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Photohydroionization Reduces Shiga Toxin-Producing *Escherichia coli* and *Salmonella* on Fresh Beef with Minimal Effects on Meat Quality

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Abstract: The photohydroionization (PHI) technology utilizes a combination of UV light and low-level oxidizers to produce antimicrobial action, and thus, is a potential intervention to control pathogen contamination on surface of fresh beef. The objectives of the study were 1) to evaluate the effect of PHI on reduction of selected *Escherichia coli* (*E. coli*) O157:H7, non-O157 Shiga toxin-producing *E. coli* (STEC; O26, O45, O103, O111, O121, O145), antimicrobial resistant (AMR) and non-AMR *Salmonella* strains inoculated on beef flanks, and 2) to evaluate the effect of PHI treatment on the lean color and lipid oxidation of beef during refrigerated storage. Inoculated beef flanks were exposed to PHI treatment for 0 (control), 15, 30, or 60 s at 4°C. Exposure to PHI for 15 s reduced ($P \leq 0.05$) pathogens on the surface of fresh beef ranging from 0.3 to 0.9 log CFU/cm^2. Increasing the exposure time to 60 s did not improve ($P > 0.05$) reductions over 15 s for the majority of the selected pathogens, but yielded pathogen reductions ranging from 0.5 to 1.1 log CFU/cm^2. Over all storage times when beef samples were exposed to PHI for 75 s, no difference ($P > 0.05$) was detected on lean a* value (24.67 versus 24.95), of treated and control fresh beef tissues, respectively. The highest TBARS values after storage for 14 d at 4°C was 0.33 mg MDA/kg of meat indicating that no oxidative rancidity occurred for treated beef samples. The PHI technology with 15 to 75 s exposure time was effective in controlling STEC and *Salmonella* contaminated on surface of fresh beef without causing adverse effects on fresh beef quality while reducing water and energy use. Further study of PHI treatment parameters under commercial plant conditions and ultimate validation of those parameters will be necessary for commercial implementation.

Keywords: beef, interventions, meat quality, photohydroionization, *Salmonella*, STEC
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Introduction

Foodborne pathogens are estimated to cause over 37 million illnesses per year in the U.S., resulting in 229,000 hospitalizations and 2,600 deaths due to consumption of contaminated food (CDC, 2018a; Scallan et al., 2011). *Salmonella* is the most common foodborne bacteria and is responsible for more than a million foodborne illnesses and 452 deaths each year (CDC, 2018b). Additionally, Shiga toxin-producing *Escherichia coli* (STEC) cause over 265,000 infections annually, leading to 3,700 hospitalizations and 30 deaths (CDC, 2018b; Scallan et al., 2011).

Salmonella and STEC may contaminate beef carcasses during cattle harvest, which involves removal of hides and intestines (Koohmaraie et al., 2005). Subsequent fabrication and processing steps may spread bacteria to other products. Thus, antimicrobial interventions are needed to reduce bacterial populations, including spoilage bacteria and pathogens. Numerous antimicrobial interventions have been used

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in the beef industry during harvesting including hot water, diluted organic acids, and oxidizing agents to reduce pathogens from surfaces of beef carcasses (Acuff, 2005; Ayebah et al., 2005; Barkate et al., 1993; Cutter and Siragusa, 1994; Geornaras et al., 2012; Gill and Badoni, 2004; Kalchayanand et al., 2008; Kalchayanand et al., 2009; Kalchayanand et al., 2012; King et al., 2005; Penney et al., 2007). With washing and decontamination throughout the processing steps, the amount water used can range from 1,181 to 2,275 L per 454 Kg live weight for processing of beef cattle (Johns, 1995). According to Ziara et al. (2016), the antimicrobial interventions used 15.7% of the total water usage, approximately 22.2 to 42.6 L per 454 Kg of animal live weight. In addition, a great amount of wastewater is generated during meat processing ranging from 1,644 to 5,670 L per 454 Kg live weight (EPA, 2004), while only 16% of wastewater is reused (José et al., 2013). Therefore, water and energy conservation has become an issue for the meat industry. With growing water and sewer charges and effluent regulations, these figures put an economic strain on processors (Ziara et al., 2016). The meat industry is looking for non-water based as well as non-thermal based antimicrobial systems that are effective for decontamination of foodborne pathogens on surfaces of fresh meat to reduce water usage and to limit the environmental footprint.

Photohydroionization (PHI) technologies utilize a combination of advanced oxidation (AO) gases (such as ozone, hydrogen peroxide, super oxides, and hydroxyl ions) in conjunction with germicidal ultraviolet (UV) radiation to control and reduce bacterial contamination (Grinshpun et al., 2004; Ortega et al., 2007). Ozone acts as a strong oxidizer that attacks the cellular membrane of bacterial cells (Khadre et al., 2001). Ozone auto-decomposes rapidly to produce oxygen. Therefore, ozone is an effective germicide and leaves no residue in food (Prabha et al., 2015). Ozone was approved by the Food and Drug Administration (FDA) in June 2001 (USDA-FDA, 2001) as a sanitizer for food-contact surfaces and for direct application on food products. Both AO and UV light have been used independently by food processing industry for many years (Saini et al., 2014; Sastry et al., 2000). Therefore, the combination of these 2 natural disinfectants creates an environment that is effective in reducing microorganisms on food surfaces (Saini et al., 2014; Sastry et al., 2000). The combination of AO and UV light compensates for the limitation of UV light not contacting all surfaces when there are folds or cuts that can hide bacteria.

The PHI technology is a nonthermal process and does not require the use of water nor leave any residual by-product. Therefore, PHI technology may be attractive to not only the meat industry as an alternative antimicrobial intervention, but also modern consumers demanding less processing and less chemicals. A few studies have evaluated the antimicrobial effect of PHI against bacteria in the air, on surface materials, and on food surfaces (Grinshpun et al., 2004; Ortega et al., 2007; Saini et al., 2014; Skowron et al., 2018). However, the use of PHI treatment to decontaminate STEC, and *Salmonella* on surfaces of fresh beef is very limited. To this end, the objectives of the study were to determine the effectiveness of PHI treatment on the reductions of STEC, AMR, and non-AMR *Salmonella* on beef surfaces, as well as its effect on color and lipid oxidation of treated beef during dark refrigerated storage.

Materials and Methods

Bacterial strains, growth conditions and preparation of inocula

A total of 21-strains of STEC, antimicrobial resistant (AMR) *Salmonella*, and non-AMR *Salmonella* from the U. S. Meat Animal Research Center (USMARC) culture collection, from human and cattle isolates, were used in the study. Two strains each of non-O157 STEC serogroup O26, O45, O103, O111, O121, and O145, 3 strains of *E. coli* O157:H7, 3 strains of non-AMR *Salmonella*, and 3 strains of AMR *Salmonella* were grown statically in nutrient broth (NB; Difco, Becton and Dickinson, Detroit, MI) at 37°C for 16 to 18 h. Each bacterial strain was adjusted for optical density at 600 nm (McFarland, 1907) using a spectrophotometer (Thermo Spectronic, Fisher Scientific, Pittsburgh, PA) with NB to a cell concentration of approximately 10^8 log CFU/ml. An equal volume of each strain was mixed to form 3 groups of inoculum (Table 1) based on colony colors developed on the USMARC chromogenic agar (UCA) medium (Kalchayanand et al., 2013) as follows: (a) Inoculum-1 was composed of *E. coli* O157:H7 (strain 43895, FSIS 3, and FSIS 4), O111:NM (3007:85), O121:H19 02E-2074, and *S.* Newport (15124). (b) Inoculum-2 was composed of *E. coli* O103:H2 (strain 2421 and G5550637), O26:H11 (strain 3392 and 3819), O121:H7, *S.* Anatum (NS-127), *S.* Newport (13324), and *S.* Typhimurium (DT-104). (c) Inoculum-3 was composed of *E. coli* O45:H2 (strain 01E-1269 and O45 WDG3), O111:NM (1665), O145:NM and O145 GB, *S.* Anatum (NS-114) and *S.* Typhimurium (14249). The rationale behind the three inocula was the limitation of the UCA medium to dif-

Table 1. Bacterial strains and inoculum information

Bacterial species	Serotype	Strain/source[1]	Antibiotic resistance phenotype and profile[2]	Colony color on USMARC chromogenic agar	Inoculum group	Volume in inoculum group
Escherichia coli	O157:H7	ATCC 43895/Hum.	N/A	Green	1	0.33 ml
Escherichia coli	O157:H7	FSIS 3/Hum.	N/A	Green	1	0.33 ml
Escherichia coli	O157:H7	FSIS 4/Hum.	N/A	Green	1	0.33 ml
Escherichia coli	O26:H11	3891/Hum.	N/A	Turquoise	2	0.50 ml
Escherichia coli	O26:H11	3392/Hum.	N/A	Turquoise	2	0.50 ml
Escherichia coli	O45:H2	01E-1269/Hum.	N/A	Light blue green	3	0.50 ml
Escherichia coli	O45	WDG3/Bov.	N/A	Light blue green	3	0.50 ml
Escherichia coli	O103:H2	2421/Hum.	N/A	Light green	2	0.50 ml
Escherichia coli	O103:H2	G5550637/Hum.	N/A	Light green	2	0.50 ml
Escherichia coli	O111:NM	1665/Hum.	N/A	Dark blue green	1	1.00 ml
Escherichia coli	O111:NM	ECRC3007:85/Hum.	N/A	Small dark blue green	3	1.00 ml
Escherichia coli	O121:H7	GB/Bov.	N/A	Light blue gray	3	1.00 ml
Escherichia coli	O121:H19	:02E-2074/Hum.	N/A	Purple	1	1.00 ml
Escherichia coli	O145:NM	GS5578620/Hum.	N/A	Purple	3	0.50 ml
Escherichia coli	O145	GB/Bov.	N/A	Purple	3	0.50 ml
Salmonella	N/A	Anatum NS-114/Bov.	Susceptible	Colorless	3	0.50 ml
Salmonella	N/A	Anatum NS-127/Bov.	MDR: CSSuT	Colorless	2	0.33 ml
Salmonella	N/A	Newport 15124/Bov.	Susceptible MDR:	Colorless	1	1.00 ml
Salmonella	N/A	Newport 13324/Bov.	AmApFT(Ax)CSSuTe	Colorless	2	0.33 ml
Salmonella	N/A	Typhimurium14249/Bov.	Susceptible	Colorless	3	0.50 ml
Salmonella	N/A	Typhimurium DT-104/Bov.	MDR: ACSSuTe	Colorless	2	0.33 ml

[1]Source; Hum., human isolate; Bov., bovine isolate. N/A, not applicable.

[2]MDR, multi-drug resistant; Am, amoxicillin–clavulanic acid; Ap, ampicillin; F, cefoxitin; T, ceftiofur; Ax, ceftriaxone; C, chloramphenicol; S, streptomycin; Su, sulfisoxazole; Te, tetracycline. Antibiotics in parentheses indicate intermediate resistance (i.e., the MIC was increased but was below the resistance breakpoint).

ferentiate each serogroup due to diversity of non-O157 STEC. For example, serogroups O45, O103, and O157 have similar colony color, making harder differentiation among the serogroups. Thus, 3 inoculum groups were created to ensure accurate identification and simulate multiple serogroups contamination. All three inocula were placed in an ice bath to restrict further growth before inoculation on surfaces of fresh beef.

Fresh beef sample preparation and inoculation

Frozen vacuum-packaged beef flanks (cutaneous trunci muscle; previously collected from a local beef packing plant) were thawed at 4°C overnight, aseptically cut into 5 × 5 cm^2 sections, and stored at 4°C until use for inoculation. A total of 108 meat sections were individually placed on sterile Petri dishes inside a biological safety cabinet. A 50-µL aliquot of each inoculum was inoculated on the surface of individual 25 cm^2 sections and spread using a sterile cell spreader to a final cell concentration approximately 10^5 to 10^6 CFU/cm^2. Inoculated meat sections were maintained on sterile Petri dishes at room temperature for 15 min to resuscitate the bacterial cells from the cold stress and to allow bacterial attachment to the surfaces of fresh beef. For each replication, 3 meat sections were inoculated individually with 3 inocula and were exposed to 4 PHI treatment times (0, 15, 30, and 60 s). Three independent repetitions were conducted for the study. Therefore, the total meat samples were 108 sections (3 × 3 × 4 × 3 = 108).

For determination of color and lipid oxidation, there was no bacterial inoculation. Frozen vacuum-packaged beef flanks were thawed at 4°C overnight and cut into 10 × 10 cm^2 sections. A total of 24 beef sections with lean exposed surfaces were used for color analysis, while another 24 beef sections with external fat surfaces were used for lipid oxidation analysis

Photohydroionization (PHI) Treatment

Meat samples were placed in a Food Pure chamber (RGF Environmental Group, Inc., Riviera Beach, FL). The Food Pure is composed of 2 germicidal components (Fig. 1) as follows: a) 4 UV-C lamps (254 nm) on the top of the Food Pure chamber, and b) Photohydroionization (PHI) System located inside the control unit. The PHI is an advanced oxidation (AO) technology developed and owned by RGF Environmental Group. It consists of a broad spectrum high intensity UV light bulb targeted on a quad metallic (rhodium, titanium, silver, and copper)

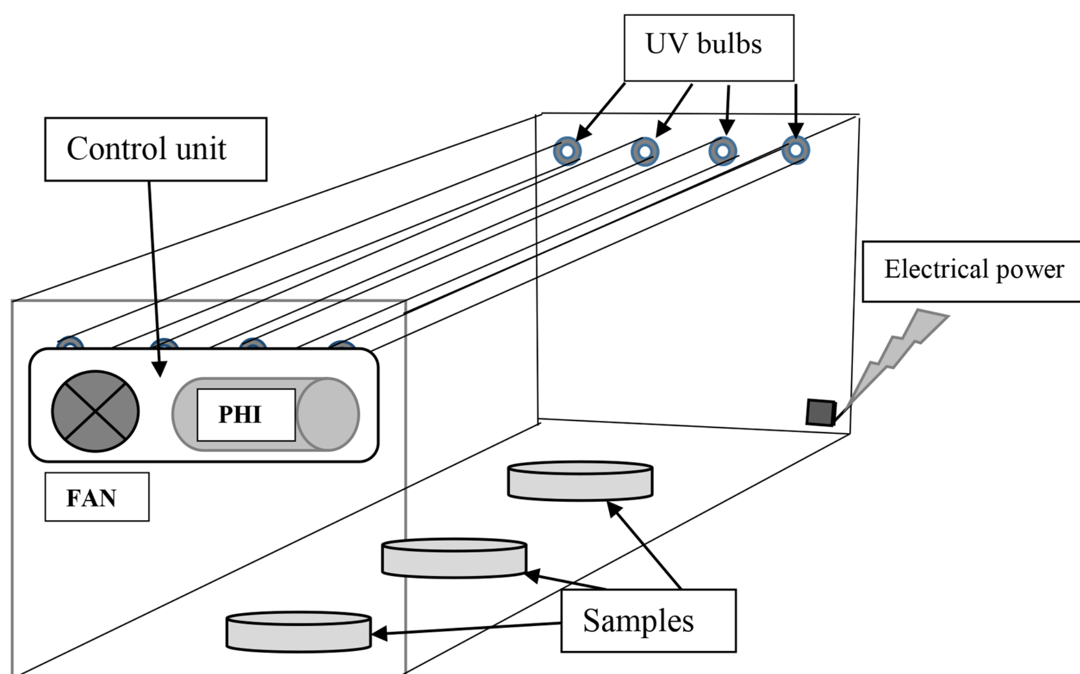

Figure 1. Food Pure chamber diagram.

catalysts ultraviolet (UV) target in a low-level ozone and moist atmosphere. This creates an advanced oxidation process providing user friendly oxidizers such as hydrogen peroxides, super oxide ions, and hydroxide ions that revert back to oxygen and hydrogen. The distance between the surface of meat samples and the UV source was 12.7 cm. Energy delivered by the UV source at this distance was 0.0042 J/cm^2 using a UV meter (Blak-Ray, Upland, CA). The UV dose was calculated from 0.0042 J/cm^2 × exposure time in seconds. The ozone and hydrogen peroxide levels generated within the chamber according to manufacturer's specifications were 200 to 300 μg/kg and 150 to 200 μg/kg, respectively. Before subjecting inoculated fresh beef sections to the PHI treatments, three inoculated beef samples were randomly picked and placed individually into three filtered bags (Whirl-Pak, Nasco, Fort Atkinson, WI) to serve as untreated controls (0 s). The rest of inoculated beef samples were randomly assigned to one of the PHI treatments (3 samples per inoculum per treatment) and exposed to PHI for 15, 30, or 60 s at 4°C. Both control and PHI treated samples were enumerated for the survivors. Three independent repetitions were conducted for the study.

Microbiological analysis

Control and PHI treated samples were placed individually in filter bags (Whirl-Pak, Nasco) and 50-mL buffered peptone water (BPW, Difco, BD) was added. After 2-min homogenization with a stomacher (BagMixer 400, Interscience, Weymouth, MA), a 1-mL aliquot of each sample was 10-fold serially diluted in maximum recovery diluent (MRD, Difco, BD). Appropriate dilutions were spiral plated (Spiral Biotech, Norwood, MA) on UCA (chromogenic selective medium; Kalchayanand et al., 2013) to enumerate STEC and *Salmonella* colony counts and brain heart infusion agar (BHA, non-selective medium) to enumerate for total aerobic plate count (APC). The UCA plates were incubated at 37°C for 24 h and set for 1 h at room temperature for full color development as described by Kalchayanand et al. (2013). The BHA plates were incubated at 37°C for 24 h. Colonies were counted, recorded, and transformed as log CFU/cm^2 for statistical analysis. The detection limit of using the spiral plater was 60 CFU/cm^2. Two presumptive colonies for each STEC serogroup and *Salmonella* per plate were picked for confirmation with multiplex PCR (Perelle et al., 2004; Kim et al., 2006; DebRoy et al., 2005; Feng et al., 2005). The D-value, the time to kill 90% or 1 log reduction after the PHI exposure, was calculated from the absolute value of the inverse of the slope from linear regression between logarithm of survivors and exposure times (Bradshaw et al., 1987).

Instrumental color measurement

The average calculated D-value of STEC and *Salmonella* on surfaces of fresh beef exposed to PHI was

74 s. Thus, the exposure time was set to 75 s to determine the effect of PHI treatment on the meat quality traits of color and lipid oxidation of fresh beef. On each sampling day before subjecting beef sections to PHI treatments, 6 samples were randomly picked and placed individually into 6 bags (Whirl-Pak, Nasco) to serve as untreated controls. Other 6 beef samples were exposed to PHI treatment for 75 s at 4°C. Both control and PHI treated beef samples were stored aerobically in the dark at 4°C for 0, 1, 3, 7, and 14 d. Immediately following PHI treatment, both control and treated samples were measured for color of each beef sample using a spectrophotometer (MiniScan EZ; Hunter Association Laboratory Inc., Reston, VA). Surface lean color was measured using illuminant A/10° observer at three different locations and the 3 values were averaged to obtain a single value for each beef sample. Instrumental color was expressed as CIE L* (lightness), a* (redness) and b* (yellowness) values. Before measurement, the spectrophotometer was standardized using white and black tile standards. Two independent repetitions were conducted for the study.

Thiobarbituric Acid Reactive Substances (TBARS) analysis

On each sampling day before subjecting beef sections to PHI treatments, 6 samples were randomly picked and placed individually into 6 bags (Whirl-Pak, Nasco) to serve as untreated control. Other 6 beef samples were exposed to PHI treatment for 75 s at 4°C, placed individually into the bags, and stored aerobically at 4°C for 1, 3, 7, and 14 d. After each storage period, surface fat tissue (10 g) of each sample was excised using a sterile scalpel and added to 10 mL chilled phosphate buffered saline (pH 6.5), then homogenized for 2 min at 20,000 rpm using a homogenizer (Tekmar Tissumizer, SDT-181059, Tekmar Co., Cincinnati, OH). The TBARS analysis was used to measure lipid oxidation from the tissue homogenate. Thiobarbituric acid reacts with the oxidation products of fat to form malonaldehyde (MDA), which was determined according to manufacturer's instructions using a Quantichrom TBARS Assay Kit (DTBA-100, BioAssay Systems, Hayward, CA). On different sampling days, surface fat was excised from different locations on the surface of the same beef flank sections. No location was sampled twice. The results were expressed as mg MDA/kg. Two independent repetitions were conducted for the study.

Statistical analysis

If the strains within the same STEC O serotype were used in different inoculum groups, the counts for that STEC serotype was the average counts of the strains regardless of inoculum group. All the survivor counts were log transformed prior to analysis. Experimental design for the study was randomized complete block designs with PHI treatment as a fixed effect. Analysis of variance (ANOVA) was performed using Proc Mixed (SAS version 9.4, SAS Inst. Inc., Cary, NC). The least squares means were calculated and pairwise comparisons of means were determined with Tukey-Kramer test method to compare microbial counts of surviving STEC and *Salmonella* on untreated control and PHI treated samples within each tested pathogen inoculum group. A separate ANOVA was conducted to determine whether AMR and non-AMR *Salmonella* were differentially sensitive to the PHI treatment at different exposure times. For color and lipid oxidation, the analysis was a 2 × 5 factorial arrangement with repeated measures. The PHI treatment and storage time were the 2 fixed effects. Analysis of variance procedure was performed using nlme R package (Pinheiro et al., 2011). One PHI treated sample was an extreme outlier and was removed from the statistical analysis for lipid oxidation. Each repetition within the experiment was considered as a blocking factor in a randomized complete block design. The significance of differences was defined as $\alpha = 0.05$.

Results and Discussion

Antimicrobial effect of photohydroionization (PHI) treatment

The least squares means survivors and reductions of pathogenic and aerobic bacteria on surfaces of fresh beef after exposure to PHI unit for 15, 30, and 60 s compared with controls are presented in Table 2. The PHI treatment reduced ($P \leq 0.05$) both pathogenic and aerobic bacteria with all treatment times (except non-AMR *Salmonella* at 15 s). Non-O157 STEC (serogroups O26, O45, O103, O111, O121, and O145) and *E. coli* O157:H7 reductions on surfaces of fresh beef after exposure to PHI for 30 or 60 s ranged from 0.7 log to 1.1 log. This range of reduction indicated that the minimally effective exposure time was from 30 to 60 s, which is similar to the findings from Saini's group (Saini et al., 2014). The PHI treatment on surfaces of ready-to-eat turkey contaminated with *Listeria monocytogenes* reduced approximately 1 log after exposure for 30 or 60 s (Saini et al., 2014). The PHI treatment reduced AMR *Salmonella* by 0.9 log after exposure for 15 s, while only 0.3 log reduction was found

Table 2. Populations[1] of STEC, *Salmonella* (AMR and non-AMR), and aerobic bacteria (APC) on fresh beef flank before (control) and after exposure to photohydroionization (PHI)

	Survivors of pathogens and aerobic bacteria (log CFU/cm^2)[2]									
PHI Treatment	O26	O45	O103	O111	O121	O145	O157	non-AMR[3] *Salmonella*	AMR[3] *Salmonella*	APC
Control	5.5 (0.1)^{A}	5.4 (0.1)^{A}	5.3 (0.1)^{A}	5.5 (0.1)^{A}	5.1 (0.1)^{A}	5.5 (0.1)^{A}	5.5 (0.1)^{A}	5.2 (0.1)^{A}	5.4 (0.3)^{A}	6.4 (0.1)^{A}
15 s	4.7 (0.1)^{B}	4.8 (0.1)^{B}	4.4 (0.1)^{B}	4.9 (0.1)^{B}	4.4 (0.1)^{B}	4.9 (0.1)^{B}	4.9 (0.1)^{B}	4.9 (0.1)^{AB}	4.5 (0.3)^{B}	5.5 (0.1)^{B}
	[0.8][4]	[0.6]	[0.9]	[0.6]	[0.7]	[0.6]	[0.6]	[0.3]	[0.9]	[0.9]
30 s	4.6 (0.1)^{B}	4.5 (0.1)^{BC}	4.3 (0.1)^{B}	4.8 (0.1)^{B}	4.3 (0.1)^{B}	4.7 (0.1)^{B}	4.7 (0.1)^{B}	4.7 (0.1)^{B}	4.4 (0.3)^{B}	5.5 (0.1)^{BC}
	[0.9]	[0.9]	[1.0]	[0.7]	[0.8]	[0.8]	[0.8]	[0.5]	[1.0]	[0.9]
60 s	4.5 (0.1)^{B}	4.4 (0.1)^{C}	4.3 (0.1)^{B}	4.8 (0.1)^{B}	4.4 (0.1)^{B}	4.4 (0.1)^{B}	4.8 (0.1)^{B}	4.7 (0.1)^{B}	4.4 (0.3)^{B}	5.3 (0.1)^{C}
	[1.0]	[1.0]	[1.0]	[0.7]	[0.7]	[1.1]	[0.7]	[0.5]	[1.0]	[1.1]
Overall[5]								4.8 (0.12)^{X}	4.4 (0.13)^{Y}	

$^{A-C}$ LSmeans in the same column for each target bacterium bearing a common letter are not significantly different ($P > 0.05$).

$^{X-Y}$ LSmeans in the same row for each *Salmonella* bearing a common letter are not significantly different ($P > 0.05$).

[1]Inoculum-1: *E. coli* O157:H7 (strain 43895, FSIS 3, and FSIS 4), O111:NM (3007:85), O121:H19 02E-2074, and Newport (15124). Inoculum-2: *E. coli* O103:H2 (strain 2421 and G5550637), O26:H11 (strain 3392 and 3891), O121:H7, Anatum (NS-127), Newport (13324), and Typhimurium (DT-104). Inoculum-3: *E. coli* O45:H2 (strain 01E-1269 and O45 WDG3), O111:NM (1665), O145:NM and O145 GB, Anatum (NS-114) and Typhimurium (14249).

[2]Values are least squares means (LSmeans) and the number in parentheses represents standard errors of means.

[3]Non-AMR *Salmonella*, Anatum NS-114, Newport 15125, and Typhimurium 14249 are pan susceptible to antibiotics; AMR *Salmonella*, Anatum NS-127, Newport 13324, and Typhimurium DT-104 are resistant to CSSuT, AmApFT(Ax)CSSuTe, and ACSSuTe, respectively (Am, amoxicillin–clavulanic acid; Ap, ampicillin; F, cefoxitin; T, ceftiofur; Ax, ceftriaxone; C, chloramphenicol; S, streptomycin; Su, sulfisoxazole; Te, tetracycline). Antibiotic in parenthesis indicates intermediate resistance (i.e., the MIC was increased but was below the resistance breakpoint).

[4]The number in brackets represents log reductions (CFU/cm2) between control and treated fresh beef samples.

[5]Each datum of non-AMR and AMR was pooled together regardless of treatment times before ANOVA to determine the effect of PHI treatment. The number in parentheses represents standard error of means.

for non-AMR *Salmonella* with the same exposure time (Table 2). Increasing the treatment time from 15 to 60 s did not affect any reduction except non-O157 STEC serogroup O45. Thus, the reduction data for AMR and non-AMR *Salmonella* were pooled together for each group regardless of exposure times and inoculum groups. The result indicated that non-AMR *Salmonella* was less sensitive to PHI treatment compared to AMR *Salmonella* (Table 2). One of the germicides of PHI is UV light. The mode of action of UV light against bacteria is targeted to damage their DNA/RNA which inhibits replication and transcription and subsequently prevents the cells from multiplying (von Sonntag et al., 2004). However, various microorganisms respond differently to the doses of UV light (Chevrefils et al., 2006; Hijnen et al., 2006). For example, non-AMR *Salmonella* populations were less sensitive to UV treatment (exposed to PHI for 60 s) compared to STEC (Table 2). Not only different species show differences in sensitivity to UV treatment, but also several researchers found that different strains of the same species such as *E. coli* have different UV sensitivity (Sommer et al., 1998; Sommer et al., 2000; Malley et al., 2004; Chintagari et al., 2015). Besides differences in bacterial species and strains, there are several factors that affect efficiency of PHI treatment as follows: The inactivation of PHI treatment also differed on different surface materials, and on the forms of bacterial cells such as biofilm and planktonic cells (Skowron et al., 2018). The authors also reported that the biofilm was harder to eliminate than the planktonic form on the tested surface materials. In addition, the chemical composition of treated samples played an important role in bacterial resistance and survival, which produced different inactivation results when exposed to UV light (Hamidi-Oskouei et al., 2015). For example, the reduction of *L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7 inoculated on the surface of agar plates was higher than the inoculated the pathogens on surfaces of fresh beef (Kim et al., 2014). The differences in surface contour and porosity are another factor in efficiency of UV light produced from PHI treatment. Wong et al. (1998) reported that the antimicrobial efficacy of UV light was greater against pathogens on pork skin than pork muscle surfaces.

The PHI treatment reduced aerobic bacteria approximately 1 log, when exposed to the treatment for 15, 30, or 60 s (Table 2). Aerobic bacteria were enumerated using non-selective medium (BHA). However, no difference in bacterial reduction was detected among 15, 30, or 60 s exposed to PHI treatment. This indicates that no additional injured cells were recovered after the treatment. Similar findings were reported for PHI treated cheese inoculated with *L. monocytogenes* (Saini et al., 2014). The authors

enumerated *L. monocytogenes* using a selective medium (modified oxford medium agar, MOX) compared to using a nonselective medium for resuscitation of the injured cells followed by overlaying with MOX and found no difference in the detection of *L. monocytogenes*. This may be part of the action of ozone generated from PHI treatment. Ozone attacks bacterial cells and causes cell lysis within a few seconds. Therefore, the exposed bacteria have very little chance to develop resistance to ozone (Pope et al., 1984). The oxidizing potential of ozone is 2.07V, which is higher than that of chlorine (1.36V; Manley and Niegowski, 1967) making ozone a strong disinfecting agent. Several studies have been conducted using ozone as an antimicrobial intervention to reduce bacteria on surfaces of fresh meat. However, the efficacy results of ozone treatment have been highly variable, basically due to factors changing reactivity and antimicrobial efficacy, for example temperature and pH (Bablon et al., 1991; Achen and Yousef, 2001). Since ozone is a strong oxidizer, the presence of organic substances may compete with microorganisms for ozone (Emerson et al., 1982; Priyanka et al., 2014). Aqueous ozone had a 2.5 log reduction of aerobic bacteria on beef tissue using 0.5% ozone concentration (Gorman et al., 1997), but the ozone concentration was much higher than the Food Pure chamber used in our study. Another study reported that gaseous ozone reduced aerobic bacteria and coliforms from pork 0.45 to 1.04 log and 0.26 to 0.30 log reduction, respectively (Jeong et al., 2007). Dipping beef trimmings into 1% ozone solution for 7 or 15 min effectively reduced *E. coli* and *S.* Typhimurium (Stivarius et al., 2002). However, other studies reported that aqueous ozone treatment did not reduce *E. coli* O157:H7 and *Salmonella* on surfaces of beef carcasses and bovine head and cheek meat (Castillo et al., 2003; Kalchayanad et al., 2008). The ineffectiveness of ozone is probably due to decomposition of ozone to oxygen on warm beef carcasses and bovine head and cheek meat. Similar findings were reported when *E. coli* O157:H7-contaminated apples were treated with ozone at 4, 22, and 45°C, and the least effective temperature of ozone for reducing *E. coli* O157:H7 was 45°C (Achen and Yousef, 2001).

Decimal reduction time (D-value)

The rate of inactivation of O157:H7 STEC with PHI treatment is presented in Fig. 2 with correlation coefficient (*r*) of 0.7991. The rates of inactivation curves of non-O157 STEC and *Salmonella* were similar to the curve in Fig. 2 (data not shown). The inactivation curves indicated that PHI treatment deviated from the first-order disinfection kinetics where no further increase in inactivation occurred at higher dose of PHI (Hijnen et al., 2006). Although the rates of inactivation of pathogens studied were not linear with exposure times, an attempt was made to calculate the estimated D-values from the linear regressions for non-O157 STEC serogroups O26, O45, O103, O111, O121, O145, *E. coli* O157:H7, and non-AMR and AMR *Salmonella*. Absolute values of the inverse slope of linear regressions between survivors (log CFU/cm^2) and exposure time were used for D-value calculation (Bradshaw et al., 1987). The D-values due to PHI treatment are presented in Table 3. Among the STEC and *Salmonella*, D-values with PHI treatment ranged between 65 and 92 s. The D-value of O157:H7 STEC on surfaces of fresh beef exposed to UV treatment was 85 s. The average D-values from five strains of *E. coli* O157:H7 in phosphate buffer saline solution were 68.2 and 15 s after exposure at 0.00104 and 0.00302 J/cm^2, respectively (Chintagari et al., 2015). This indicated that the chemical composition of the treated samples played an important role in pathogen resistance and survival. For example, fresh beef versus phosphate buffered saline produced greatly different inactivation results when exposed to UV treatment. Overall, the average D-value of STEC and *Salmonella* on surfaces of fresh beef exposed to PHI was 74 s. Thus, the exposure time was set to 75 s to determine the effect of PHI treatment on the meat quality traits of color and lipid oxidation of fresh beef. Based on estimated D-values in Table 3, exposed for 75 s to PHI treatment would inactivate 1 log reduction of STEC strains and *Salmonella* except serogroups O111, O121, O157:H7, and non-AMR *Salmonella* on surface of fresh beef.

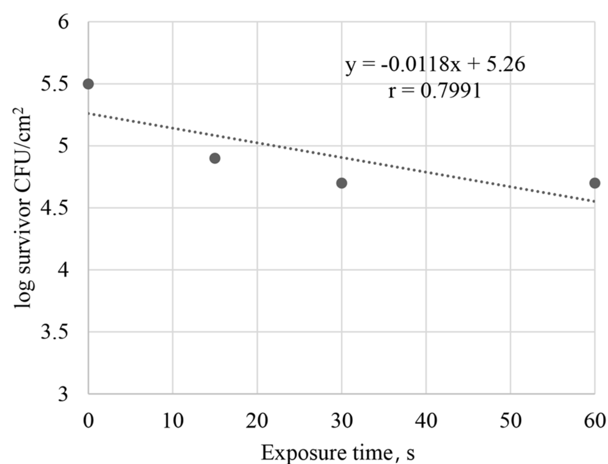

Figure 2. Rate of inactivation of E. coli O157:H7a on surfaces of fresh beef after RCI treatment. Each survivor point was the average of 3 strains of *E. coli* O157:H7 (43893, FSIS 3, FSIS4).

In this study, the inactivation of PHI treatment did not follow the first order disinfection kinetics. The inactivation of target organisms started with a sharp reduction rate and less reduction rate at higher PHI dosages. This

Table 3. Estimated D values[1] of pathogens inoculated on surfaces of fresh meat following photohydroionization (PHI) treatment

	D-value (s)	
Pathogen[2]	PHI Treatment	r[3]
O26	70	0.80
O45	65	0.88
O103	72	0.73
O111	92	0.77
O121	90	0.72
O145	58	0.91
O157	85	0.80
Non-AMR Sal	65	0.99
AMR Sal	72	0.73
Overall average[4]	74	

[1]The D value was calculated from the absolute value of the inverse of the slope from linear regression between logarithm of survivors and exposure time.

[2]O26, STEC serogroup O26; O45, STEC serogroup O45; O103, serogroup O103; O111, serogroup O111; O121, serogroup O121; O145, serogroup O145; O157, STEC O157:H7; Non-AMR Sal, non-antimicrobial resistant *Salmonella*; AMR, antimicrobial resistant *Salmonella*.

[3]r, Correlation coefficient indicates the linear correlation between survivors and exposure times.

[4]D-values for each PHI treatment were pooled together regardless of bacterial species to represent natural contaminations. The average D-value was used as an exposure time for the effect of PHI treatment on color and lipid oxidation.

deviation is called "tailing". The tailing effect also was reported with the study of UV treatment of various STEC strains (Chintagari et al., 2015). Tailing effect normally starts after at least 99% of initial population are inactivated (Hijnen et al., 2006). The causes of tailing effect are still unknown, but have been hypothesized as clumping of microorganisms, resistance of the subpopulation of microorganisms, and food surface topography that may block the UV irradiation (Hijnen et al., 2006; McLeod et al., 2017). Stermer et al. (1987) reported that the rough surfaces of fresh beef tended to shield the bacteria from UV treatment. Similarly, the UV treatment reduced more bacteria on smooth surfaces such as stainless steel, rather than on chicken meat (Kim et al., 2002). The shielded bacteria should be inactivated by reactive oxygen species (ROS) from the AO gases (Ortega et al., 2007). However, several bacterial pathogens produce superoxide dismutase (SOD) and catalase, 2 enzymes that degrade reactive oxygen species (Fierer et al., 2017).

Effect of photohydroionization (PHI) treatment on instrumental color and lipid oxidation of beef

Hunter tristimulus color values (L*, a*, and b*) of PHI treated fresh beef are presented in Table 4. No interactive effect of treatment and storage time was identified ($P > 0.05$) on lean color and lipid oxidation for beef flanks. Over all storage times, the lean L* values were higher ($P < 0.05$) for PHI treated samples than for control samples, indicating that PHI treated lean had a lighter appearance. In the present study, no difference ($P > 0.05$) was detected on lean a* value, suggesting that both control and treated samples appeared to be a similar red color. However, in the study conducted by Cárdenas et al. (2011), a* values were lower for beef exposed to gaseous ozone for 3 h at 4°C in comparison with those of the untreated control. Across storage times at 4°C, the L* values increased through d 3, then declined by d 14 to the d 7 levels. The a* values increased slightly on d 1 to d 7, then declined to the lowest level at d 14. The b* values increased on d 1 to 7, then declined to d 0 levels at d 14 (Table 4).

The oxidative changes in the food products caused by reactive oxygen species from the AO gases are the

Table 4. Least squares means (standard error of means) for lean color traits of control and photohydroionization (PHI) treated fresh beef tissues during storage at 4 °C

Traits[1]	L*	a*	b*	TBARS
Treatment[2]				
Control	53.17 (2.83)^{B}	24.95 (1.40)^{B}	20.19 (0.41)^{B}	0.19 (0.06)^{B}
PHI	55.19 (2.83)^{A}	24.67 (1.40)^{B}	21.09 (0.41)^{B}	0.19 (0.06)^{B}
Storage time[3]				
Day 0	51.15 (2.83)^{C}	24.54 (1.43)^{B}	18.40 (0.46)^{C}	0.12 (0.06)^{C}
Day 1	53.08 (2.83)^{B}	26.53 (1.43)^{A}	21.31 (0.46)^{B}	0.17 (0.06)^{B}
Day 3	58.44 (2.83)^{A}	25.49 (1.43)^{AB}	21.44 (0.46)^{AB}	0.15 (0.06)^{BC}
Day 7	54.38 (2.83)^{B}	27.09 (1.43)^{A}	22.69 (0.46)^{A}	0.18 (0.06)^{B}
Day 14	53.86 (2.83)^{B}	20.40 (1.43)^{C}	19.37 (0.46)^{C}	0.33 (0.06)^{A}

[1]L*, the lightness of meat color; a*, the redness; b*, the yellowness TBARS, thiobarbituric acid reactive substances is expressed as mg malondialdehyde per kilogram (MDA/kg) of meat.

[2]A-B LSmeans for treatment main effect within the same trait bearing a common letter are not significantly different ($P > 0.05$).

[3]A-C LSmeans for storage time main effect in the same column for a trait bearing a common letter are not significantly different ($P > 0.05$).

key components in the PHI process. Therefore, the thiobarbituric acid reactive substances (TBARS) analysis which is widely accepted as evidence of oxidation of polyunsaturated fatty acids (Pimpa et al., 2009) was measured to determine if deleterious oxidation on the meat occurred that could cause off-flavors. The TBARS values did not differ ($P > 0.05$) for control and PHI treated samples (Table 4). The TBARS values of fresh beef slowly increased ($P \leq 0.05$) during storage at 4°C for 0, 1, 3, 7, and 14 d (Table 4). The maximum TBARS value for all the samples on d 14 was 0.33 (± 0.06) mg MDA/kg, which was much lower than the minimal TBARS value to cause strong off-odor development at 2 mg MDA/kg (Campo et al., 2006). Thus, PHI treatment at the levels used in this study would not cause oxidation related off-odors or off-flavors. The present study results agreed with Cárdenas et al. (2011); Saini et al. (2014); Korhonen et al. (1981), that the use of UV or gaseous ozone did not affect lipid oxidation for beef or turkey.

Conclusion

The PHI technology was effective in controlling non-O157 STEC, *E. coli* O157:H7, and *Salmonella* contaminated on surface of fresh beef, when exposed to PHI for 15 to 60 s. This technology which uses no heat or water can be integrated as an effective processing aid or part of a hurdle approach to enhance the safety of meat products without impairing color or creating lipid oxidation off-flavor issues. The studies were performed under controlled conditions, rather than in a commercial situation, and therefore the validation of the PHI technology under actual in-plant conditions will ultimately be necessary.

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