EVALUATION OF RADIANT CATALYTIC IONIZATION IN REDUCING *ESCHERICHIA COLI, LISTERIA INNOCUA* AND *SALMONELLA TYPHIMURIUM* ON REPRESENTATIVE FOOD CONTACT SURFACES

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Abstract.–*Escherichia coli, Listeria* spp. and *Salmonella typhimurium* are common food pathogens and responsible for frequent and widespread outbreaks of foodborne illness annually. This study examines the potential of radiant catalytic ionization (RCI) as a food decontamination technology through its reduction of inoculations of these bacteria on representative food items (apples, cantaloupes, and spinach). RCI exposure resulted in ≥99% reduction in the recovery of these bacteria within a 90-min exposure, with two exceptions (*E. coli* and *S. typhimurium* inoculated on cantaloupe: 94% reduction; 88% reduction, respectively). When *E. coli*, *L. innocua* and *S. typhimurium* were inoculated onto apple slices, the percentages remaining after exposure to RCI for 0, 30, 60 and 90 min were: 100, 2, 0, 0; 100, 30, 9, 0.01; and 100, 21, 0.004, 0.02, respectively. When *E. coli*, *L. innocua* and *S. typhimurium* were inoculated onto the rough outer skin of cantaloupes, the percentages remaining after exposure to RCI for 0, 30, 60 and 90 min were: 100, 19, 15, 6; 100, 9, 10, 1; and 100, 24, 22, 12, respectively. When *E. coli*, *L. innocua* and *S. typhimurium* were inoculated onto spinach leaves, the percentages remaining after exposure to RCI for 0, 30, 60 and 90 min were: 100, 16, 0.001, 0.002; 100, 19, 11, 0.005; and 100, 0.007, 0, 0, respectively (*SE* = ± 0.1 maximum). These results indicate that RCI is an effective technology for reducing foodborne pathogens.

Keywords: radiant catalytic ionization, *Escherichia*, *Listeria*, *Salmonella*, food pathogens

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Foodborne illness outbreaks linked to fresh products are becoming more frequent and widespread, with food pathogens such as *Escherichia coli* 0157:H7, *Listeria* spp., and *Salmonella* spp. developing into growing concerns (Ortega et al. 2007; CDC 2013;

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WHO 2015). In addition, spoilage microorganisms that reduce product shelf life cost food producers millions of dollars in lost products every year (Sahu & Bala 2017). The USDA once estimated the costs associated with foodborne illnesses to be between $2.3 and $4.6 billion a year (USDA 2001). The CDC estimates that each year 48 million people become sick due to foodborne pathogens, of which 128,000 are hospitalized and 3,000 die (CDC 2016). The National Institutes of Health released values on the per person cost for foodborne illness, an average of $1,626, which correlates to $77.7 billion a year the U.S. government spends to help those infected with foodborne illness (NIH 2017).

New microbial control technologies have emerged in recent years such as ozonation of water and UV light exposure treatments that are being used in a multitude of places to decontaminate contact surfaces (e.g., Sommers et al. 2010) and foods (e.g., Sharma et al. 2003; Bialka & Demirci 2007; Sopher et al. 2007; Vurma et al. 2009; Blogoslawaki & Stewart 2011; Sommers et al. 2017). Another technology that is becoming accepted within the food processing industry, radiant catalytic ionization (RCI) was originally developed by NASA in the mid-1990s to purify air on the International Space Station due to an over production of ethylene gas by plants taken aboard the space station for food supplies (Space Foundation 2017). Ethylene gas is highly detrimental to plant maturation and fruit growth. RCI products interact with ethylene gas to produce viable nutrients for plant growth and maturation (Space Foundation 2017). RCI technology is now used in air handling systems within hospitals and offices as a method for indoor air purification (to eliminate "sick-building syndrome") (e.g., Grinshpun et al. 2007), where rates for pathogens on surfaces have been reduced up to 98%. Application of RCI technology for food microbial control by food processors and retailers would appear to be most useful immediately prior to packaging and transport and/or immediately prior to, or during, retail stocking and display. RCI technology uses a UV phototube to generate reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), hydroxyl radical (OH•) and ozone (O_3), that interact with DNA, lipids, and proteins within cells (Ortega et al. 2007), specifically through lipid peroxidation, amino acid

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degradation, interference with RNA transcription and protein synthesis, and DNA replication (e.g., Davies et al. 1987; Fridovich 1998; Gaunt et al. 2006). RCI has since been demonstrated to be an effective organic form of treatment to disinfect food contact surfaces through the actions of ROS (e.g., Davies et al. 1987; Fridovich 1998; Ortega et al. 2007).

The focus of this study is an examination of the reduction by exposure to RCI of *Escherichia coli*, *Listeria innocua*, and *Salmonella typhimurium*, which were inoculated onto skin samples of apples, cantaloupes and spinach leaves; and the potential of RCI to become incorporated into the regime of food microbial control techniques by the food processing industry and food retailers.

MATERIALS & METHODS

Bacterial isolates.–Bacterial strains of *Escherichia coli* (ATCC 25922), *Listeria innocua* (ATCC 33090; used in place of the pathogenic and more dangerous *Listeria monocytogenes*) and *Salmonella typhimurium* (ATCC 14028) were obtained from Fisher Scientific Company (Pittsburgh, PA). Single-species inoculations were used in all experiments.

Bacterial growth and preparation of food item samples.–Bacterial isolates were cultured independently in 5 mL Tryptic Soy Broth (TSB) using 16-mm sterile glass tubes at 37°C and 250 rpm for 24 h (to reach the stationary phase at 10^9 cells mL^{-1}) using a New Brunswick Scientific Model 12500 Series Incubation Shaker (New Brunswick; Edison, NJ). Following removal from the shaker, 1-mL aliquots of the cultures were placed into sterile 1.5-mL microfuge tubes. The bacteria were sedimented by centrifugation for 30 sec in a Marathon Micro A centrifuge (Fisher Scientific Company; Pittsburgh, PA) and suspended in 1 mL of sterile M9 medium. After a second centrifugation, the supernatant was removed and 1 mL of sterile M9 medium was subsequently added to each tube. A 1:100 dilution of the suspended cells was then prepared using sterile M9 medium. Samples of apple skin were prepared by peeling the skin from Granny Smith apples and then using a sterile 1000-mL plastic disposable pipet tip to cut out uniform

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8-mm diameter circular skin samples. Similar procedures were used to obtain uniform samples of spinach leaves and the rough exterior skin of cantaloupes.

Inoculation of samples and exposure to reactive oxygen species using RCI.–Sterile polyester swabs (serving as a standardized, reference contact surface; catalog number 23-400-122, Fisher Scientific Company; Pittsburgh, PA) and samples of the representative food items were inoculated with 50-µL aliquots (approximately 10^7 cells) of *E. coli*, *L. innocua* or *S. typhimurium* immediately prior to exposure to RCI for 0, 30, 60 or 90 min, with three replications per swab and per bacterium per food item (i.e., each experimental point). An additional series of controls, also with three independent replications per bacterium per food item (i.e., each experimental point), were performed with inoculated food item samples set outside the RCI chamber (i.e., without RCI exposure) for 0, 30, 60 or 90 min. The bacterial suspensions were vortexed for 30 sec using a Fisher Scientific Touch Mixer, Model 231(Fisher Scientific Company; Pittsburgh, PA) immediately prior to inoculation. Preparation of food item samples and inoculation of same were performed immediately prior to exposure of the samples to RCI.

A custom built RCI apparatus was used to expose the inoculated food samples (Green Tech Environmental: Allen Johnston, personal communication). Production of ROS by RCI involves the action of ultraviolet (UV) light (produced by a UV phototube) interacting with metal catalysts. The exposure chamber consists of a rectangular, galvanized steel box (standard heating and cooling duct material), 46 cm (w) by 46 cm (h) by 95 cm (l). A commercial fan is installed at one end of the chamber to provide air flow for the ROS produced by RCI from a BLS HVAC Air Purification System (Best Living Systems, LLC; probe model, 8H-D11; cell model, MCI48K; www.BestLivingSystems.com). Immediately in front of the BLS HVAC is a 40 cm by 40 cm stainless steel mesh platform (accessed through a side panel) for placement of the samples. Beneath the platform is an enamel dissecting pan filled with water to raise the humidity of the chamber; increased humidity reportedly improves the

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effectiveness of ROS on bacterial reduction/inactivation (Acton 2013). The nominal temperature and relative humidity (RH) in the RCI chamber averaged 28.4°C (±0.718 *SE*; $n = 20$) and 92.7% RH (±2.06 *SE*; $n = 20$). The chamber is evacuated at the far end by a flexible conduit leading to a standard fume hood. The RCI chamber was activated and allowed to run for 10 min prior to exposing a swab or food item sample, according to instructions by the manufacturer, to insure the establishment of ROS prior to placement of samples into the chamber.

Recovery of inoculated samples.–Immediately following their exposure to RCI, the polyester swab heads and the food samples were removed from the exposure chamber and placed into a microfuge tube containing 1000 µL of M9 medium and vortexed for 30 sec. The cells were then serially diluted and 100 µL of the diluents were plated onto TSA plates. The plates were incubated overnight at 37°C and then observed for CFUs. SigmaPlot 12.5 (Systat Software, Inc.; https://systatsoftware.com/products/sigmaplot/) was used for statistical analyses.

Analysis of ozone (O_3) and hydrogen peroxide (H_2O_2) in the presence of RCI.–To verify the production of ROS and determine their nominal amounts (with reference to recommended human safety standards), concentrations of O_3 and H_2O_2 were measured using Dräger™ colorimetric tubes (Fisher Scientific Company; Pittsburgh, PA) according to the manufacturer's instructions.

RESULTS & DISCUSSION

Results of the effectiveness of radiant catalytic ionization (RCI) in the reduction of the bacterial species inoculated on sterile polyester swabs are summarized in Table 1. Exposure to RCI resulted in a significant reduction at the end of 90 min for *E. coli* ($\chi^2 = 8.535$; $df = 2$; $P < 0.02$), *L. innocua* ($\chi^2 = 9.603$; $df = 2$; $P < 0.01$), and *S. typhimurium* ($\chi^2 = 6.421$; $df = 2$; $P < 0.05$), compared to the initial concentrations. Additionally, there was also a significant reduction for

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Table 1. Reduction of colony forming units (CFUs) of bacteria inoculated on sterile polyester swabs following exposure to radiant catalytic ionization (RCI).

RCI Exposure Time (min)	Mean Number of CFUs (±*SE*; *n* = 3)	Percentages Remaining
Escherichia coli		
0*	6.0 (±0.66) x 10^2	100.00
30	2.6 (±0.58) x 10^2	44.00
60	0.69 (±0.63) x 10^2	11.50
90	0.01 (±0.01) x 10^2	<0.01
Listeria innocua		
0*	7.4 (±0.77) x 10^2	100.00
30	2.3 (±0.33) x 10^2	31.20
60	0.05 (±0.04) x 10^2	0.02
90	0.01 (±0.001) x 10^2	<0.01
Salmonella typhimurium		
0*	6.1 (±0.21) x 10^2	100.00
30	1.5 (±0.07) x 10^2	25.40
60	0.13 (±0.003) x 10^2	<0.01
90	0.13 (±0.003) x 10^2	<0.01

*Controls, with no RCI exposure.

all three strains at each individual time point (0, 30, 60, and 90 min) when comparing +RCI and –RCI conditions (χ^2, $P < 0.01$; $df = 2$, for each test).

Similar reduction curves were obtained for bacteria inoculated on the representative food items. In all but two instances, exposure to RCI resulted in a ≥99% reduction in the recovery of the three bacterial species used in this study within a 90 min RCI exposure on each food item sample (Table 2). The two exceptions were *E. coli* and *S. typhimurium* inoculated on cantaloupe (94% reduction; 88% reduction, respectively). There are, in addition, some apparent differences in the rate of reduction among the bacteria and among the food items. On the apple slices, *E. coli* is almost completely eliminated after only a 30 min exposure; such was not the case for *L. innocua* or *S. typhimurium*, which are not essentially eliminated until after 60 min exposure. Conversely, *S. typhimurium* is most quickly eliminated on spinach leaves (after a 30 min exposure), compared to *E. coli* (after 60 min exposure) and *L. innocua* (after 90 min exposure). The cause for these

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Table 2. Reduction of bacterial cell counts following exposure to radiant catalytic ionization (RCI) (–RCI = no exposure to RCI (controls); +RCI = exposure to RCI. Values are percentages remaining at end of stated times, with 100% = approximately 10^7 CFU ml^{-1}. For each experiment, $n = 3$; maximum *SE* approximately ±0.1).

Exposure	Apple		Cantaloupe		Spinach	
Time (Min)	-RCI	+RCI	-RCI	+RCI	-RCI	+RCI
Escherichia coli						
0	100.00	100.00	100.00	100.00	100.00	100.00
30	79.00	2.00	85.00	19.00	90.00	16.00
60	14.00	0.00	70.00	15.00	13.00	<0.01
90	4.00	0.00	59.00	6.00	3.00	<0.01
Listeria innocua						
0	100.00	100.00	100.00	100.00	100.00	100.00
30	55.00	30.00	63.00	9.00	23.00	19.00
60	35.00	9.00	54.00	10.00	15.00	11.00
90	15.00	0.01	44.00	1.00	7.00	<0.01
Salmonella typhimurium						
0	100.00	100.00	100.00	100.00	100.00	100.00
30	76.00	21.00	83.00	24.00	81.00	<0.01
60	19.00	<0.01	79.00	22.00	18.00	0.00
90	6.00	0.02	59.00	12.00	18.00	0.00

differing rates of reduction on the two food item surfaces is not readily apparent. For all three bacteria (but much less so for *L. innocua*), resistance to reduction is evident on the cantaloupe skin, probably due to the roughened surface of cantaloupe compared to apple skin and spinach leaves, which may have provided some shielding effect from reactive oxygen species (ROS) due to the cantaloupe's surface topology, especially for *E. coli* and *S. typhimurium*. Further studies are needed to determine if greater reduction in the numbers of bacteria can be achieved on such rough-skinned fruits. In addition, because significant killing is observed on the trend lines for each data set, it is expected that each time-point within a data set might also result in significant killing, which is the case here, in that that there is a statistical difference at each of the time-points between –RCI and +RCI in each data set as indicated by Chi-square analyses (χ^2, $P < 0.01$; $df = 2$, per test). Results of the analysis of ozone and hydrogen peroxide concentrations are presented in Table 3. The concentrations of both

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Table 3. Analysis of ozone (O_3) and hydrogen peroxide (H_2O_2) production in the presence of radiant catalytic ionization using Dräger™ colorimetric tubes. Average readings are based on sample sizes of $n = 3$.

Dräger™ tube type	Average reading (ppm ± *SD*)	OSHA Safe Standards (ppm)
O_3 0.05–0.7 ppm	0.15 ± 0.020	0.08–0.1 (averaged over 8 h)
H_2O_2 0.1–3 ppm	0.40 ± 0.265	1.0

ROS conform to the permissible exposure limits (PELs) set by OSHA (CDC 1978; OSHA 2006).

CONCLUSIONS

The CDC has recommended the use of intervention technologies for reducing the risk of foodborne illnesses (CDC 2013; 2016). These results indicate the effectiveness of commercially used and relatively inexpensive radiant catalytic ionization (RCI) in the reduction of *Escherichia coli*, *Listeria innocua*, and *Salmonella typhimurium* on apple, cantaloupe and spinach contact surfaces. In general, RCI results in a ≥99% reduction in the recovery of these bacteria within a 90 min exposure (with two exceptions noted earlier). The reductions of *E. coli*, *L. innocua*, and *S. typhimurium* are also consistent with the use of RCI and Breeze AT ozone generation in reducing other foodborne pathogens (e.g., *Bacillus globigii, Staphylococcus aureus, Candida albricans, Stachybotrys chartarum, Pseudomonas aeruginosa, Streptococcus pneumoniae,* and *L. monocytogenes* [Ortega et al. 2007]) and of ultraviolet irradiation (UV-C) in reducing *L. monocytogenes, S. aureus* (Sommers et al. 2010)*, E. coli* and *Salmonella* spp. (unpublished data of the authors).

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Education (PASSHE), but is strictly for the purpose of providing specific information.

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