Service, Wyndmoor, Pa. (*E. coli* O157:H7 F4546, *E. coli* O157:H7 SEA13B88, *E. coli* O157:H7 C7927, *S. enterica* Avatum F4317, *S. enterica* Stanley H0558, *S. enterica* Newport H1275, and *S. enterica* Infantis F4319).

Culture identity and purity were verified with Gram stains and reactions on the GNI card (Gram-Negative) of Vitek AMS Automicrobic System (bioMérieux Vitek, Inc., Hazelwood, Mo.) (1, 14). All cultures were maintained on tryptic soy agar (Difco Laboratories, Detroit, Mich.) slants at 4°C. Prior to use, tryptic soy broth (Difco) cultures were prepared and were kept at 4°C. The day prior to the inoculation, each isolate was cultured separately in 100 ml of tryptic soy broth in a 500-ml baffled flask with agitation at 150 rpm on a rotary shaker for 18 h at 37 ± 1°C. The cultures were harvested by centrifugation, were resuspended in buffered peptone water (Difco), and were combined in equal volumes to make a 400-ml inoculum cocktail.

**Inoculation of sprouts for determination of radiation *D* values.** Aseptic techniques were used throughout the inoculation procedure. The irradiated sprouts (approximately 1 kg) were placed in a tray, and the 400-ml pathogen cocktail was added and gently mixed for 1 min. The sprouts were placed in a colander to drain the excess liquid, and approximately 25-g samples were placed in polyester/polyethylene filter stomacher bags (Spiral Biotech, Inc., Bethesda, Md.). Three samples for each dose level were prepared.

**Determination of radiation *D* values and radiation source.** The inoculated sprout samples received absorbed radiation doses of 0, 0.27, 0.54, 0.79, 1.06, 1.31, 1.58, 1.84, 2.07, 2.36, and 2.62 kGy at 19 ± 1°C. The mean deviation of the absorbed dose from the target dose was 0.048 kGy, with a standard error of 0.005

kGy. Each study included three samples per dose and was repeated twice. A <sup>137</sup>Cs self-contained gamma radiation source (Lockheed Georgia Company), with a strength of approximately 109,159 Ci and a dose rate of 0.105 kGy min<sup>-1</sup>, was used for this study. The dose rate was established using National Institute of Standards and Technology (Gaithersburg, Md.) alanine transfer dosimeters. Variations in doses absorbed by experimental samples were minimized by placement within a uniform area of the radiation field. The actual dose was verified with alanine dosimeters using an electron paramagnetic resonance analyzer (EMS 104 EPR, Bruker, Rheinstetten, Germany). The sample temperature was monitored continuously during irradiation and was maintained by injecting the gas phase from liquid nitrogen into the irradiation chamber.

**Microbiological analysis for the radiation *D*-value determination.** The uninoculated irradiated sprouts were assayed along with the samples from the radiation *D*-value determination. The samples were diluted with buffered peptone water and were stomached for 1 min using a Stomacher 400 (Tekmar Co., Cincinnati, Ohio). After serial dilution in 0.1% peptone water (Difco), the samples were plated on tryptic soy agar by using an automatic spiral plater (Autoplate 4000, Spiral Biotech). The plates were incubated for 24 h at 35 ± 1°C before the number of CFUs was determined with a spiral laser colony scanner (Model 500A, Spiral Biotech).

**Microbiological analysis of irradiated sprouts grown from naturally contaminated seeds.** Alfalfa seeds, naturally contaminated with *S. enterica* Mbandaka, were obtained from K and F Seeds (Division of Fefield Land Co., Inc., Brawley, Calif.). Using aseptic techniques, 25 g of seeds was sprouted and mixed before packaging in filter stomacher bags. The sprouts were irradiated at 19 ± 1°C with a radiation dose ranging from 0 to 3.0 kGy in increments of 0.5 kGy. Total plate counts using tryptic soy agar and *Salmonella* determinations were performed. In addition, seeds were irradiated with a dose of 2 kGy at 20°C and were sprouted, and *Salmonella* determinations were performed on the sprouts. Unirradiated seeds, seeds irradiated to 2 kGy, and the sprout water from each germination tray were analyzed for *Salmonella*.

After a 1:10 dilution with buffered peptone water of the sprouts and seeds, the sprout and seed samples and the sprout water were incubated in Rappaport-Vassiliadis broth (Difco) at 37°C and tetrathionate broth (Difco) at 42°C and then were streaked on both XLT4 (Difco) and brilliant green (Difco) agars. An overnight enrichment step at 37°C for all samples was also performed before the selective enrichment and streaking on the agars.

**Statistical analysis.** For analysis, the average surviving CFU per gram was divided by the average of the six zero-dose values to give a survivor value. Subsequent calculations used the log of the survivor values. To avoid possible shoulder effects, the zero-dose values were not used, and a minimum of five values in the linear portion of the inactivation curves was used to calculate each regression (20). The results from the two independent replicate studies were pooled, and the slope of the inactivation curve was determined by least square analysis. The radiation *D* values were calculated from the slopes. Statistical calculations were performed using the general linear models procedure of the SAS statistical package (20). The regressions were tested for significant differences by analysis of covariance.

## RESULTS AND DISCUSSION

To date, there are no reports for the effect of radiation on the survival of the pathogens used in this study on raw

TABLE 1. Radiation *D* value (kGy) of *E. coli* O157:H7 and *Salmonella* spp. cocktail on sprouts

| Sprout                | Microorganism          | Meat isolates            | Vegetable isolates       |
|-----------------------|------------------------|--------------------------|--------------------------|
| Radish <sup>a</sup>   | <i>E. coli</i> O157:H7 | 0.34 ± 0.01 <sup>b</sup> | 0.30 ± 0.02 <sup>b</sup> |
| Alfalfa <sup>c</sup>  |                        | 0.27 ± 0.01              |                          |
| Broccoli <sup>c</sup> |                        | 0.26 ± 0.01              |                          |
| Radish <sup>a</sup>   | <i>Salmonella</i>      | 0.54 ± 0.02 <sup>b</sup> | 0.46 ± 0.02 <sup>b</sup> |

<sup>a</sup> Results of two trials.

<sup>b</sup> There was a significant difference ( $P > 0.05$ ).

<sup>c</sup> Results of one trial.

sprouts. The alfalfa, broccoli, and radish sprouts were inoculated with the cocktail of the meat isolates of *E. coli* O157:H7. During the handling of the inoculated alfalfa and broccoli sprouts, the contaminated seed coats were difficult to contain. The results of the radiation *D*-value determinations were the same for both alfalfa and broccoli (0.27 ± 0.02 kGy) and were within the range found for the radish (Table 1). Therefore, based on these results, the remainder of the study was done using only radish sprouts.

The radiation *D* values in kilograys for the four pathogen cocktails inoculated on raw radish sprouts are listed in Table 1. There was a significant difference in radiation *D* values between the *E. coli* O157:H7 and the *Salmonella* spp. meat and vegetable isolates. However, from a real-world point of view, they would not be considered different. The average *D* value for all of the determinations for both *E. coli* O157:H7 and *Salmonella* spp. (Figs. 1 and 2) is consistent with those results reported earlier by Thayer et al. (23, 24). They reported an average of 0.53 ± 0.02 kGy for the meat isolates of the *Salmonella* spp. on chicken, turkey, beef, pork, lamb, bison, ostrich, alligator, and caiman meat (23–25). Thayer et al. (24) reported that the radiation *D* values obtained for *E. coli* O157:H7 suspended

in meat ranged from 0.29 ± 0.04 kGy to 0.32 ± 0.02 kGy, which is consistent with the results from this study.

*Salmonella* was not detected by enrichment culture on sprouts grown from alfalfa seeds naturally contaminated with salmonellae after the sprouts were irradiated to a dose of 0.5 kGy or greater (Table 2). We confirmed that these alfalfa seeds were positive for *Salmonella* by enrichment culture of the seed, by cultural examination of the sprouts grown from the seeds, and by culture of the water in which the seeds were sprouted (Table 2). Further studies are needed to determine the effect of irradiation on controlling the pathogen on the seeds. The initial microbial population on the control sprouts using tryptic soy agar plates was >9 log CFU/g, and only a 4-log reduction was observed. These results were consistent with the dose required to sterilize the radish sprouts for the radiation *D*-value determinations. There was a mixture of colony morphologies observed on these plates, but no identification was done. The water remaining in the trays after growing the sprouts for 4 days was positive for *Salmonella*.

Ionizing radiation to a dose of 1 kGy is an approved process to prevent the sprouting of tubers and for insect deinfestation (2). Drake and Neven (7) showed that low-dose electron-beam irradiation (<0.6 kGy) was effective for pest control and did not affect the quality of sweet cherries. The use of ionizing radiation was reported to increase the keeping quality and to reduce *Listeria monocytogenes* on pre-cut vegetables with a dose of 1 kGy with no loss of vegetable quality (9, 10). Kader (13) reported that the potential use of ionizing radiation on fresh fruits and vegetables to control fungal and bacterial diseases depends both on the organism and the type of plant tissue. We observed that the radiation *D* value on the radish sprouts for both the *E. coli* O157:H7 strains and the *Salmonella* serotypes used were similar to previously reported values and that the radish sprouts used for the radiation *D*-value determinations

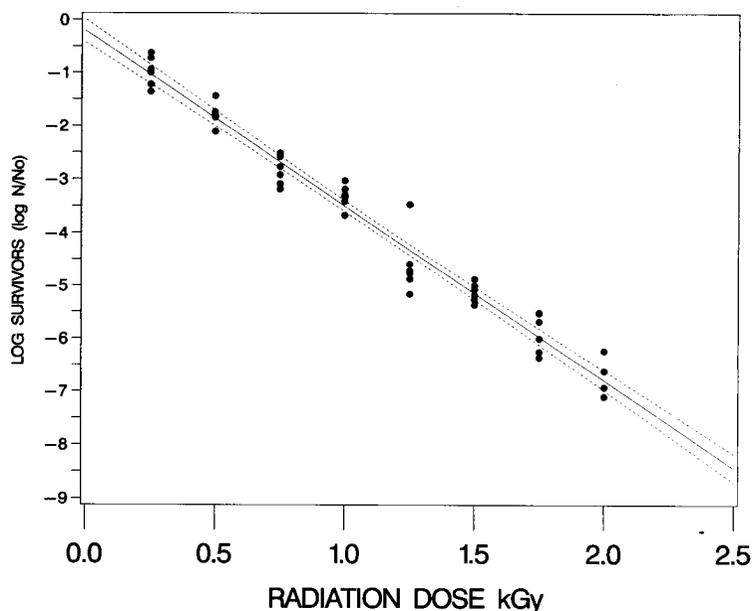


FIGURE 1. Survival of a mixture of *E. coli* O157:H7 F4546, *E. coli* O157:H7 SEA13B88, and *E. coli* O157:H7 C7927 on raw radish sprouts after gamma irradiation.

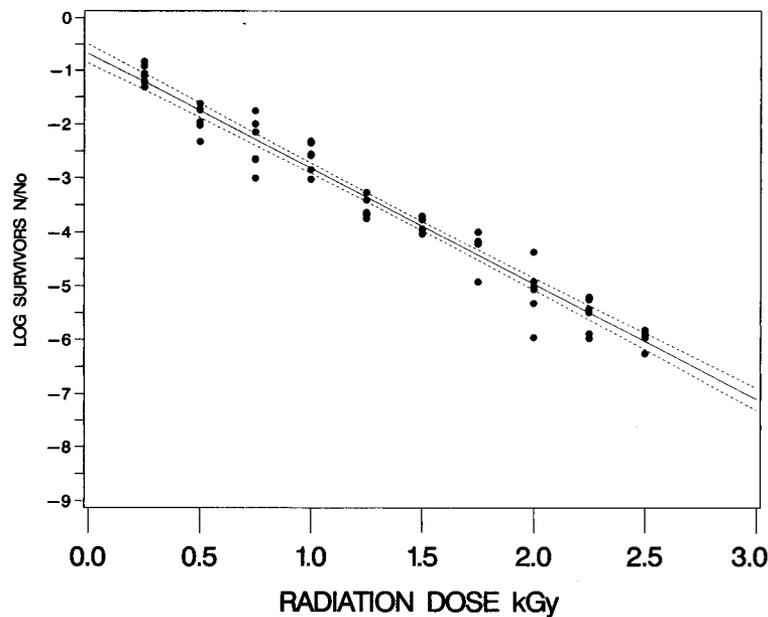


FIGURE 2. Survival of a mixture of *Salmonella Avatum*, *Salmonella Stanley*, *Salmonella Newport*, and *Salmonella Infantis* on raw radish sprouts after gamma irradiation.

maintained their structure after being sterilized and reirradiated. Further research is necessary to determine the effect of ionizing radiation on the structure and keeping quality of the various sprouts.

Cut raw vegetables have been found to have microbial contamination both on the surface and within the plant tissue (11, 16). The effectiveness of various wash solutions were evaluated for their ability to remove or reduce the bacterial populations. The effectiveness of washing with water, calcinated calcium, organic acids, and chlorine was studied (3, 6, 8, 18, 21). None of these treatments was able to produce an edible, pathogen-free product at the treatment levels used. The commercial use of low-level ionizing radiation (<2 kGy) to control pathogenic microorganisms on raw produce, like sprouts, is a possibility. The shelf life of bean sprouts was improved by modified atmosphere packaging (27). Further studies are needed to determine whether combinations of treatments using ionizing radiation would

be effective in controlling pathogens. Such studies would include determining the effect of combining an effective wash and low-dose ionizing radiation on the produce packaged under modified atmosphere to control pathogens on fresh fruits and vegetables. In addition, the effect of low-dose ionizing radiation on the nutrient content of the fresh fruits and vegetables needs to be studied.

Based on the results that no detectable *Salmonella* was found on the sprouts grown from the naturally contaminated seeds following irradiation and that the radiation *D* values for *Salmonella* spp. and *E. coli* O157:H7 were similar to previously reported values on moist products, irradiation is a process that can be used to inactivate foodborne pathogens on sprouts.

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TABLE 2. Population of aerobic microorganisms and the presence of *Salmonella* following irradiation of the sprouts grown from naturally contaminated alfalfa seeds

|              | Dose (kGy) | Population (CFU/g) <sup>a</sup> | <i>Salmonella</i> <sup>b</sup> |
|--------------|------------|---------------------------------|--------------------------------|
| Sprouts      | 0          | 9.45                            | +                              |
|              | 0.5        | 8.30                            | -                              |
|              | 1.0        | 7.23                            | -                              |
|              | 1.5        | 6.37                            | -                              |
|              | 2.0        | 5.89                            | -                              |
|              | 2.5        | 5.44                            | -                              |
|              | 3.0        | 5.27                            | -                              |
| Sprout water | 0          | 9.40                            | + <sup>c</sup>                 |

<sup>a</sup> Total plate counts reported as log CFU/g.

<sup>b</sup> Enrichment test for presence of *Salmonella*.

<sup>c</sup> Two positive out of four trials.

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