

Ultraviolet Light (254 nm) Inactivation of *Listeria monocytogenes* on Frankfurters That Contain Potassium Lactate and Sodium Diacetate

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ABSTRACT: *Listeria monocytogenes*, a psychrotrophic foodborne pathogen, is an occasional postprocess contaminant on ready-to-eat meat (RTE) products including frankfurters. Ultraviolet C light (UVC) is an FDA-approved technology for the decontamination of food surfaces. In this study, the ability of UVC to inactivate *L. monocytogenes* on frankfurters that contained potassium lactate (PL) and sodium diacetate (SDA), either before or after packaging, was investigated. UVC irradiation of frankfurters that were surface-inoculated with *L. monocytogenes* resulted in a 1.31, 1.49, and 1.93 log reduction at doses of 1, 2, and 4 J/cm², respectively. UVC treatment had no effect on frankfurter color or texture at UVC doses up to 4 J/cm². Frankfurter meat treated with UVC doses up to 16 J/cm² did not increase mutagenesis in bacterial or human cells, either with or without exogenous metabolic activation. UVC treatment of single-layer frankfurter packs at a dose of 2 J/cm² resulted in a 0.97 (±0.14) log reduction of *L. monocytogenes*. Following 8 wk of refrigerated storage *L. monocytogenes* levels decreased by only 0.65 log in non-UVC-treated frankfurter packs compared with 2.5 log in the UVC-treated packs. Because the numbers of *L. monocytogenes* associated with contaminations of ready-to-eat meats are typically very low, the use of UVC in combination with potassium lactate and sodium diacetate has the potential to reduce the number of frankfurter recalls and foodborne illness outbreaks.

Keywords: frankfurters, *Listeria monocytogenes*, ultraviolet light

Introduction

Listeria monocytogenes is an occasional postprocess contaminant on ready-to-eat (RTE) meat products, including frankfurters, and a number of foodborne illness outbreaks have been attributed to *L. monocytogenes* (Barnes and others 1989; Anonymous 1998; Mead and others 1999; Anonymous 2001; FDA 2001), and the incidence of listeriosis has been persistent (Anonymous 2006; Denney and McLauchlin 2008). *L. monocytogenes* is capable of growth at refrigerated temperatures and in high-salt environments, which allows it proliferate during long-term cold storage (Smith 1996). Because of the high mortality rate associated with infection, especially among at-risk populations such as the very young, very old, pregnant, and immuno-compromised, *L. monocytogenes* is given a zero tolerance in RTE meat products in the United States (USDA 1989; Gerba and others 1996). In the last 5 y (2003 to 2008), there have been over 10 Class I recalls of frankfurters, not including other RTE meats, due to contamination with *L. monocytogenes* (USDA FSIS 2008).

While the health consequences of *Listeriosis* are serious it has been noted that low levels of *L. monocytogenes* in foods equal low risk (Chen and others 2003). In a recent survey of RTE food products, *L. monocytogenes* was detected in 0.89% of deli meat products. However, 39% of the samples were contaminated with less than 0.1 CFU/g *L. monocytogenes*, 73% contained less than 1.0 CFU/g of

the bacterium, and 87% contained less than 10 CFU/g of *L. monocytogenes* (Gombas and others 2003). Therefore, use of intervention technologies that produce even modest reductions in bacterial number has the potential to reduce the number of frankfurter recalls and *L. monocytogenes*-associated foodborne illness outbreaks.

To prevent the contamination of frankfurters by *L. monocytogenes*, and to ameliorate the consequences due to consumption of accidentally contaminated frankfurters, the USDA FSIS has recommended thorough plant sanitation and testing for the presence of *Listeria* spp. as part of an effective hazard analysis and critical control point (HACCP) plan. Postprocess interventions, either alone or in combination with antimicrobial compounds may be used to inactivate and prevent the proliferation *L. monocytogenes* on RTE meat products. Both potassium lactate (PL) and sodium diacetate (SDA) have been approved by the U.S. FDA for use in RTE meat products (FDA 2000a) and combinations of the two can effectively inhibit the growth of *L. monocytogenes* on RTE meats during long-term refrigerated storage (Mbandi and Shelef 2001; Seman and others 2002; Sommers and others 2003). UVC irradiation (254 nm), which exerts its bactericidal effect by production of cyclobutane pyrimidine dimers and 6-4 photoadducts in the bacterial chromosome, is an FDA-approved intervention that can be used for the surface decontamination of foods (FDA 2000b; Reardon and Sancar 2005).

The purpose of this study was to (1) determine the ability of UVC light to inactivate *L. monocytogenes* on the surfaces of frankfurters that contain PL and SDA, either before or after packaging, (2) to determine the growth potential of *L. monocytogenes* on frankfurters during refrigerated storage, (3) to determine the effect of UVC irradiation on frankfurter color and texture, and (4) to evaluate the mutagenic potential of UVC-irradiated frankfurters that contain PL and SDA.

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Materials and Methods

Frankfurters

Freshly manufactured frankfurters were purchased from a local manufacturer. The frankfurters consisted of beef, pork, water, salt, flavoring, paprika, sodium phosphate, SDA (0.07%), PL (1.13%), sodium erythorbate, sodium nitrate, and 25% fat. Frankfurters were frozen to -20°C and then gamma irradiated (dose rate 0.95 kGy/min) using a temperature-controlled cesium-137 self-contained irradiator (Lockheed Martin, Marietta, Ga., U.S.A.) to a dose of 10 kGy to inactivate background microflora.

UVC irradiation

A custom-made UVC irradiator containing four 24-inch UVC emitting bulbs (Atlantic Ultraviolet, White Plains, N.Y., U.S.A.) was used. The apparatus delivered a UVC dose rate of $10\text{ mW/cm}^2/\text{s}$ as determined by calibrated UVX Radiometer (UVP, Inc., Upland, Calif., U.S.A.) at a distance of 20 cm from the bulbs. It should be noted that $1\text{ W} \times \text{s} = 1\text{ J}$, and the Joule is the SI unit for UVC dose. Because frankfurters possess an overall cylindrical shape, single frankfurters were irradiated by rotating them $90^{\circ} \times 4$ times during the exposure to UVC. Therefore a frankfurter exposed to 4 J/cm^2 UVC received $4 \times 1\text{ J/cm}^2$ exposures. UVC treatment did not increase the surface temperature of the frankfurters to greater than 20°C . Single-layer frankfurter packs were exposed to 2 J/cm^2 UVC on each side.

L. monocytogenes

Three *L. monocytogenes* strains isolated from RTE meats (F4561, H7762, and H7764) were obtained from the Centers for Disease Control and Prevention (Atlanta, Ga., U.S.A.). The strains were propagated on Tryptic Soy Agar (BD-Difco Laboratories, Sparks, Md., U.S.A.) at 37°C and maintained at 0 to 2°C until ready for use. Identity of *Listeria* was confirmed by Gram Stain followed by analysis with Gram-positive identification (GPI) cards using the Vitek Automatic Microbic System (bioMerieux Vitek, Inc., Hazelwood, Mo., U.S.A.).

UVC inactivation of *L. monocytogenes*

Each *L. monocytogenes* strain was cultured independently in 30 mL Tryptic Soy Broth (Difco) in baffled 50 mL sterile tubes at 37°C (150 rpm) for 18 h . The cultures were then sedimented by centrifugation ($1735 \times g$ for 10 min) and resuspended as a mixture in 9 mL of Butterfield's phosphate buffer (BPB) (Applied Research Inst., Newtown, Conn., U.S.A.). Refrigerated gamma-irradiated frankfurters were then placed on a sterile polynylon bag (Uline, Inc., Philadelphia, Pa., U.S.A.) and rolled 4 to 5 times in 0.2 mL of inoculum (approximately 10^8 cells), from end to end, and allowed to dry in the refrigerator (4°C) for approximately 30 min prior to UVC irradiation.

Following UVC irradiation, the samples were assayed for CFU's by standard pour plate procedures. One hundred milliliters of sterile BPB were added to a Nr 400 stomacher bag (Seward Ltd., London, U.K.) that contained a frankfurter and shaken manually for 1 min (Sommers and Thayer 2000). The samples were then serially diluted in BPB, using 10-fold dilution, and 1 mL of diluted sample was pour plated using Tryptic Soy Agar or Tryptic Soy Agar amended with 4% sodium chloride to assess cell membrane integrity (Novak and Juneja 2001). Three 1-mL aliquots were plated per dilution. The TSA plates were then left in a lighted room at room temperature for approximately 4 h to allow for photoreactivation and resuscitation (see Sancar and Sancar 1988; Weber 2005). The plates were then incubated for 48 h at 37°C prior to enumeration.

Storage study

L. monocytogenes was cultured as previously described. Thirty-three microliters of each *L. monocytogenes* culture were diluted into a single 99 mL volume of BTB to make the inoculum. Refrigerated non-gamma-irradiated frankfurters were placed, 4 to a pack (144 g), in sterile UV transmissible polynylon bags (Uline, Inc.), and $200\text{ }\mu\text{L}$ of inoculum were pipetted on to the frankfurter surfaces to yield a final *L. monocytogenes* concentration of approximately 1500 CFU/g *L. monocytogenes*. UV transmissibility of the bags was verified by placing the detector of the UVX Radiometer (UVP, Inc.) inside the bag and placing the bag in the UVC source. The frankfurter packs were then vacuum-packed (30 mB) and refrigerated for approximately 30 min prior to UVC irradiation. The frankfurter packs were then exposed to 2 J/cm^2 UVC on each side. UVC irradiated and nonirradiated frankfurter packs were then stored at 0 to 4°C for the duration of the study. The samples were assayed every 2 wk for 8 wk using the methodology previously described with the exception that Palcam medium was used to select for *L. monocytogenes*.

Scanning electron microscopy

Frankfurters were inoculated as described previously. Thin (1 mm), centimeter-size slices of the surfaces of hot dog samples were excised with a stainless steel razor blade and immersed in approximately 20 mL of a 2.5% glutaraldehyde– 0.1 M imidazole buffer solution ($\text{pH } 7.2$). The samples were then stored for a few days in sealed vials. For sample processing, slices were washed in imidazole buffer then, dehydrated by exchange with 20 mL volumes of graded ethanol solutions (50%, 80%, absolute ethanol), with 2 changes at each concentration. Subsequently, samples were critical point dried from liquid CO_2 and dry slices were glued with Duco cement (ITW Performance Polymers, Riviera, Fla., U.S.A.) and colloidal silver adhesive (Electron Microscopy Sciences, Hatfield, Pa., U.S.A.) to specimen stubs. Finally, the mounted samples were sputter coated with gold and digitally imaged using a model Quanta 200 FEG scanning electron microscope (FEI Co., Inc., Hillsboro, Ore., U.S.A.) operated in the high vacuum, secondary electron imaging mode.

Confocal laser scanning fluorescence microscopy

Frankfurters were inoculated as described previously. Thin (2 to 3 mm wide) slices of the surface of hot dog samples were immersed in 1 mL volumes ($3\text{ }\mu\text{L}$ of A + B components/ mL imidazole buffer solution) of the nucleic acid staining, viability dye kit L-7012 (Molecular Probes, Eugene, Ore., U.S.A.). After 20 min of incubation in the dye solution, concentrated glutaraldehyde was added to a final concentration of 2.5%, samples were transferred into $50\text{ }\mu\text{L}$ staining solution in a glass bottom microwell dish (MatTek Corp., Ashland, Mass., U.S.A.), and surfaces were imaged with a model IRBE optical microscope and $63\times$ water immersion lens coupled to a TCS-SP confocal fluorescence attachment (Leica Microsystems, Exton, Pa., U.S.A.). Fluorescence was excited by the 488 nm line of an Argon laser and emission was collected simultaneously as stacks of optical sections into 2 channels, 500 to 540 nm for glutaraldehyde-induced autofluorescence, marking the superficial matrix of the frankfurter and fluorescence from the viability dye, Syto 9, labeling the microbial cells and 640 to 680 nm for the "nonviable" dye, propidium iodide. Maximum projection images of the 2 stacks of optical sections in the 2 channels were overlaid and compared for the abundance of viable and nonviable cells on the convoluted surface features of treated and nontreated samples.

Color analysis

Color analysis was then performed using a Hunter Lab Miniscan XE Meter (Hunter Laboratory, Inc., Reston, Va., U.S.A.) (Sommers

and others 2003). The meter was calibrated using white and black standard tiles. Illuminate D65, 10° Standard observer, and a 2.5-cm port/viewing area were used. Results were from 3 independent experiments, with 12 readings taken per experiment.

Texture analysis

Cutting force of the frankfurters was measured using a Texture Technologies Corp. (Scarsdale, N.Y., U.S.A.) TA-XT2 texture analyzer. A TA-7 Warner–Bratzler blade was used with a test speed of 2.0 mm/s, 55 mm distance, and 20 g autotrigger (Sommers and others 2003). Maximum shear force (g) results were from 3 independent experiments, with 12 readings taken per experiment.

Bacterial mutagenicity tests

Salmonella typhimurium and *Escherichia coli* tester strains TA100, TA1535, TA98, TA1537, WP2[pKM101], and WP2 *uvrA*[pKM101] were obtained from Moltox, Inc. (Boone, N.C., U.S.A.), as were 50X Vogel-Bonner Salts, 0.5 mM histidine/Biotin and tryptophan solutions, S9 fraction from Aroclor 1254 induced rats, S9 fraction buffers, 2-aminoanthracene (2-AA) and methyl methanesulfonate (MMS). The positive controls were dissolved in ethanol (Sigma-Aldrich Chemical Co., St. Louis, Mo., U.S.A.). The positive controls, 2-AA and MMS, were tested at 5.0 and 600 µg/plate, respectively.

Nonirradiated frankfurters were finely diced (1 mm) using a sterile knife. Subsequently, 0.5 gram of diced frankfurter was placed in a sterile Petri dish and UV irradiated (uncovered) to doses of 0, 4, 8, and 16 J/cm². The frankfurter samples were then placed in a stomacher bag with 10 mL of ethanol and mixed for 2 min to emulsify the sample. One hundred microliters of emulsion (5 mg of frankfurter) were used to assess the mutagenic potential of the UVC-irradiated frankfurters as described by Maron and Ames (1983). Each experiment was conducted independently 3 times, with and without exogenous metabolic activation.

Generation of 6-thioguanine resistant mutants in TK6 cells

The ability to generate 6-thioguanine resistant mutants was carried out using a microtiter plate based method (Oberly and others 1997). The TK-6 (*hprt*+/-) cells were initially cultured (5% CO₂/high humidity) in RPMI 1640 medium supplemented with 10% FBS, penicillin/streptomycin solution, and HAT medium supplement for 2 passages to suppress the generation of spontaneous 6-TG resistant mutants. The TK6 cells were then propagated for 2 passages without HAT medium supplement prior to exposure to the frankfurter emulsion described previously. Fifty milliliters of TK6 cells (10⁶/mL) were exposed to 0, 0.13, 2.5, or 5.0 mg of frankfurter emulsion for 4 h. Positive controls were either MMS (20 µg/mL) or 2-AA (10 µg/mL). Following the incubation viability was assessed using trypan blue exclusion method. The frankfurter preparations did not reduce the viability of the TK6 cells below 90%, either with or without exogenous metabolic activation. The cells were then

pelleted by centrifugation (200 × g), resuspended in RPMI 1640 medium supplemented with 10% FBS and penicillin/streptomycin solution, and allowed to incubate for 2 passages to allow fixation of the *hprt*- mutations. The TK6 cells were then adjusted to a concentration of 10⁶ cells/mL in medium with and without 6-TG (1 µg/mL) and plated (150 µL per well/approximately 10⁴ cells) in sterile 96 well microtiter plates. The microtiter plates were then incubated for 2 to 3 wk and the number of negative (not containing viable cells) wells per microtiter plate was scored by microscopy. The methods of Oberly and others (1997) and Sommers and others (1995) were used to determine the mutation frequency (MF), the fraction of 6-thioguanine resistant (*hprt*-/-) cells. The experiment was conducted 3 times.

Statistical analysis

Each experiment was conducted independently 3 times. Statistical analysis functions of MS Excel (Microsoft Corp., Redmond,

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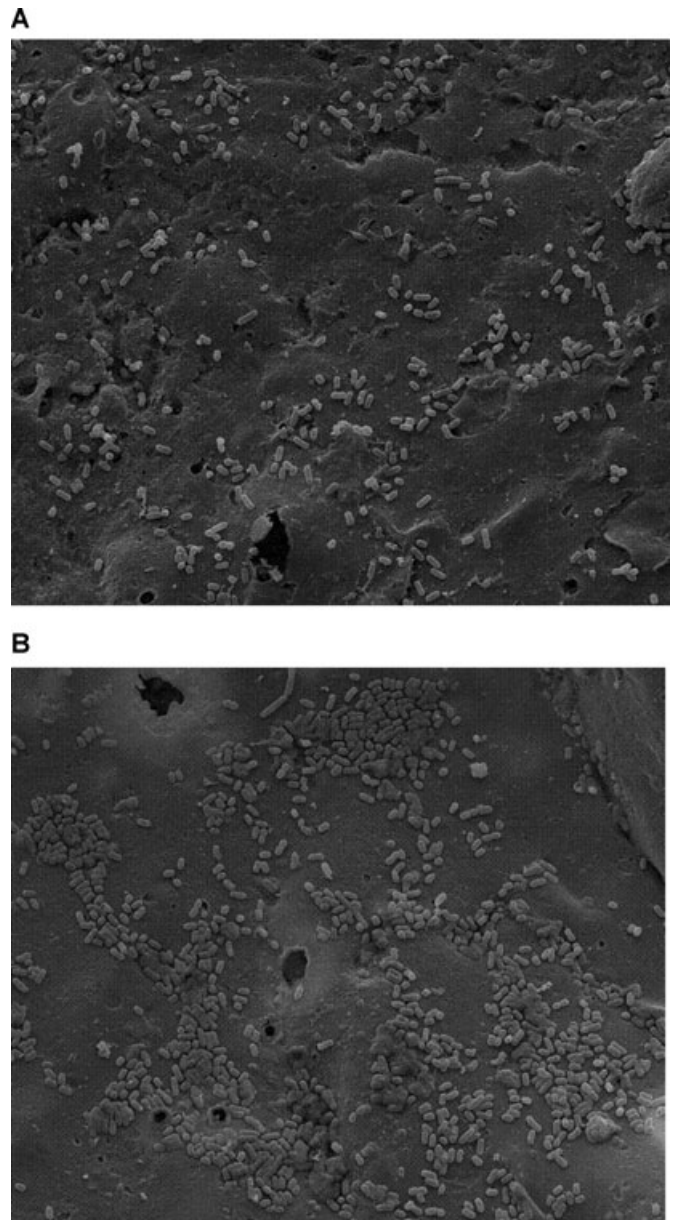


Figure 1—Scanning electron microscopy of frankfurter surfaces inoculated with *Listeria monocytogenes* before (A) and after (B) UVC irradiation.

Table 1—Inactivation (log reduction) of *Listeria monocytogenes* surface-inoculated onto frankfurters by UVC radiation.

% Salt	0 J/cm ²	1 J/cm ²	2 J/cm ²	4 J/cm ²
0%	0.00 (±0.00)	1.31 (±0.06)	1.49 (±0.07)	1.93 (±0.13)
4%	0.00 (±0.00)	1.33 (±0.06)	1.44 (±0.14)	1.97 (±0.15)

Each experiment was conducted independently 3 times. Standard errors of the mean are shown in parentheses. There was no difference in log reduction between *L. monocytogenes* grown on Tryptic Soy Agar or Tryptic Soy Agar amended with 4% sodium chloride as determined by ANOVA (*n* = 3, α = 0.05).

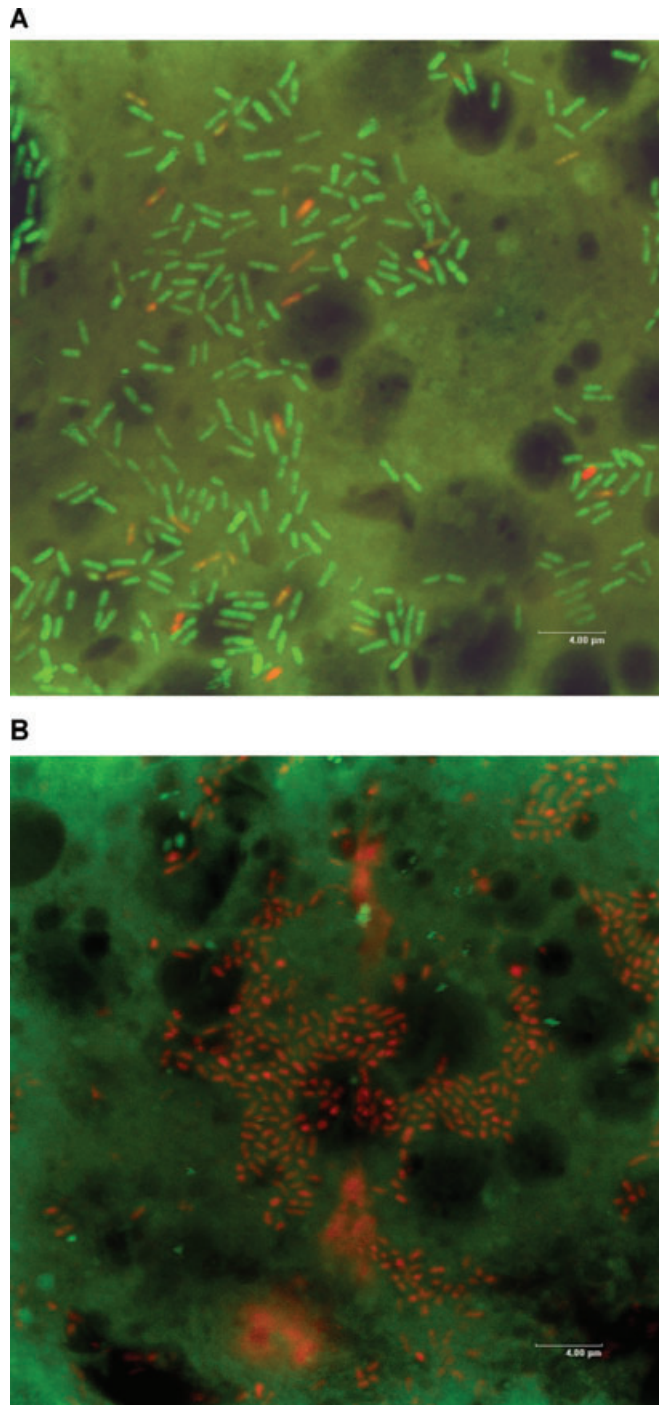


Figure 2—Confocal laser scanning fluorescence microscopy of frankfurter surfaces inoculated with *Listeria monocytogenes* before (A) and after (B) UVC irradiation. Live cells appear green and dead cells appear red.

Table 2—Color and texture analysis of frankfurters exposed to UVC radiation.

	0 J/cm ²	1 J/cm ²	2 J/cm ²	4 J/cm ²
<i>a</i> value	13.7 (±0.57)	13.6 (±0.85)	13.1 (±0.62)	13.9 (±0.83)
<i>b</i> value	26.1 (±1.15)	26.6 (±1.93)	25.6 (±0.79)	27.3 (±1.12)
<i>L</i> value	53.1 (±1.25)	52.4 (±1.35)	53.6 (±1.32)	52.8 (±0.91)
Max shear force (g)	1722 (±126.7)	1667 (±215.8)	1655 (±198.6)	1635 (±198.6)

Each experiment was conducted independently 3 times. Standard errors of the mean are shown in parentheses. There was no difference in color or texture of frankfurters treated with UVC radiation as determined by ANOVA ($n = 4$, $\alpha = 0.05$).

Wash., U.S.A.) were used for routine calculations, descriptive statistics, and analysis of variance (ANOVA).

Results and Discussion

In this study, the ability of UVC to inactivate *L. monocytogenes* on the surfaces of frankfurters that contain PL and SDA was investigated. As shown in Table 1, a modest UVC dose of 1 J/cm² was able to inactivate 1 log of the pathogen, while a UVC dose of 4 J/cm² was able to inactivate 1.93 log of *L. monocytogenes* on frankfurters prior to packaging. Kim and others (2002) found that a UV dose of 1.5 J/cm² inactivated either 0.48 or 0.46 log *L. monocytogenes* inoculated onto raw chicken, with or without skin, respectively. Stermer and others (1987) found that UVC radiation inactivated 1 log of bacteria on the surfaces of beef. Yousef and Marth (1988) found that UVC effectively inactivated *L. monocytogenes* in liquid media and on agar surfaces. Doyle (1999) lists the resistance of microorganisms to be *L. monocytogenes* > *Staphylococcus aureus* > *Salmonella enteritidis* > *E. coli*. Sommers and Geveke (unpublished data) found that a UV dose of 4 J/cm² inactivated 0.37 log of *L. innocua* on the surface of turkey ham. It should be noted that the surface topology of frankfurters is much different than that of a whole muscle meat such as turkey ham, with the bacteria much more exposed to UVC radiation on the frankfurter (Figure 3). In addition, no antimicrobial was applied to either the turkey ham or the chicken in those studies.

Because this is the 1st study to investigate the use of UVC light in combination with PL and SDA, damage to cell membranes was also assessed. Differential plating of *L. monocytogenes* on salt-amended growth medium (Novak and others 2003) detected no evidence of sublethal injury to cell membranes. In addition, examination of UVC and non-UVC-irradiated cells inoculated onto frankfurter surfaces by scanning electron microscopy failed to resolve any obvious microstructural or nanostructural differences in cellular topography (Figure 1). In contrast, confocal laser scanning fluorescence microscopy of UVC and non-UVC-irradiated *L. monocytogenes* revealed mostly red fluorescent or dead cells on the UVC-treated frankfurters, but not on non-UVC-treated frankfurters, as determined by the use of the viability, live–dead stain (Figure 2). While not quantified, results obtained using confocal laser scanning fluorescence microscopy were in general agreement with the log reduction values for *L. monocytogenes* obtained using standard microbiological isolation and enumeration procedures.

To assess the effect of UVC irradiation on frankfurter quality UVC- and non-UVC-treated frankfurters were subjected to color and texture analysis, and assessment of mutagenic potential. There was no effect of UVC irradiation on frankfurter redness (*a* value), yellowness (*b* value), and brightness (*L* value) as determined by Hunter color analysis (Table 2). There was no effect of UVC irradiation on texture as measured by maximum shear force (Table 2).

In recent years, there has been increasing emphasis concerning appropriate toxicological risk analysis of foods treated using non-thermal processing technologies, as there are little data available in peer-reviewed scientific publications. Consumer groups such

Table 3 – Mutagenicity of frankfurters exposed to UVC radiation.

Strain	S9	0.0 J/cm ²	4.0 J/cm ²	8.0 J/cm ²	16 J/cm ²	Pos. control
TA100	0%	93.5 (±6.89)	86.0 (±5.51)	91.8 (±5.93)	102 (±7.07)	728 (±8.67)
	10%	96.3 (±8.52)	99.5 (±6.64)	100 (±5.63)	102 (±7.07)	954 (±8.95)
TA1535	0%	14.0 (±1.0)	11.8 (±1.59)	12.0 (±1.32)	16.3 (±7.62)	509 (±55.4)
	10%	13.3 (±0.14)	13.3 (±0.28)	14.8 (±4.19)	10.8 (±3.32)	169 (±13.7)
TA98	0%	28.3 (±2.83)	21.3 (±3.83)	20.2 (±1.17)	25.8 (±0.33)	309 (±29.2)
	10%	27.0 (±4.44)	22.8 (±3.48)	19.0 (±4.31)	20.0 (±3.79)	616.5 (±42.7)
TA1537	0%	10.0 (±1.61)	9.50 (±2.31)	8.00 (±1.61)	6.50 (±1.04)	120 (±4.86)
	10%	6.75 (±0.72)	8.25 (±1.59)	5.50 (±0.29)	7.50 (±0.58)	120 (±8.70)
WP2 [pKM101]	0%	7.56 (±2.45)	12.1 (±3.32)	6.86 (±4.33)	8.41 (±1.67)	368 (±12.7)
	10%	10.4 (±1.93)	7.84 (±2.56)	12.6 (±2.04)	10.7 (±2.54)	56.6 (±7.35)
WP2 <i>uvrA</i> [pKM101]	0%	33.8 (±2.98)	28.4 (±4.22)	35.3 (±3.57)	29.6 (±3.81)	386 (±19.6)
	10%	24.5 (±2.47)	31.3 (±3.11)	26.2 (±2.73)	30.4 (±3.30)	463 (±24.8)
TK6	0%	3.33 (±0.10)	3.36 (±0.89)	2.38 (±0.39)	2.44 (±0.17)	17.6 (±3.42)
	3%	2.27 (±0.18)	2.50 (±0.33)	2.28 (±0.52)	2.02 (±0.29)	33.6 (±1.33)

Each experiment was conducted independently 3 times. Values for *Salmonella* tester strains TA100, TA98, TA1535, and TA1537 are shown as the mean number of histidine revertant colonies per plate, while the values for *Escherichia coli* tester strains WP2 [pKM101] and WP2 *uvrA* [pKM101] are shown as the number of tryptophan revertant colonies per plate. Mutation frequency in TK cells is per 10⁶ viable cells. Standard errors of the mean are shown in parentheses. There was no difference in the mutagenicity of frankfurters with UVC radiation compared with that of the untreated controls as determined by ANOVA ($n = 3, \alpha = 0.05$).

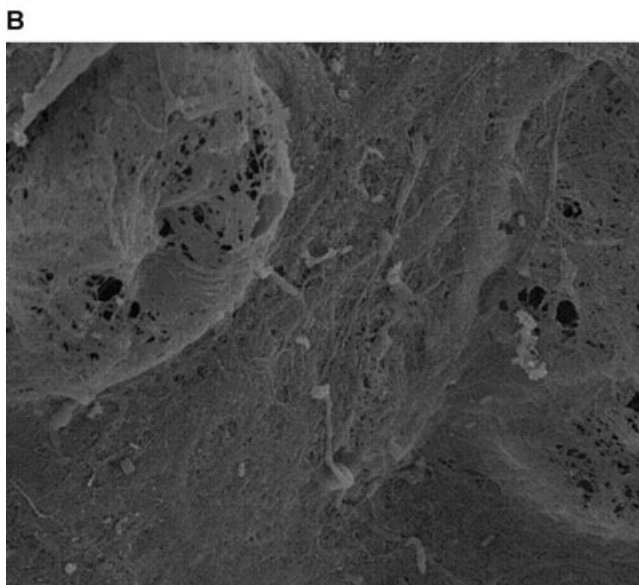
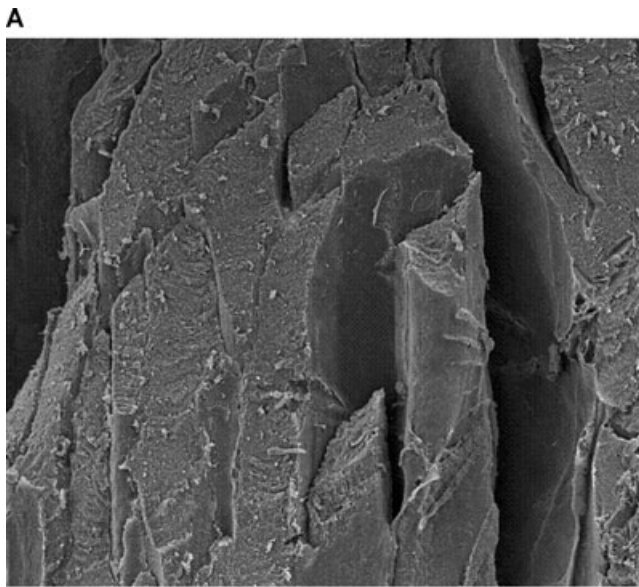


Figure 3 – Scanning electron microscopy of turkey-ham surfaces: (A) 100 μm; (B) 10 μm.

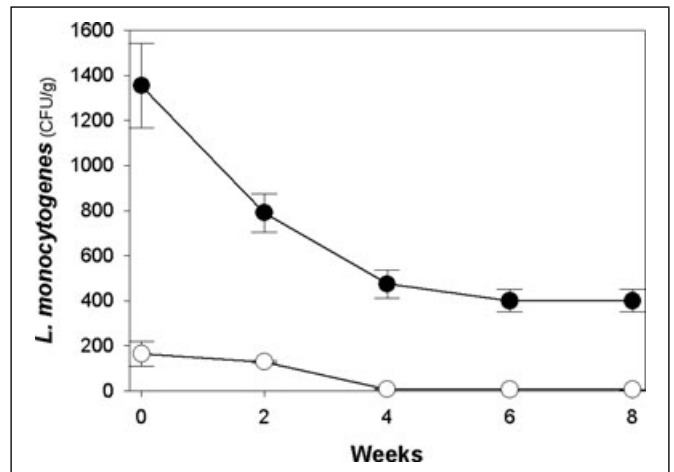


Figure 4 – Proliferation of *Listeria monocytogenes* during 8 wk refrigerated storage on frankfurters that contain potassium lactate and sodium diacetate with (open circles) and without (closed circles) UVC irradiation. Each experiment was conducted independently 3 times, with the standard error of the mean shown by error bars.

as Food and Water Watch have requested that food treated using emerging nonthermal intervention technologies be subjected to standard genotoxicity tests. To assess mutagenic potential, frankfurter meat UVC irradiated to a dose of 16 J/cm² were tested in bacterial mutagenicity tests, both with and without exogenous metabolic activation. No increase in point mutations (TA100 and TA1535, WP2 [pKM101], and WP2 *uvrA* [pKM101]) or frame-shift mutations (TA98 and TA1537) was detected, either in the presence or absence of 10% S9 fraction (Table 3). No increase in mutation frequency was detected in human TK6 lymphoblasts. UVC-treated frankfurter meat did not affect the viability of bacterial or human cells over that of the untreated controls as determined by assessment of the bacterial lawn turbidity, or trypan blue exclusion for TK6 lymphoblasts.

The ability of UVC light to inactivate *L. monocytogenes* in single-layer frankfurter packs and the growth potential of *L. monocytogenes* on UVC-irradiated frankfurters that contained PL and SDA during long-term refrigerated storage (0 to 4 °C) was examined. UVC irradiation (1 W/cm²) applied to each side of single-layer frankfurter packs inoculated with approximately 1400 CFU/g of *L.*

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monocytogenes resulted in a 0.97 log reduction of the pathogen, or to a level of approximately 165 CFU/g (Figure 4). While UVC would not be able to inactivate *L. monocytogenes* in between frankfurters, pathogen levels decreased by an additional log by week 4 of the refrigerated storage period. In contrast, only 0.65 log of *L. monocytogenes* was inactivated on the non-UVC-treated frankfurter packs during the 8-wk storage period (Figure 4). Based on these results, UVC irradiation of single-layer or individually packaged frankfurters that contain PL and SDA would be feasible, although UV of prepackaged single frankfurters would be desirable to allow exposure of the entire frankfurter to UVC.

The 2.6 log reduction of *L. monocytogenes* on frankfurters that contained PL and SDA following 8 wk of refrigerated storage is not surprising. Sommers and Boyd (2006) found that gamma irradiation of a frankfurter on a roll, a prepackaged "heat and eat" sandwich product, resulted in increased lethality of *L. monocytogenes* during a 2-wk refrigerated storage at 10 °C. Sommers and others (2003) found that gamma irradiation of *L. monocytogenes* inoculated onto beef bologna resulted in an additive inactivation of the pathogen, and that the growth of *L. monocytogenes* was inhibited for 2 mo at an abuse temperature of 10 °C. Exposure of *L. monocytogenes* to DNA damage inducing intervention technologies such as gamma irradiation and UVC, in combination with PL and SDA, results in additional lethality during refrigerated storage.

Conclusions

UVC irradiation is an FDA-approved process that can be used to inactivate *L. monocytogenes* on the surface of foods and food contact surfaces that does not require additional labeling, as does ionizing radiation. PL and SDA are U.S. FDA-approved generally recognized as safe (GRAS) additives that are currently in use by the meat processing industry. The results of this study indicate that UVC irradiation, an inexpensive intervention technology, in combination with the antimicrobials PL and SDA, given the low levels of *L. monocytogenes* associated with contaminated frankfurters and other RTE meats, has the potential to significantly reduce the number of frankfurter recalls and foodborne illness outbreaks. While UVC was able to penetrate the packaging material used in this study, perhaps the best application of the technology is application of UVC to frankfurter surfaces immediately prior to packaging as many materials are pre-labeled, which could interfere with UVC penetration of the product packaging.

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