Research Note

Use of Electron Beam Radiation for the Reduction of *Salmonella enterica* Serovars Typhimurium and Tennessee in Peanut Butter

A. L. HVIZDZAK, S. BEAMER, J. JACZYNSKI, AND K. E. MATAK*

Animal and Nutritional Sciences, West Virginia University, Morgantown, West Virginia 26506, USA

MS 09-291: Received 7 July 2009/Accepted 2 October 2009

**ABSTRACT**

Peanut butter and peanut paste products were implicated as the vehicle of contamination in an outbreak of *Salmonella Typhimurium*, which began in September 2008, and in the November 2006 outbreak of *Salmonella Tennessee*. Therefore, this study evaluated the effectiveness of electron beam (e-beam) radiation for the reduction of *Salmonella* serovars Tennessee (ATCC 10722) and Typhimurium (ATCC 14028) in creamy peanut butter. Each strain was studied independently. Peanut butter samples were inoculated with approximately 8.0 log CFU/g of *Salmonella*, and exposed to e-beam doses ranging from 0 to 3.1 kGy. Doses were confirmed with film dosimetry. Survivors were enumerated by standard spread plating on nonselective tryptic soy agar (TSA) and selective xylose-lysine-desoxycholate agar (XLD) media. *Salmonella Tennessee* was more susceptible to e-beam radiation, with 5.00- and 6.75-log reduction of cells on TSA and XLD, respectively, at the approximate e-beam dose of 3.0 kGy. *Salmonella Typhimurium* was reduced by 4.19 and 4.85 log on TSA and XLD, respectively, at the approximate e-beam dose of 3.0 kGy. *D₀*-values show that *Salmonella Typhimurium* was more resistant (0.82 ± 0.02 and 0.73 ± 0.01 kGy on TSA and XLD, respectively) than was *Salmonella Tennessee* (0.72 ± 0.02 and 0.60 ± 0.01 kGy on TSA and XLD, respectively) to e-beam radiation (P < 0.05). The recovery on growth and selective media were different (P < 0.05), indicating cell injury. The results of this study demonstrate that e-beam radiation may be an effective processing step for the nonthermal inactivation of *Salmonella* in peanut butter.

Recent salmonellosis outbreaks that implicate peanut butter prove to be unique, since peanut butter provides an environment in which organisms are able to survive because of the high fat (up to 55%) and low water activity (a₀), which can range from 0.17 to 0.50 (3, 12, 15). Burnett et al. (3) found that *Salmonella* survives in peanut butter and peanut butter spreads for at least 24 weeks, and believe that it is likely to survive for the duration of their expected shelf lives (3). The production of peanut butter begins with peanuts harvested from the farm and shipped to a manufacturing facility, where they are shelled and cleaned. They are then roasted in ovens at temperatures around 180°C for 50 min, which should be adequate to kill *Salmonella* (25). When roasting is complete, the peanuts are blanched, ground into a paste, and mixed with other ingredients such as salt, oil stabilizers, and/or sugar. Prior to packaging, peanut butter typically undergoes another heat treatment at temperatures between 70 and 75°C; however, *Salmonella* cells are heat resistant in high-fat and low-a₀ environments, and are able to survive in peanut butter at temperatures as high as 90°C (15). Microbial reductions are not improved by longer treatment; in fact, higher temperatures and longer exposure times would decrease the quality of the peanut butter by eliciting taste and texture changes (10, 15). The recent *Salmonella* outbreaks associated with peanut butter and peanut butter products highlight the need for better process controls. Without a new method of effective microbial reduction, peanut butter and peanut butter products will continue to be a food safety concern.

Electron beam (e-beam) irradiation is a process by which food products are exposed to ionizing radiation to destroy microorganisms, viruses, or insects. Consumer safety of irradiated foods has been studied for many years, with no documented adverse health effects being seen (11). It is approved by the U.S. Food and Drug Administration for use on meat, poultry, spices, fresh fruits, and vegetables (23). E-beam radiation is generally characterized by its low penetration and high dosage rates; the beam is a concentrated, highly charged stream of electrons generated by the acceleration and conversion of electricity. The main assets of this technology include high lethality, fewer detrimental effects on food quality as compared with thermal methods, and it does not require the use of radioactive isotopes to generate gamma rays.

The mechanism of inactivation for microorganisms by e-beam radiation is believed to be due to the direct interaction of the radiation with cell components and to the indirect action from free radicals generated by water radiolysis (1, 22). Approximately 50 to 70% of the bacterial cell mass is composed of water; therefore, when bacteria are present in the food being irradiated, the energy from the e-
beam radiation is transferred to the water and the other molecules within the organism. As a result, hydroxyl radicals and hydrated low-energy electrons are produced, and are responsible for DNA damage and defects in the genetic instructions (8). Unless the cell is capable of repair, it will die due to reproductive death (22). Therefore, the direct effect of e-beam on microbial inactivation is independent of the medium in which it is suspended. Water subjected to ionizing radiation undergoes a radiolysis reaction in which water molecules are split and free radicals are generated. The surface of the cell is damaged by these free radicals, causing leakage from cell membranes and ultimately death (1). Therefore, the indirect effect of e-beam radiation on microbial inactivation is dependent on water availability in a product, which may influence the effectiveness of this technology to reduce pathogens in low-water foods like in peanut butter.

The effectiveness of e-beam radiation as a nonthermal process for the reduction of pathogenic bacteria in peanut butter has not been reported; therefore, the objective of this research was to investigate the efficacy of e-beam for reduction of *Salmonella enterica* subsp. *enterica* serotypes Tennessee and Typhimurium in peanut butter.

**MATERIALS AND METHODS**

**Bacterial cultures.** *Salmonella Typhimurium* ATCC 14028 and *Salmonella* Tennessee ATCC 10722 were revived in tryptic soy broth (TSB; unless otherwise stated, all media were from Difco, Becton Dickinson, Sparks, MD), incubated at 37°C for 18 to 24 h and twice transferred. Working stocks of these cultures were spread onto sterile slants of tryptic soy agar (TSA), incubated at 37°C for 18 to 24 h, and stored at 4°C.

**Inoculum preparation.** Each strain of *Salmonella* was twice transferred into 100 ml of TSB and incubated at 37°C for 24 h in a rotating incubator (Classic C24, New Brunswick Scientific Co., Inc., Edison, NJ) at 150 rpm. Cells were harvested by centrifugation twice at 10,000 × g for 10 min at 5°C (Sorvall RC-SB refrigerated super-speed centrifuge, Du Pont, Wilmington, DE). After the first centrifugation, the supernatant was poured off. The cells were washed with 100 ml of ddH₂O, and then recentrifuged with the same procedure. The supernatant was poured off, and the remaining cells were used to inoculate the peanut butter samples.

**Peanut butter preparation.** A commercial brand of creamy peanut butter was purchased at a local grocery store (Morgantown, WV). The composition of the peanut butter included (in the order listed on the product label) roasted peanuts, sugar, hydrogenated vegetable oils, salt, and partially hydrogenated cottonseed oil. aw (=0.51) was confirmed with a bench top humidity temperature indicator (model Hygrolab 3, Rotronic Instrument Corp., Huntington, NY); pH (6.28) was confirmed with an Oakton pH 11 series handheld pH meter (Oakton Instruments, Vernon Hills, IL). All equipment used to handle the peanut butter was sanitized by spraying with 70% ethanol and by drying under UV light (254 nm). The inocula of *Salmonella* were placed into separate, labeled, large stomacher bags (Fisherbrand, Hampton, NH), each containing 375 g of peanut butter, and then pummeled by hand for 1 min to ensure even distribution of the culture. The target initial inoculum level for each strain was 10⁶ CFU/g. The inoculated peanut butter was further divided into 10-g aliquots, placed into SealPAK bags (Kapak Corp., Minneapolis, MN), and labeled to receive one of six different e-beam radiation doses: 0 (control), 0.5, 1, 1.5, 2, and 2.5 kGy. The sample bags were individually sealed and placed into a larger SealPAK bag to reduce the possibility of leakage or contamination. The peanut butter was spread smoothly (~1 mm thick) in the bag to promote even e-beam absorption. The bags were packed in Ziploc plastic containers (9.5 cups [2.25 liters]; SC Johnson Products, Racine, WI) and stored at 22°C in a rotating incubator (Classic C24, New Brunswick Scientific Co., Inc.) until transportation to the irradiation facility. Samples were maintained at ambient temperatures for the duration of the study, as per manufacturer’s recommendations for optimum product quality. Preliminary studies (data not shown) showed no significant microbial reductions for either strain in inoculated control samples maintained at this temperature for the length of the study.

**E-beam radiation.** Samples were shipped overnight to an e-beam processing facility (Sterigenics International, San Diego, CA). At the irradiation facility, samples were maintained at room temperature (20 to 24°C) until treatment with one-sided e-beam radiation, with energy fixed at 10 MeV. The following target doses were applied: 0 (control), 0.5, 1, 1.5, 2, and 2.5 kGy. The actual absorbed doses were confirmed with film dosimetry (FWT-60 series radiochromatic dosimeters, Far West Technology, Inc., Goleta, CA). On conclusion of e-beam treatment, the samples were repacked and shipped overnight at room temperature. Once back at West Virginia University, samples were placed and stored in an incubator at 22°C. The total time from inoculation of the samples until commencement of microbial analyses was 6 days (144 h). Microbial survival over this time was confirmed, with no significant (P > 0.05) reduction in control samples.

**Microbiological analysis.** Microbial analyses commenced on day 2 after e-beam exposure (day 0). Samples were serially diluted in 0.1% buffered peptone water, and 0.1-ml aliquots of the appropriate dilution were spread plated in duplicate onto TSA and xylose-lysine-deoxycholate (XLD; Remel, Lenexa, KS) agars, and then incubated for 18 to 24 h at 35°C. Nonselective medium (TSA) was used to facilitate repair of injured cells for comparison of cells recovered on selective medium (XLD). Discrete *Salmonella* colonies were counted. Preliminary studies (data not shown) determined background flora to be fewer than 100 CFU/g, and *Salmonella* was not detected on XLD (detection limit was <10² CFU/g).

**Statistical analysis.** The experiment was replicated three separate times. All microbiological analyses were performed in duplicate. Microbial counts (expressed in CFU per gram) were converted to logarithmic values, and then analyzed by linear regression by using Microsoft Office Excel software (Microsoft Corp., Redmond, WA). The D₁₀-value was calculated with the following equation: \( \log(N/N_0) = -1/D \times t \), where \( N \) is the number of survivors at the particular e-beam dose, \( N_0 \) is the initial microbial concentration, \( D \) is the \( D_{10} \)-value (decimal reduction dose), and \( t \) is radiation dose (7). One-way analysis of variance and Tukey’s honestly significant difference test were used to determine differences (P < 0.05).

**RESULTS AND DISCUSSION**

The effectiveness of e-beam irradiation as a nonthermal process for the reduction of *Salmonella Typhimurium* and *Salmonella* Tennessee in peanut butter was tested, and the results are presented in Table 1. Significant (P < 0.05) microbial reductions were seen after 0.5-kGy e-beam doses. Calculated \( D_{10} \)-values in Table 2 show that *Salmonella*
Salmonella Survival in E-Beamed Peanut Butter

Typhimurium was more resistant than was Salmonella Tennessee to e-beam radiation ($P < 0.05$). $D_{10}$-values were significantly ($P < 0.05$) different between Salmonella serovars, and between cells recovered on selective and nonselective media.

The $a_w$ of the peanut butter used in this study was relatively low (0.51), and the fat content relatively high (53%). This likely had an effect on $D_{10}$-values because cell death due to the indirect effects of e-beam radiation would have been limited. For example, Black and Jaczynski (1) looked at the effect of product temperature on the reduction of Escherichia coli O157:H7 in meat by e-beam irradiation, and found that the frozen samples (−20°C) had greater $D_{10}$-values than had samples not frozen, regardless of temperature (4°C and 22°C). They concluded that the physical state of water (frozen or unfrozen) in a product has a major effect on microbial inactivation, due to water radiolysis (1). Thayer and Boyd (18) reported significantly greater $D_{10}$-values for both E. coli O157:H7 and Staphylococcus aureus in ground beef when irradiated at subfreezing temperatures. As the temperature of the frozen ground beef was incrementally reduced from 0 to −20°C, the inactivation of both pathogens followed Arrhenius kinetics (18). This is likely due to reduction of $a_w$ as temperatures are lowered past freezing. Thayer and others (20) reported $D_{10}$-values of a five-strain Salmonella cocktail in various meats, which ranged from 0.51 kGy in pork to 0.71 kGy in turkey meat; they hypothesized that the differences in responses were likely associated with differences in chemical or physical properties of the meats (20). In a separate study, Black and Jaczynski (2) showed that even a slight reduction (i.e., from 1.00 to 0.99) in unbound water from a food product would significantly increase the survival of E. coli. Therefore, it follows that the effectiveness of e-beam processing to reduce bacterial load would be greatly affected by the availability of unbound water, due to limitations of the indirect mechanism of bacterial inactivation.

When the radiation sensitivity of Salmonella was tested in low-water foods, $D_{10}$-values ranged from 0.81 kGy in alfalfa seeds (19) to 1.35 kGy on radish seeds (24). The radiation $D_{10}$-values for Salmonella in peanut butter were less than these values. When Rajkowski and others (13, 14) compared the radiation sensitivity of Salmonella and E. coli O157:H7 between sprouts and broccoli seeds, they found that the $D_{10}$-values for broccoli seeds were much greater (0.46 versus 1.10 kGy, respectively). They speculated that the much lower water content of the seeds, versus the fresh sprouts, had an effect on the radiation sensitivity of Salmonella, which resulted in greater $D_{10}$-values (13). In contrast, Thayer and others (21) reported $D_{10}$-values of Salmonella on alfalfa seeds (0.46 > $a_w$ > 0.52) to be 0.97 kGy, and offered that factors other than moisture content of the seeds may have had an influence on radiosensitivity. It is possible that the $D_{10}$-values generated by the aforementioned studies with seeds (broccoli, radish, and alfalfa) were greater than those reported on peanut butter because the pathogen was predominantly located on or around the seed scar (hilum) (18), which would make the $a_w$ inside the seed less of a factor.

Studies that directly compare the effect of e-beam radiation on the recovery of the two Salmonella serovars tested are limited. Our findings showed Salmonella Tennessee to be more sensitive to radiation than was Salmonella Typhimurium. This is in contrast to the findings by Sherry and others (16), who compared the inherent

### TABLE 1. Survival of Salmonella Tennessee and Salmonella Typhimurium in peanut butter plated on nonselective TSA and selective XLD

<table>
<thead>
<tr>
<th>E-beam dose (kGy)</th>
<th>No. observed</th>
<th>Salmonella Tennessee</th>
<th>Salmonella Typhimurium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TSA</td>
<td>XLD</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>8.20 ± 0.09 A</td>
<td>8.20 ± 0.09 A</td>
</tr>
<tr>
<td>0.5</td>
<td>3</td>
<td>5.43 ± 0.05 a</td>
<td>4.30 ± 0.11 a</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>4.19 ± 0.13 c</td>
<td>4.13 ± 0.07 b</td>
</tr>
<tr>
<td>1.5</td>
<td>2</td>
<td>4.42 ± 0.01 c</td>
<td>4.36 ± 0.08 b</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>4.37 ± 0.20 c</td>
<td>4.05 ± 0.38 b</td>
</tr>
<tr>
<td>2.5</td>
<td>3</td>
<td>3.62 ± 0.13 d</td>
<td>2.91 ± 1.05 bc</td>
</tr>
<tr>
<td>3.0</td>
<td>2</td>
<td>3.21 ± 0.01 e</td>
<td>1.45 ± 0.02 c</td>
</tr>
</tbody>
</table>

$^a$ Doses were confirmed with film dosimetry. Actual values were within ±0.25 kGy.

$^b$ Values are means ± standard deviations.

$^c$ Values designated with the same letter within a column are not significantly different ($P > 0.05$) as determined by Tukey’s honestly significant difference test.

$^d$ Value significantly ($P < 0.05$) different as determined by Tukey’s honestly significant difference test.

### TABLE 2. $D_{10}$-values of Salmonella serotypes Tennessee and Typhimurium in peanut butter exposed to e-beam radiation, based on confirmed e-beam doses

<table>
<thead>
<tr>
<th>Salmonella serotype</th>
<th>$D_{10}$-value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TSA</td>
</tr>
<tr>
<td>Tennessee</td>
<td>0.72 ± 0.02 A a</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>0.82 ± 0.02 B a</td>
</tr>
</tbody>
</table>

$^a$ Values with different capital letters within columns indicate significant ($P < 0.05$) differences in recovery between the two Salmonella serotypes. Values with different lowercase letters within rows indicate significant ($P < 0.05$) differences in recovery on selective versus nonselective media.
resistance of 40 S. enterica serovars to an applied radiation dose of 1.5 kGy at 20°C. Salmonella Tennessee was more resistant, with a <2-log reduction, than were each of the six strains of Salmonella Typhimurium tested, which had reductions between 2 and 3.25 log (16). In the aforementioned study, Salmonella cells were suspended in TSB plus 0.6% yeast extract and exposed to radiation during the stationary-growth phase; whereas in our study, a 24-h stationary-phase culture was used to inoculate peanut butter, yet irradiation was not conducted until ~72 h later. During this time, it is likely that cell adaptation to the stresses of the peanut butter environment occurred; the extent of cell adaptation may have influenced the response of each serotype to irradiation treatment. Adaptation and the development of microbial resistance to one environmental stressor enhance tolerance to other stressors (6). It is possible that Salmonella Typhimurium was better able to adapt to the stresses of the peanut butter environment and, in turn, rendered it more resistant than was Salmonella Tennessee to e-beam radiation.

There was a significant (P < 0.05) difference in recovery on nonselective and selective media. Nonselective media like TSA facilitates the recovery of injured cells because it contains vital nutrients required for bacterial growth; whereas selective media like XLD favors the recovery of noninjured cells, due to the inclusion of agents that ensure the survival of only cells with certain attributes. Since the D_{10} values for both Salmonella serovars were significantly greater on TSA than on XLD, this indicates that e-beam radiation caused significant cell injury. This is in agreement with the findings by Tesfai and others (17), who looked at the recovery of Salmonella Typhimurium in liquid whole egg after sublethal exposure to e-beam radiation. They found that not only was there significant cell injury, as shown by greater recovery on TSA, but also that injured cells that were able to repair and recover became more radiation resistant (17). In contrast to our findings, Sherry and others (16) did not see a difference in recovery of various S. enterica serovars on selective or growth media. This may have been due to differences in the composition of the media (TSA plus yeast extract [TSAYE] versus peanut butter) in which the bacteria were subjected to the e-beam treatment. The high a_{oe} of TSAYE would have favored production of free radicals, which would cause significant damage to the cell membrane and imminent death to infected cells. The low a_{oe} of peanut butter would have minimized the indirect effect of radiation, yet cells would have still sustained injury by the direct interaction of radiation with intracellular components such as genetic material. The source of radiation (gamma [^{60}Co] versus e-beam) may have had an effect on the recovery of injured cells; however, studies that directly compare the effect of the two radiation sources on the inactivation of bacteria are limited. It is possible that cell injury was more apparent with e-beam radiation, because the radiation dose is applied at a much faster rate than gamma radiation is (i.e., e-beam radiation’s rate is 10^3 to 10^5 Gy/s; gamma radiation’s rate is 0.01 to 1 Gy/s) (9). The effect of dose rate on cell injury and inactivation is an area that should be explored further.

Peanut butter and peanut butter products have been implicated as the vehicle of multistate outbreaks of Salmonella Tennessee and Typhimurium (4, 5). Current processing methods include heat treatment, yet peanut butter continues to harbor infectious Salmonella cells. Salmonella has been shown to survive in peanut butter for long periods. E-beam irradiation is a nonthermal technology that is effective for the reduction of Salmonella and Typhimurium in peanut butter. D_{10} values for Salmonella in peanut butter are greater than those values reported in other commodities. It is possible that the unique composition of peanut butter, such as a low a_{ue} and high fat content, could influence the sensitivity of Salmonella to irradiation. The increased radiation resistance could also be a result of adaptation of the cells to the peanut butter environment.

ACKNOWLEDGMENTS

We extend special thanks to Sterigenics International for allowing us to use their e-beam facility. Our appreciation extends to Richard C. Vallejo and Carl A. Zain of Sterigenics for invaluable technical expertise with e-beam.

REFERENCES

inoculated broccoli seeds and effects of irradiation on broccoli sprout keeping quality and seed viability. J. Food Prot. 66:760–766.


