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ULTRAVIOLET LIGHT (254 NM) INACTIVATION OF FOODBORNE PATHOGENS ON FOODS AND STAINLESS STEEL SURFACES

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ABSTRACT

Ultraviolet Light (254 nm) is a U.S. Food and Drug Administration approved nonthermal intervention technology that can be used for decontamination of food surfaces. In this study the use of Ultraviolet Light (UV) at doses of 0.5 to 4.0 J/cm^2 to inactivate Salmonella *spp.,* Listeria monocytogenes*, and* Staphylococcus aureus *surface-inoculated (10^2-10^5 CFU/g) on the surfaces of frankfurters, bratwurst, shell eggs, chicken drumsticks, boneless skinless chicken breasts, boneless pork chops, tomatoes, and Jalapeno peppers was investigated. The pathogens displayed similar UV sensitivities to foodborne pathogens on individual food products. Pathogen reductions ranged from approximately 0.5 log CFU/g on raw meat and poultry to almost 4 log CFU/g on tomatoes, while the pathogens were not recovered from stainless steel at a UVC dose of 0.4 J/cm^2. Use of UVC light should be given serious consideration as a technology for routine surface decontamination of food contact surfaces and appropriate food products.*

PRACTICAL APPLICATIONS

Ultraviolet light (UVC) is an FDA approved intervention technology that can be used to inactivate pathogenic bacteria in liquid foods and water, food contact surfaces, and food surfaces. This work indicates than UVC would be a an effective technology for inactivation of foodborne pathogens on the surfaces of frankfurters and sausages immediately prior to packaging, shell eggs immediately prior to cracking in the production of liquid egg products, and smooth skinned produce such as tomatoes and jalapeno peppers prior to further processing. This work provides pathogen inactivation kinetics for food processors and government regulatory agencies.

Keywords: Ultraviolet, UVC, Salmonella, *Listeria, S. aureus*.

INTRODUCTION

The last in depth analysis of foodborne illness in the U.S. by Mead *et al.* (1999) indicates that consumers suffer with approximately cases of foodborne illness, 325,000 hospitalizations, and 5,000 deaths each year. While improvements have been made in reducing incidence of foodborne illness caused by specific pathogens have leveled off (Denny and McLauchlin 2008; Anonymous 2006, 2008), which indicates that new approaches may be helpful in reducing the risk of foodborne illness.

Ultraviolet light (254 nm-UVC) is an FDA approved technology that can be used for decontamination of food and food contact surfaces (U.S. FDA 2000). UVC irradiation exerts its bactericidal effect by production of cyclobutane pyrimidine dimers and 6-4 photoadducts in the bacterial chromosome (Reardon and Sancar 2005), either killing the bacteria or rendering them unable to reproduce.

While many scientific studies have been conducted to assess the efficacy of UVC for inactivation of foodborne pathogens, many are limited in scope, use UVC sources with limited intensity, or assess the survival of only a single species of pathogenic bacteria on a single food product. The purpose of this study was: (1) to determine the ability of UVC to inactivate multiple foodborne pathogens including *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus* (2) to examine the log reductions obtained following inoculation of the pathogens on multiple food products including raw meat and poultry, precooked sausages, shell eggs, and fruits and vegetables, and (3) to examine the efficacy of UVC for decontamination of stainless steel surfaces as a food contact surface.

MATERIALS AND METHODS

Foods and Stainless Steel Coupons

Precooked bratwurst, fat-free frankfurters, chicken breasts, chicken drumsticks, boneless skinless pork chops, shell eggs, Roma tomatoes, and Jalapeno peppers were purchased from local retailers or farmers markets. Bead-blasted and electroplated stainless steel coupons were provided by Dr. Carmen Moraru (Cornell University, Ithaca, NY).

Bacteria

Listeria monocytogenes strains H7762, H7962, H7969 were obtained from the Centers for Disease Control and Prevention (Atlanta, GA). *Staphylococcus aureus* strains 25923, 13565, and 14458; *Salmonella* Enteriditis 13076, *S*. Typhimurium and *S*. Newport 6962, *Y. enterocolitica* 51871were obtained from the American Type Culture Collection (Manassa, VA). Identity of the isolates was confirmed by Gram Stain, followed by analysis with Gram Positive or Negative Identification cards using the Vitek Automicrobic System (bioMerieux Vitek, Inc., Hazelwood MO). The bacterial strains were cultured on Tryptic Soy Agar (TSA) (BBL/Difco, Inc., Sparks, MD) at 37C and maintained at 0-4C, until use.

UVC Inactivation of Pathogens

Each bacterial isolate was cultured independently in 30-mL Tryptic Soy Broth (Difco) in baffled 50-mL sterile tubes at 37 °C (150 rpm) for 18 h. Aliquots (333 µl) of each culture were then added to a single volume of Butterfield's Phosphate Buffer to a total volume of 100 mL. All food products were then individually dip inoculated for 10 seconds using sterile polynylon bags (Uline, Inc., Philadelphia, PA) and allowed to dry in a biological safety cabinet for approximately 30 min. A dip inoculation was chosen over a spot inoculation to simulate potential washing or spraying of foods prior to final processing or packaging, and resulted in inoculation levels of 10^2-10^5 CFU/cm^2. For instance tomatoes were inoculated with higher pathogen levels than raw poultry based on initial studies. Following drying the food products were placed in a refrigerator (4C) for approximately 1 hr, and then exposed to UVC light.

After UVC irradiation the samples were assayed for CFU's by standard pour plate procedures, which were conducted in a lighted room, to allow for photoreversal of cyclobutane pyrimidine dimers. Fifty-mL of sterile BPB was added to a No. 400 Stomacher bag that contained a food product and shaken manually for 1 min (Sommers and Thayer 2000). The samples were then serially diluted in BPB, using tenfold dilutions, and 1-mL of diluted sample was pour plated using Salmonella selective Hektoen Agar, *Listeria* selective Palcam Agar, and *S. aureus* selective Baird-Parker Agar (BD-Difco, Detroit, MI). Shell eggs were inoculated, stored at 25C for 48 hrs, irradiated, rinsed, cracked, the shells macerated in a sterile tube, and the macerated shell incubated with pre-warmed (42C) BPB as described by Musgrove *et al.* (2005), and then serially diluted in BPB. In the case of stainless steel coupons 100 µl if inoculums (10^6 CFU/ml) was placed on the surface of the stainless steel coupon, spread on the coupon using a sterile pipet tip, and allowed to dry for 30 minutes in a biological safety cabinet prior to irradiation. Three 1-mL aliquots were plated per dilution. The agar plates were then left in a lighted room at room temperature for approximately 6 h to allow for photo-reactivation of UV induced DNA adducts and resuscitation. The plates were then incubated for 48 h at 37C prior to enumeration. In preliminary experiments growth and incubation of the pathogens at 25-30C or use of tryptic soy agar versus selective media had no effect on the experimental outcome.

UVC Irradiation

A custom made UVC irradiator containing four 18 inch UVC emitting bulbs (Atlantic Ultraviolet, White Plains, NY) made from UV reflective electroplated stainless steel was used. The apparatus delivered a UVC dose rate of 10 $mW/cm^2/s$ as determined by calibrated UVX Radiometer (UVP, Inc, Upland, CA) at a distance of 20 cm from the bulbs. It should be noted that a $J= W \times s$. Frankfurters, bratwurst, eggs, chicken drumsticks, tomatoes, and jalapeno peppers irradiated by rotating them $90^o \times 4$ times during the exposure to UVC. Relatively flat products including pork chops and boneless skinless chicken breasts were treated on each flat" side, therefore 2×2 J/cm^2=a total UVC dose of $4J/cm^2$.

Statistical Analysis

Each experiment was conducted independently 3 times. Descriptive statistics and Analysis of Variance (ANOVA) were performed using Microsoft Excel, Microsoft Corp (Redmond, WA).

RESULTS AND DISCUSSION

Ultraviolet light (UVC-254 nm) is an FDA approved technology that can be used for inactivation of bacteria in liquid foods such as juices, decontamination of air and water (U.S. FDA). While it has been used extensively for these purposes, the use of UVC light for decontamination of actual solid food surfaces has been somewhat limited because it is a non-penetrating form of irradiation. In addition, pathogen inactivation data available in the scientific literature is often limited to single foodborne pathogens on single food products. With the recognition that many contaminations of foods are due to relatively low numbers of bacteria, and that low pathogen numbers equals lower risk (Chen *et al.* 2003), meaning that exposure to lower numbers of pathogens reduces the risk of contracting foodborne illness. UVC light leaves no harmful chemical residue on food or food contact surfaces, and could be applied prior to packaging or further processing.

The USDA FSIS has recently published procedures for adoption of emerging non-thermal food safety technologies including UVC light, that call for evidence of efficacy prior to installation of decontamination equipment in processing facilities (USDA FSIS 2008). Therefore, in order to provide the food processing industry and regulatory agencies information as to the efficacy of UVC Light for food product and food contact surface decontamination the ability for the technology to inactivate *Salmonella* spp., *L. monocytogenes*, and *S. aureus* on the surfaces of a variety of foods, including pork chops, boneless skinless chicken breasts, chicken drumsticks, shell eggs, bratwurst, fat-free frankfurters, Roma tomatoes, Jalapeno peppers, and two types of stainless steel was determined.

Sausages (Table 1): Previous research has indicated that UVC light is capable of inactivating 1.5-2.0 log of *L. monocytogenes* on the surface of frankfurters using a spot inoculation procedure, and that UVC had no effect on frankfurter color and texture (Sommers *et al.* 2008; Sommers and Geveke 2006). In this study UVC light inactivated 1.4-2.0 log of the three pathogens at 2 J/cm^2, and 1.8-2.0 of the 3 pathogens at 4 J/cm^2 on both on fat-free frankfurters. Inactivation of 1.4-2.0 log of the pathogens were obtained at 2 J/cm^2, and 1.8-2.0 at 4 J/cm^2 on bratwurst. Use of UVC immediately prior to packaging and application of antimicrobials such as sodium diacetate, potassium lactate, of lauric arginate ester, may enhance inactivation and/or prevent outgrowth of foodborne pathogens (Sommers *et al.* 2008). There was no difference in the UV resistance of the 3 pathogen species when inoculated onto the sausage surfaces, as determined by ANOVA (n=3, α=0.05).

Raw meat and poultry (Table 1): UVC inactivated between 0.5 and 1.0 log of the 3 pathogen species inoculated onto the surfaces of boneless pork chops, boneless skinless chicken breasts, or chicken drumsticks at UVC doses of 2-4 J/cm^2. Again, there was no difference in the UV resistance of the 3 pathogens species when inoculated onto raw meat and poultry surfaces as determined by ANOVA. Lyon *et al.* (2007) found obtained a 2 log reduction of *L. monocytogenes* on raw poultry using an inoculum of 10^9 cfu/ml. Kim *et al.* (2003) reported a 0.5 log reduction of *L. monocytogenes* on raw chicken using a dip inoculation similar to what was used in this study. Wong *et al.* (1998) obtained a 1.5-2.0 log reduction of *Escherichia coli* and Salmonella *Senftenberg* on pork muscle and pork skin using inoculation levels of 6 log cfu/cm^2. Kim *et al.* (2002) found that UVC light could inactivate approximately 1 log of *L. monocytogenes*, S. Typhimurium, or *E. coli* O157:H7 on the surfaces of skin on or skin off chicken meat. Stermer *et al.* (1987) was able to inactivate 2 log of microorganisms on raw meat, and found the cut edge of raw meat products was capable of shielding microorganisms from UVC light. Sommers and Geveke (2006) who obtained a 1.5-2.0 log reduction of *L. innocua* on frankfurters, found that UVC inactivated only 0.5 log reduction of the microorganism on turkey ham surfaces. Sumner and others (1996) found a 2-3 log reduction of S. Typhimurium on poultry skin following a UVC exposure of 2000 uW/cm^2/s.

Shell eggs: Rodriguez and Romo (2005) previously reported a 2.5 log reduction of Salmonella *Enterica* on shell eggs that were surface contaminated to a density of approximately 10^6 CFU/g at a UVC treatment of 2,500 μW/cm^2 for 5 min. Kuo *et al.* (1997) obtained a 2.9-4.6 log reductions of Salmonella *Typhimurium* inoculated onto shell eggs using an inoculum of 10^8-10^9 CFU/ml at UVC intensities of 620 uW/cm^2 at treatment times of 1.0-7.0 min. In our study log reductions of 0.3-0.5 and 0.8-1.2 of the 3 pathogens inoculated onto shell eggs were obtained at 2 and 4 J/cm^2, respectively. It should be noted that in this study the eggs were stored for 2 days prior to exposure to ultraviolet light, which may have allowed protective biofilms to be formed. Unlike produce, eggs are subjected to a thermal pasteurization process when made into liquid eggs, or typically cooked by consumers prior to consumption (American Egg Board, 2008). UVC may be useful in reducing the need for chemical washes during egg production.

Roma Tomatoes and Jalapeno Peppers (Stem off): Yuan *et al.* (2004) obtained a 2.2 log reduction of *Salmonella* spp. surface inoculated onto tomatoes to a density of 10^3 CFU/g at maximum UV dose of 25 mW (25 mJ)/cm^2. The authors were unable to find any reports regarding UVC inactivation of pathogens on Jalapeno peppers by ultraviolet light. In this study a UVC dose of 0.5 J/cm^2 inactivated 2.6-3.1 log of the 3 pathogens on the surface of Roma tomatoes, while a UVC dose of 4 J/cm^2 inactivated 3.6-3.8 log. Similar results were obtained for Jalapeno peppers, with 3.0-3.1 log inactivated at 0.5 J/cm^2, and 3.3-3.8 log inactivated at

4.0J/cm^2. The higher log reductions at 4.0 J/cm^2 were at the limit of detection for the inoculation, recovery and plating methodology used in this study.

Stainless Steel: In this study >5 log reduction of all three pathogens was observed at a UV dose of 0.4 J/cm^2 as indicates by the lack of recovery of the microorganisms from the electroplated or bead blasted stainless steel coupons. Sterility of the stainless steel coupons cannot be claimed, as no enrichment was performed for the 3 pathogens. Kim *et al.* (2002) obtained >5 log reductions of *L. monocytogenes*, S. Typhimurium, and *E. coli* O157:H7 following treatment of stainless steel for 3 min at a UVC intensity of 1,500 μW/cm^2. Results of this study and the Kim *et al.* (2002) study are in agreement regarding the efficacy of UVC for the inactivation of foodborne pathogens on stainless steel.

CONCLUSIONS

The degree of effectiveness of UVC light to inactivate *L. monocytogenes*, *Salmonella* spp., and *S. aureus* was observed as follows: stainless steel>Roma tomatoes and Jalapeno peppers> frankfurters and bratwurst > shell eggs > raw meat and chicken. The UVC resistances of the three pathogens were equivalent when inoculated onto the same food or food contact surface. UVC may be an effective means to reduce foodborne pathogen levels on precooked sausages, smooth skinned fruits and vegetables prior to further processing (slicing/dicing), and shell eggs prior to cracking in the production of liquid egg products. The efficacy and utility of UVC for decontamination of raw meat and poultry remains uncertain, although inactivation of spoilage bacteria and the effect on product shelf-life and pathogen proliferation at refrigerated temperatures was not examined. UVC would be an effective technology for decontamination of stainless steel conveyors and surfaces in food production environments.

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TABLE 1.

INACTIVATION (LOG REDUCTION) OF PATHOGENS SURFACE-INOCULATED ONTO VARIOUS FOOD PRODUCTS BY ULTRAVIOLET LIGHT

		0.5 J/cm^2	1 J/cm^2	2 J/cm^2	4 J/cm^2
Salmonella spp.	Fat Free Franks	1.56 (±0.05)a	1.96 (±0.12)a	1.66 (±0.19)a	2.19 (±0.16)b
S. aureus		1.27 (±0.22)a	1.62 (±0.37)a	1.46 (±0.15)a	1.97 (±0.30)b
L. monocytogenes		1.50 (±0.04)a	1.98 (±0.04)b	1.78 (±0.11)b	2.14 (±0.19)b
Salmonella spp.	Bratwurst	1.14 (±0.26)a	1.30 (±0.15)a	1.32 (±0.14)a	1.51 (±0.07)a
S. aureus		1.10 (±0.16)a	1.29 (±0.06)a	1.22 (±0.10)a	1.38 (±0.10)a
L. monocytogenes		1.42 (±0.16)a	1.53 (±0.07)a	1.61 (±0.14)a	1.78 (±0.10)a
Salmonella spp.	Drumsticks	0.39 (±0.10)a	0.37 (±0.14)a	0.54 (±0.06)a	0.45 (±0.04)a
S. aureus		0.42 (±0.17)a	0.37 (±0.08)a	0.42 (±0.10)a	0.42 (±0.17)a
L. monocytogenes		0.48 (±0.02)a	0.35 (±0.03)a	0.65 (±0.13)a	0.63 (±0.07)a
Salmonella spp.	Shell Eggs	0.43 (±0.21)a	0.31 (±0.20)a	0.53 (±0.52)a	0.98 (±0.55)a
S. aureus		0.12 (±0.11)a	0.30 (±0.17)a	0.31 (±0.24)a	0.81 (±0.42)a
L. monocytogenes		0.28 (±0.26)a	0.50 (±0.36)a	0.53 (±0.53)a	1.16 (±0.54)a
Salmonella spp.	Chicken Breast	0.33 (±0.04)a	0.36 (±0.12)a	0.44 (±0.15)a	0.32 (±0.13)a
S. aureus		0.33 (±0.06)a	0.31 (±0.04)a	0.46 (±0.11)a	0.44 (±0.05)a
L. monocytogenes		0.25 (±0.03)a	0.26 (±0.06)a	0.40 (±0.03)a	0.37 (±0.09)a
Salmonella spp.	Pork Chop	0.43 (±0.09)a	0.50 (±0.10)a	0.56 (±0.12)a	0.53 (±0.11)a
S. aureus		0.50 (±0.05)a	0.61 (±0.08)a	0.49(±0.06)a	0.49 (±0.09)a
L. monocytogenes		0.61 (±0.07)a	0.58 (±0.05)a	0.63 (±0.07)a	0.65 (±0.08)a

		0.5 J/cm^2	1 J/cm^2	2 J/cm^2	4 J/cm^2
Salmonella spp.	Roma Tomato	3.08 (±0.07)a	3.36 (±0.02)a	3.51 (±0.02)a	3.82 (±0.05)b
S. aureus		3.13 (±0.10)a	3.63 (±0.07)b	3.60 (±0.13)b	3.62 (±0.03)b
L. monocytogenes		2.59 (±0.30)a	3.43 (±0.13)b	3.55 (±0.08)b	3.60 (±0.10)b
Salmonella spp.	Jalapeno Pepper	3.02 (±0.06)a	3.31 (±0.05)b	3.54 (±0.06)b	3.79 (±0.11)b
S. aureus		3.09 (±0.13)a	3.41 (±0.08)a	3.73 (±0.10)b	3.33 (±0.12)b
L. monocytogenes		3.11 (±0.04)a	3.33 (±0.10)a	3.63 (±0.09)b	3.72 (±0.19)b

Results are presented in log reductions per J/cm^2. The standard error of the mean is in parenthesis. Each experiment was conducted independently 3 times (n=3, α=0.05). Within each row values with the same letter are statistically similar as determined by ANOVA.

TABLE 2.

INACTIVATION (LOG REDUCTION) OF PATHOGENS SURFACE-INOCULATED ONTO STAINLESS-STEEL BY UVC RADIATION

		0.05 J/cm^2	0.10 J/cm^2	0.20 J/cm^2	0.40 J/cm2
Salmonella spp.	Electroplated	2.52 (±0.19)	2.70 (±0.21)	3.34 (±0.15)	NR*
S. aureus		1.86 (±0.15)	2.02 (±0.20)	2.58 (±0.16)	NR*
L. monocytogenes		2.27 (±0.14)	2.29 (±0.24)	2.89 (±0.23)	NR*
Salmonella spp.	Bead Blasted	3.05 (±0.32)	3.90 (±0.31)	4.00 (±0.34)	NR*
S.aureus		2.88 (±0.32)	3.60 (±0.45)	4.18 (±0.23)	NR*
L. moncytogenes		3.03 (±0.35)	3.62 (±0.48)	4.63 (±0.21)	NR*

*None recovered. Results are presented in log reductions per J/cm^2. The standard error of the mean is in parenthesis. Each experiment was conducted independently 3 times (n=3, α=0.05). Within each row values with the same letter are statistically similar as determined by ANOVA.