Germicidal Ultraviolet Light to Lower Numbers of *Listeria monocytogenes* on Broiler Breast Fillets[1]

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ABSTRACT Raw broiler breast fillets were subjected to germicidal ultraviolet (UV) light (dose of 1,000 $\mu W/cm^2$ for 5 min at a wavelength of 254 nm) to evaluate its potential to reduce *Listeria monocytogenes* numbers on raw product before shipment to a poultry further-processing plant. Boneless, skinless breast fillets were inoculated with 4 different strains of *L. monocytogenes* 5 min before treatment. After the UV treatment, breast fillets were stored at 4°C for 24 h. Enumeration of remaining *L. monocytogenes* was performed using the spread plate method on modified Oxford agar. An approximate 2-log reduction in viable *L. monocytogenes* was observed with all 4 strains on UV-treated breast fillets as compared with the nontreated breast fillets. The UV treatment caused only slight changes in meat color (lightness, redness, and yellowness) on day of treatment or after 7 d of storage. This study suggests that UV treatment of raw breast fillets at a slaughter plant can significantly reduce *L. monocytogenes* without negatively affecting meat color. This process could be used to reduce the negative effect of raw poultry as a transmission vector of *L. monocytogenes* into a poultry further-processing plant.

Key words: *Listeria monocytogenes*, ultraviolet light, poultry processing, meat color

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INTRODUCTION

Listeria monocytogenes is an important foodborne pathogen that can result in a fatality rate from 20 to 30% (Slutsker and Schuchat, 1999). Two of the deadliest outbreaks of foodborne disease in America were a result of listeriosis (CDC, 1999a; Ryser, 1999). In 2002, fully cooked poultry products were involved in a multistate listeriosis outbreak in the northeast United States, resulting in 46 confirmed cases, 7 deaths, and 3 stillbirths or abortions (CDC, 2002). The responsible company voluntarily recalled 27.4 million pounds of processed chicken and turkey meat, the largest meat recall ever in the United States.

Listeria monocytogenes is ubiquitous in nature, and the broiler slaughter plant environment can become a reservoir (Cox et al., 1997). Cox et al. (1997) noted that the percentage of broiler samples positive for *L. monocytogenes* increased from 1.3% (prescald) to 40% (postchill) as the carcasses moved through the processing plant. Contaminated raw poultry can be a vector for *L. monocytogenes* entry into a poultry further-processing plant (Berrang et al., 2002, 2005). The same subtypes of *L. monocytogenes* found on raw product coming into a poultry further-processing plant were also isolated from both the raw and cook sides of the plant on the same sampling day by Berrang et al. (2005). A subtype isolated from raw poultry meat can become persistent throughout a further-processing plant where contamination of fully cooked product becomes a concern (Berrang et al., 2005).

Ultraviolet (**UV**) light from 200 to 300 nm has been reported to have germicidal properties on the surface of fresh meats including fresh poultry (Stermer et al., 1987; Wallner-Pendleton et al., 1994). Ultraviolet irradiation is a nonthermal process that can reduce the presence of pathogens (Sumner et al., 1996) while not significantly affecting the color or rancidity of fresh poultry (Wallner-Pendleton et al., 1994). Another benefit to the poultry processor is that UV irradiation does not result in any chemical or radioactive residues on the meat (Anonymous, 1999). Several studies have shown UV light to be an effective bactericide on meat (Stermer et al., 1987; Kim et al., 2002) and poultry skin (Wallner-Pendleton et al., 1994; Sumner et al., 1996). Wallner-Pendleton et al. (1994) and Sumner et al. (1996) reported a 61 and 80.5% reduction, respectively, in *Salmonella* Typhimurium on chicken meat with skin. Stermer et al. (1987) reported an approximate 2-log reduction in bacteria on fresh beef.

A reduction in *L. monocytogenes* on raw chicken entering a poultry further-processing plant could reduce the presence of the pathogen in the plant environment, lessening the potential for cross-contamination of cooked poultry.

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[1]Mention of trade names or commercial products in this publication is solely to provide specific information and does not imply recommendation or endorsement by the USDA.

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The objectives of this research were as follows: 1) to evaluate the effect of germicidal UV light on the recovery of *L. monocytogenes* from fresh boneless, skinless chicken breasts and 2) determine the effect of UV light treatment on the color of breast fillets.

MATERIALS AND METHODS

L. monocytogenes Cultures and Fillet Inoculation

Four isolates of *L. monocytogenes*, each of different molecular subtypes and different antibiotic resistance profiles, were chosen from 161 isolates that were originally recovered from a commercial poultry further-processing plant (Berrang et al., 2005). Isolates were selected to include different antibiotic resistance profiles to 4 specific antibiotics (ceftriaxone, oxacillin with 2% NaCl, ciprofloxacin, and tetracycline) as determined by the broth microdilution method using a commercial panel according to the instructions of the manufacturer (Trek Diagnostic Systems Inc., Cleveland, OH). Susceptibility breakpoints were as described by the Clinical and Laboratory Standards Institute (Wayne, PA) for gram-positive organisms. The 4 subtypes were labeled A, B, C, and D. Subtype A was resistant to both ceftriaxone and oxacillin with 2% NaCl (MIC > 32 and > 4, respectively) and sensitive to both ciprofloxacin and tetracycline (MIC ≤ 1 and 4, respectively). Subtype B was resistant to both ceftriaxone and tetracycline (MIC > 64 and 4, respectively), intermediate to ciprofloxacin (MIC 2), and sensitive to oxacillin with 2% NaCl (MIC < 2). Subtype C was intermediate to ceftriaxone (MIC 16) and sensitive to oxacillin with 2% NaCl, ciprofloxacin, and tetracycline (MIC 2, <0.5, and 2). Subtype D was sensitive to ceftriaxone and oxacillin with 2% NaCl (MIC < 2 for both) and resistant to ciprofloxacin and tetracycline (MIC > 16 for both). Cultures of the 4 subtypes were maintained on brain heart infusion agar slants (Criterion, Hardy Diagnostics, Santa Maria, CA).

Before inoculation of meat, a sterile inoculating needle was used to transfer culture from a slant to a 5-mL brain heart infusion broth tube, incubated at 35°C for 24 h. After 24 h of incubation, the inoculum contained approximately 1.0×10^9 cfu/mL. One serial dilution of the original inoculum was then prepared in a 9-mL PBS dilution blank. Ten microliters of diluted cell suspension was placed on the center of each breast (skin side). The inoculum was spread evenly across the surface of each breast with a sterile inoculating loop. Using this concentration, each breast received 10^6 cfu. The dilution blank with the inoculum remained on ice throughout the procedure. Cell count in the inoculum was confirmed by plating serial dilutions on the surface of modified Oxford agar plates (**MOX**; Oxford medium base plus modified antimicrobic supplement, Remel Inc., Lenexa, KS).

UV Equipment

A shortwave UV lamp (model XX-15S, UVP Inc., Upland, CA) with 2 bulbs generating 254 nm of wavelength, 115 V, 60 Hz, and 0.68 amp was used in these experiments. The lamp was fitted with adjustable shelves for different levels of exposure. The entire unit was placed in a hood with a clear glass sliding door that was UV light impenetrable but that still allowed easy access to the UV unit. Ultraviolet intensity was measured in microwatts per square centimeter ($\mu W/cm^2$) using an UV intensity meter (model J-225, Blak-Ray, UVP Inc.). The shelves were adjusted so that the inoculated surface of each sample received a UV intensity of 1,000 $\mu W/cm^2$. Ultraviolet intensity at the expected location for the surface of the breast was measured before and after each trial for accuracy and repeatability.

UV Treatment Procedure

Fresh, raw, boneless, skinless chicken breast fillets were purchased from a supermarket. To reduce variation, multiple packages of the same brand, from the same processing plant (USDA plant number), with the same use-by date, and with similar package weights (mean package weight of 630 g) were purchased. The study was replicated 3 times for each subtype (1 replication per wk, 12 wk total).

In each of 3 replications, 20 breasts were inoculated (10 for UV treatment group and 10 for the untreated control group); 1 additional breast was tested for the initial presence of *L. monocytogenes* (negative control). Each group of 20 breast fillets was split into 5 trials. Each trial consisted of 2 UV-treated breasts and 2 non-UV-treated breasts [5 trials × (2 breasts for UV treatment + 2 breasts for control) = 20 breasts].

Following inoculation, the fillets remained at room temperature (~25°C) for 5 min to allow the inoculum to dry. Two of the 4 breasts in each trial were chosen at random for UV treatment. The corresponding 2 control fillets remained on the laboratory bench for the 5 min when the UV treatment was occurring. Following treatment, all fillets (including the uninoculated negative controls) were aseptically placed into individual sealable bags and stored for 24 h at 4°C to simulate the overnight storage and transportation to a further-processing plant.

After 24 h, 50 mL of sterile PBS was placed into each bag and vigorously shaken by hand for 60 s. Serial dilutions of the rinse were prepared in PBS. From each dilution, 0.1 mL was spread-plated onto duplicate MOX agar plates for enumeration. The MOX plates were incubated for 24 h at 35°C, and typical *L. monocytogenes* colonies were counted and recorded as colony-forming units/breast.

Color Evaluation

Thirty postchill broiler carcasses were obtained from a local poultry processing plant. The right and left breast fillets were removed from each carcass. Half of the fillets (30) were treated with UV light at a dose of 1,000 $\mu W/cm^2$ for 5 min. The 30 treated and the 30 control fillets were bagged in individual sealable bags. Breast meat color was determined using a reflectance colorimeter (Minolta

Table 1. Mean and SE of the mean base-10 logarithm colony-forming units/breast values of 4 subtypes of *Listeria monocytogenes* for ultraviolet- (UV) treated and untreated breast fillets[1]

Strain[2]	Control	UV treated	Probability
A	6.40 ± 0.02	4.52 ± 0.08	<0.0001
B	6.35 ± 0.03	4.31 ± 0.12	<0.0001
C	6.31 ± 0.02	4.70 ± 0.10	<0.0001
D	6.09 ± 0.03	4.02 ± 0.11	<0.0001

[1]n = 30.
[2]*Listeria monocytogenes* subtypes.

chroma meter, model CR-300, Minolta Co. Ltd., Ramsey, NJ), color was expressed using the Commission International D'Eclairage (1978) system of lightness (**L***), redness (**a***), and yellowness (**b***).

The colorimeter was calibrated, and breast fillet color was read through the bag material at 3 different locations on the skin side on d 0 and then again on d 7. From d 0 to 7, breasts were stored in the dark at 4°C.

Statistical Analysis

Listeria monocytogenes numbers counted on duplicate plates were averaged as colony-forming units/breast. Counts were log-transformed, and geometric means were used in statistical analysis. For the color values, reported values were averages of the 3 readings from each breast taken at d 0 and 7 (30 observations per mean). Statistical tests were performed by SAS 9.1 (SAS Institute, 2006). The GLM procedure was used to analyze both the *L. monocytogenes* recovery (for each subtype) and the color portion (treatment, UV vs. control and storage, 0 vs. 7 d) of this study.

RESULTS AND DISCUSSION

Enumeration of L. monocytogenes

No *L. monocytogenes* was recovered from any uninoculated negative control breasts. A 5-min UV light treatment (λ = 254 nm) at a dose of 1,000 $\mu W/cm^2$ was effective in decreasing the population of inoculated *L. monocytogenes* on raw, boneless, skinless chicken breasts (Table 1). All 4 subtypes subjected to UV treatment showed a highly significant ($P < 0.0001$) base-10 logarithm colony-forming units/breast reduction compared with the control group. Reduction in *L. monocytogenes* due to UV treatment ranged from 1.61 to 2.07 $\log_{10}$ cfu/breast. This represents a reduction of or near 99% for each subtype.

The use of UV irradiation as a bactericidal food safety process for fresh meats, including poultry, has been documented previously (Stermer et al., 1987; Wallner-Pendleton et al., 1994; Sumner et al., 1996; Kim et al., 2002). The approximate 2-log reduction in *L. monocytogenes* numbers in the current study is similar to the findings of Stermer et al. (1987), who evaluated the effect of UV light on bacteria on the surface of fresh beef round streak. However, Kim et al. (2002) reported less reduction when they examined the use of UV light treatment specifically against *L. monocytogenes* on poultry meat products. They reported a 0.48 $\log_{10}$ cfu/cm^2 reduction on chicken meat with skin and a 0.46 $\log_{10}$ cfu/cm^2 reduction on chicken meat without skin after UV treatment. There are several differences between the current study and the one reported by Kim et al. (2002), which could explain the difference in efficacy. Kim et al. (2002) used a lower dose (500$\mu W/cm^2$) and shorter treatment time (3 min). They also were working against an inoculum applied by submerging meat into a cell suspension, which could result in bacteria being deposited in areas protected from UV treatment. Ultraviolet light is only effective to kill bacteria on or very near the surface. Even just the cut edge of meat can provide enough protection to shield bacteria from exposure to UV light (Stermer et al., 1987).

The 10^6 cfu/breast inoculation load used in this study was more numerous than what would be expected to occur naturally on fresh breast meat. It is possible that a UV-mediated reduction of approximately 2 $\log_{10}$ cfu/breast could virtually eliminate *L. monocytogenes* cells on the surface of chicken meat. Such a process could help lessen the role of raw poultry meat as a vector for entry of the pathogen into poultry further-processing plants.

L*, a*, and b* Color Space Values

Ultraviolet light treatment caused only slight changes to breast meat color (Table 2). On the day of treatment, UV light caused no significant differences in any of the color values. After 7 d of storage at 4°C, the UV-treated breast fillets were less red (lower a* values, $P = 0.05$) and more yellow (higher b* values, $P = 0.07$) than control

Table 2. Lightness (L*), redness (a*), and yellowness (b*) color space values[1] (mean ± SE) of ultraviolet- (UV) treated[2] and untreated raw skinless, boneless chicken breast fillets

Item	Treatment	L*	a*	b*
Day				
0	Control	53.32 ± 0.8	1.50 ± 0.1	1.13 ± 0.3
0	UV	55.31 ± 0.6	1.40 ± 0.1	1.73 ± 0.2
Probability		0.07	0.59	0.12
7	Control	56.57 ± 0.5	0.63 ± 0.1	3.28 ± 0.2
7	UV	56.93 ± 0.3	0.39 ± 0.1	3.91 ± 0.2
Probability		0.56	0.05	0.07

[1]n = 30. Color values were noted immediately after UV irradiation (d 0) and 7 d later after being held at 4°C.
[2]Ultraviolet irradiation was administered at a dose of 1,000 $\mu W/cm^2$ for 5 min.

breast fillets. However, the L* values after 7 d of storage were almost identical between the UV-treated and control breasts. Overall, the effects of the UV treatment on color was minimal. The differences recorded electronically in this study would probably not be visually noticeable by most processors or consumers. Overall, color changes during 7 d of storage were similar to those reported by Petracci and Fletcher (2002) for untreated broiler meat. The current results are also in agreement with Wallner-Pendleton et al. (1994) and Stermer et al. (1987), who studied the effects of UV irradiation on chicken meat and beef.

In summary, a germicidal UV light treatment (1,000 $\mu W/cm^2$ for 5 min) applied to the surface of raw boneless skinless chicken breast fillets reduced the number of *L. monocytogenes* cells recovered. The UV irradiation process did have a small effect on a* values 7 d after UV exposure. However, this change would probably not be large enough to be noticed visually. Thus, a UV irradiation process could be administered in a raw poultry processing plant as an intervention to lessen the likelihood of *L. monocytogenes* being transferred to a further-processing plant on raw breast fillets. This could decrease the amount of *L. monocytogenes* entering a poultry further-processing plant and lessen the chances for contamination of fully cooked poultry product.

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