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Anuradha Prakash

Chapman University, prakash@chapman.edu

Nicole Johnson

Chapman University

Denise Foley

Chapman University

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Comments

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Irradiation D Values of *Salmonella* spp. in Diced Tomatoes Dipped in 1% Calcium Chloride

ANURADHA PRAKASH,¹ NICOLE JOHNSON,² and DENISE FOLEY²

ABSTRACT

Outbreaks of salmonellosis have been associated with eating raw domestic tomatoes. In this study, we examined the efficiency of combined irradiation and a 1% calcium chloride dip to reduce the population of *Salmonella enterica* strains on diced tomatoes. Tomatoes were contaminated with nalidixic acid-resistant strains of *S. Hartford*, *S. Montevideo*, or a mixture of 5 strains (*S. Hartford*, *S. Montevideo*, *S. Poona*, *S. Michigan*, *S. Gaminara*). We irradiated tomatoes at various doses up to 0.9 kGy from an electron beam source to conduct a D-value study (decimal reduction time required to eliminate 90% of the organism). Surviving *Salmonella* populations were detected by standard and recovery plating methods. D-value results ranged from 0.26 to 0.39 kGy, indicating that a 5 log₁₀ CFU/g reduction in *Salmonella* spp. in diced tomatoes would require a dose of 1.3–1.95 kGy.

INTRODUCTION

AN ESTIMATED 1.5 MILLION CASES of foodborne infections and 500 deaths per year are linked to *Salmonella* alone (Guo et al., 2001). Exposure to 10⁵–10⁸ *Salmonella* organisms results in the colonization of the small and large intestine and leads to salmonellosis (Madigan and Martinko, 2005). Symptoms can arise 8–48 hours after exposure and range from mild gastroenteritis to severe septicemia (Madigan and Martinko, 2005).

Outbreaks of salmonellosis have been associated with eating raw domestic tomatoes (CCDR, 2005; Cummings et al., 2001; Hedberg et al., 1999). Contact with salmonellae-contaminated soil can lead to infiltration of the pathogen into the parenchyma and core of the fruit by means of the stem scar or damaged skin surfaces (Burnett and Beuchat, 2001; Guo et al., 2002). Zhuang et al. (1995) showed that *Salmonella* serotypes can grow well on chopped ripe tomatoes at 20–30°C, thus furthering the risk of con-

tracting salmonellosis associated with improperly handled tomatoes.

Currently, none of the chemical or physical treatments authorized by the United States regulatory agencies can reliably eliminate all pathogenic organisms (Guo et al., 2002). The Food and Drug Administration (FDA) has determined that tomatoes should be washed in water at least 5.5°C warmer than the fruit itself to decrease the risk of pathogen infiltration of the tissue (Venkitanarayanan et al., 2002). Studies have also confirmed the efficacy of wash water containing 200–400 ppm of chlorine in reducing *Salmonella* counts 2–3 log₁₀ CFU/g. In addition, a chlorine wash can help maintain firmness, regulate ethylene production, delay senescence, and decrease pectinesterase activity (Weissinger et al., 2000; Zhuang et al., 1995).

Irradiation combined with the chlorine rinse has the potential to reduce pathogen populations to a level safe for consumption. Sherry et al. (2004) proved that *Salmonella* serovars are sensitive to gamma irradiation at a dose of 0.56

Departments of ¹Physical Sciences and ²Biological Sciences, Chapman University, Orange, California.

kGy, which resulted in a 3 log₁₀ reduction of organisms. Bari et al. (2004) also concluded that irradiation at doses of 1.5 and 2.0 kGy were successful in reducing *Salmonella* and *Escherichia coli* populations on ready-to-eat radish and mung bean sprouts, without any adherent affects on the sensory qualities. Previously, our laboratory determined that treating tomatoes with a combination of a chlorine rinse and irradiation at 0.5 kGy proved more effective in reducing plate counts and maintaining the sensory quality of the fruit over chlorine treatment alone (Prakash and Foley, 2004). Magee et al. (2003) found that a 1% CaCl₂ dip could ameliorate the irradiation-induced softening of diced tomatoes without negatively affecting sensory characteristics. Schmidt et al. (2006) reported that electron beam irradiation of fresh-cut tomato cubes at 0.95 kGy reduced counts of *S. Montevideo* by 2.2 log₁₀ CFU/g and of *S. Agona* by 2.4 log₁₀.

The objective of this study was to determine the D values of various serotypes of *Salmonella* on fresh diced tomatoes when combining a CaCl₂ rinse treatment and electron beam irradiation.

MATERIALS AND METHODS

Table-ripe hothouse tomatoes obtained from a local retailer were diced into approximately 8 × 8 × 7 mm cubes and stored in clean resealable storage bags at 4°C until contamination.

Bacterial strains and inoculation

S. Hartford H0778 and *S. Gaminara* F2712 involved in a 1995 orange juice outbreak (Cook et al., 1998) were obtained from Joy Wells, PhD, Centers for Disease Control, Washington, DC. *S. Poona* serogroup G, *S. Michigan* serogroup J, and *S. Montevideo* serogroup C1 were all isolated from patients in outbreaks associated with cantaloupe (*Poona* and *Michigan*) or raw tomatoes (*Montevideo*). These three strains were generously provided by Larry Bechat, PhD of the University of Georgia. Nalidixic acid-resistant strains were obtained by subculturing the individual strains in tryptic soy

broth (TSB) containing increasing amounts of nalidixic acid. On the first day a colony growing on a tryptic soy agar (TSA) plate was chosen and cultured in 10 mL TSB with 10 ug/mL nalidixic acid. The following day a loopful of the culture was subcultured in TSB with 20 ug/mL nalidixic acid. The process continued until the culture was growing in media with 50 ug/ml nalidixic acid. To verify stability, the culture was then passed into TSB (one loopful into 10mL) and grown overnight at 37°C in a shaking incubator. This was repeated 10 times before the culture was again placed in TSB with 50ug/mL nalidixic acid. The resistant culture was checked for proper colony appearance on Hectoen enteric agar (HE) as well as TSA plates with 50ug/mL nalidixic acid; then a culture was frozen with 15% glycerol at -80°C until needed.

Two days prior to irradiation, overnight cultures of nalidixic acid-resistant *Salmonella* were inoculated into 45 mL of TSB with 50 ug/mL of nalidixic acid and incubated for 24 hours. The cultures were centrifuged at 3000 g for 15 minutes and resuspended in Butterfield's phosphate buffer. The cells were enumerated using a hemacytometer to obtain an estimate of cell density. The required amount of each inoculum was added to deionized water to obtain approximately equal concentrations of each strain for a final concentration of 10⁸ CFU/mL.

Tomatoes were either contaminated with two individual strains of *Salmonella*, *Montevideo* or *Hartford*, or a cocktail of *S. Poona*, *S. Hartford*, *S. Gaminara*, *S. Michigan*, and *S. Montevideo*. For each treatment, 800 g of tomatoes were contaminated under a biosafety hood by submerging samples in 500 mL of 10⁸/mL diluted inocula and gently mixing for three minutes before draining with a salad spinner (Oxo International, New York, NY). The samples were again stored at 4°C in resealable bags for one hour. After one hour, the 800 g of tomatoes were gently rinsed with 1 L of 1% CaCl₂ for one minute. After draining the tomatoes in a sanitized salad spinner, individual 15 g samples were placed in sterile homogenizer bags (Interscience, St. Nom, France), and sealed with clips (Interscience). The samples were stored at 4°C until the next day when they were transported to the irradiation facility.

D-value study

On the day of irradiation, tomatoes were placed in coolers with ice packs and transported to Sterigenics, Inc. in San Diego, a trip of approximately 90 minutes. Tomatoes were treated with electron beam irradiation using a 9.5 MeV single beam mode delivering product surface doses ranging from 0.3–0.9 kGy. Alanine pellets (Harwell Dosimeters Ltd, Oxfordshire, United Kingdom) were placed on dummy bags set up in a manner identical to the sample bags to calibrate the beam. The dose absorbed by the product was within 4.4% of the target dose for each run. Six 15 g samples of tomatoes in sealed homogenizer bags were placed into 3.8 L resealable plastic storage bags. The samples were arranged in a thin layer to receive the beam. Duplicate samples of each type of inoculation were irradiated at each target dose. Each run also included nonirradiated control samples. Samples were transported back to our institution and stored at 4°C. The D-value study was replicated three times.

Microbial analysis

A 1:10 dilution of all samples with Butterfield's phosphate buffer was obtained by using an automatic diluter (Dilumat3 mk2, AES Laboratoire, Combourge, France). The samples were homogenized for 90 seconds and serial dilutions were performed to reach the desired dilution. All samples were plated in duplicate on TSA containing 50 µg/mL nalidixic acid (TSAN) and TSAN with two 7–10 mL layers of basal yeast extract (TSAN-TAL) as a recovery method (Wu and Fung, 2001). Most samples were plated using a spiral plater (Whitley Automatic Spral Plater, dw Scientific, West Yorkshire, UK): however, samples irradiated at 0.9 kGy were plated using 0.2 mL of the 1:10 dilution and a standard spread plate methodology. TSAN plates were incubated for 24 hours at 37°C before enumeration of colonies. TSAN-TAL plates were incubated at 18°C for 18 hours and then at 37°C for another 18 hours.

Statistical analysis

For all contamination studies, TSAN-TAL recovery method data were used for analysis due

to higher (although not significant) microbial counts. D-value data was transformed using the equation $\log_{10} (N/N_0)$ where $N = \text{CFU/g}$ obtained at a given dose and $N_0 = \text{counts}$ obtained for the nonirradiated control. Tests for homogeneity of variance, linear regression lines for $\log_{10} (N/N_0)$ vs. dose, and 95% confidence intervals were calculated using SPSS 11.0 (SPSS, Chicago, IL).

RESULTS

The calculated D values ranged from 0.26 to 0.39 kGy (Fig. 1, Table 1). For all strains, the test of homogeneity of variance was not significant ($P > 0.05$). D values differ with the moisture content as well as the matrix of the given food (Kwakwa and Prakash, 2006) and can differ for different *Salmonella* strains in the same medium. For example, different strains of *Salmonella* tested in orange juice were found to have D values ranging from 0.35 to 0.71 kGy, depending on the strain (Niemira et al., 2001). The different strains and the cocktail of 5 strains tested here did not display as large a range in D values as the strains tested on the orange juice. In addition, our results correlate with those of Martins et al. (2004), who calculated the D value for *Salmonella* spp. on mini-

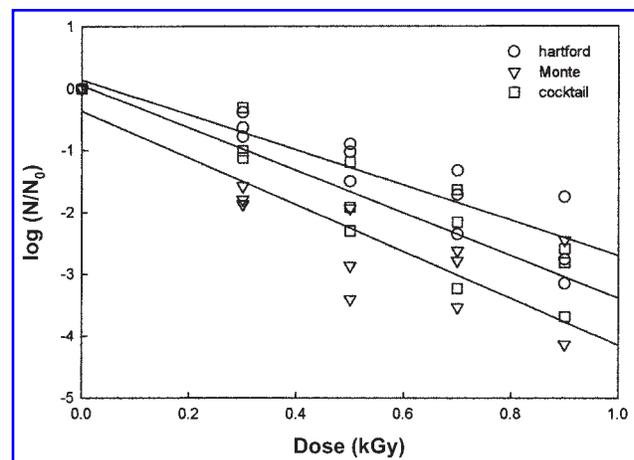


FIG. 1. D-value study linear regression models for irradiated *S. Hartford*, *S. Montevideo*, and cocktail of strains (*S. Hartford*, *S. Montevideo*, *S. Poona*, *S. Michigan*, *S. Gaminara*) plated on TSA containing 50 µg/mL nalidixic acid with two 7–10 mL layers of basal yeast extract (TSAN-TAL). Data points represent the average of duplicate samples.

TABLE 1. D VALUES, 95% CONFIDENCE INTERVALS (CI), AND R SQUARE (BEST FIT) VALUES FOR EACH *SALMONELLA* STRAIN TESTED

	<i>D value</i>	95% <i>CI</i>	<i>R square</i>
<i>S. Hartford</i>	0.39 kGy	0.33–0.47 kGy	0.925
<i>S. Montevideo</i>	0.26 kGy	0.21–0.35 kGy	0.816
Cocktail (<i>S. Hartford</i> , <i>S. Montevideo</i> , <i>S. Poona</i> , <i>S. Michigan</i> , <i>S. Gaminara</i>)	0.32 kGy	0.27–0.39 kGy	0.913

mally processed watercress to be between 0.29–0.43 kGy. Similarly, Palekar (2004) determined the D value for *Salmonella* Poona inoculated on cantaloupe surfaces to be 0.211 kGy. With a D value of 0.39 kGy, a 5 log₁₀ CFU/g reduction in *Salmonella* Hartford in diced tomatoes would require a dose of 1.95 kGy.

DISCUSSION

Nalidixic acid-resistant *Salmonella* strains were used to distinguish the specific strains used in this study from other bacteria that naturally occur on tomatoes. Recent work by Niemira and Lonczynski (2006) reported that resistance to nalidixic acid decreases the resistance of that same strain to ionizing radiation. Naturally resistant strains were more sensitive to radiation than naturally sensitive strains. Additionally, sensitive strains that were made resistant by successive culturing in progressively increasing concentrations of nalidixic acid were significantly more sensitive to irradiation than the parent strain, by 9% in buffer and 17% in orange juice. In buffer, the naturally resistant cultures had a pooled D value of 0.210 kGy, while the sensitive strains had a pooled value of 0.257 kGy. Induction of resistance in the sensitive strains caused the D values to decrease for 2 of the 3 strains tested in both buffer and orange juice and resulted in a pooled value of 0.234 kGy (Niemira and Lonczynski, 2006). Thus, it is possible that the D values reported in this paper are underestimated. However, the work by Schmidt et al. (2006) suggests that D values of approximately 0.39 and 0.43 kGy would be expected for nonadapted *S. Agona* and *S. Montevideo*, respectively. However, their values are approximations on a single dose. Since the food matrix appears to influ-

ence sensitivity of the strains to irradiation, it would be important to compare sensitivity of nalidixic acid-resistant and sensitive strains to irradiation in tomatoes. However, the technical aspects of performing this type of experiment are more difficult since, in our experience, background microflora interfere with *Salmonella* enumeration when using typical selective media such as Hectoen enteric or xylose lysine deoxycholate citrate (XLD). Sterilization of the tomato dices would be necessary prior to contamination and irradiation.

CONCLUSION

The D values for *Salmonella* spp. in diced tomatoes dipped in calcium ranged from 0.26 to 0.39 kGy, indicating that a 5 log₁₀ CFU/g reduction in *Salmonella* will require an irradiation dose of 1.3–1.95 kGy. Recent work by others indicates that nalidixic acid-resistant strains might be more sensitive to irradiation. More work needs to be done to compare the sensitivity of nalidixic acid-resistant and sensitive strains to irradiation.

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Address reprint requests to:
 Anuradha Prakash, PhD
 Department of Physical Sciences
 Chapman University
 One University Drive
 Orange, CA 92866

E-mail: prakash@chapman.edu

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