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The effectiveness of closed-circulation gaseous chlorine dioxide or ozone treatment against bacterial pathogens on produce

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ABSTRACT

The objective of this study was to examine the effectiveness of gaseous chlorine dioxide (ClO_2) or ozone (O_3) treatment against Shiga toxin-producing *Escherichia coli* (STEC), serovars of *Salmonella enterica*, and *Listeria monocytogenes* on baby-cut carrots, lowbush blueberries, and beefsteak tomatoes using a scaled-up closed-circulation treatment system. Dry ClO_2 precursors were combined in-chamber to make 0.03, 0.06, and 0.12 mg ClO_2/g produce for a 2.5 h exposure and 0.04, 0.07, and 0.15 mg ClO_2/g produce for a 5.0 h exposure time. Ozone was generated through corona-discharge of a dry oxygen feed and either 0.86 or 1.71 μg O_3/g produce concentrations were used to treat 2 kg of produce for 2.5 and 5.0 h.

Overall, ClO_2 treatment resulted in maximum bacterial reductions of >7 log observed on carrots and tomatoes and 3.7 log on blueberries. Exposure gaseous O_3 resulted in observed reductions of 1.2, 1.8, and 1.6 log and simultaneously resulted in noticeable bleaching carrot and tomato tissue as well. These results reported here indicate that gaseous ClO_2 can be a suitable treatment when implemented correctly to reduce bacterial pathogens in a storage setting.

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1. Introduction

Contamination of fresh produce can occur anywhere from farm to fork. In the pre-harvest environment, microbial contaminants can come from numerous sources including irrigation water, soil, fertilizers, and even insects. After harvest, pathogen contamination of fresh produce can occur through inappropriate handling, improperly cleaned surfaces and equipment, or from lack of suitable hygiene practices in processing or preparation environments (Yeni, Yavaş, Alpas, & Soyer, 2016). Because fresh produce is commonly eaten raw, practices that reduce levels of microorganisms during post-harvest processing (e.g. antimicrobial washing) are vital to increase shelf-life and reduce incidences of foodborne illness (Goodburn & Wallace, 2013).

Sodium hypochlorite washes with 50–200 ppm concentrations are some of the most commonly used treatments to decontaminate raw foods in the fresh produce industry (Hwang, Huang, & Wu, 2017). However,chlorinewashing suffers from reduced antimicrobial efficacy outside of limited optimal treatment conditions (e.g. pH) and in the presence of any additional organic matter. Furthermore, the occupational health hazard associated with chlorine use, negative environmental impacts, and a reported inability to reduce pathogens internalized in plant tissues has led to a trend to use safer and more effective antimicrobials to treat produce (Goodburn & Wallace, 2013). Although alternative aqueous treatments (e.g. hydrogen peroxide or peracetic acid) have been previously proposed for use in the fresh produce industry, antimicrobial washes suffer from a few key issues. First, the combination of large volumes of water with the sizable masses of plant tissue treated at a time introduces a cross-contamination risk, especially when wash-water is recycled for environmental and economic considerations. Additionally, residual moisture following treatment can also promote mold growth if a proper drying step is not implemented (Alwi & Ali, 2014; Gómez-López, Rajkovic, Ragaert, Smigic, & Devlieghere, 2009). Comparatively, gaseous treatments use little, if any, water and are capable of treating areas and irregularities on produce surfaces that aqueous antimicrobials have difficulty reaching (Gómez-López, Ragaert, Debevere, & Devlieghere, 2008). Although washing is an important step in most post-harvest practices, an additional gaseous intervention step can help complement already existing treatment practices to

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further reduce levels of microbial contaminants in post-harvest environments.

Compared to washing, gaseous intervention can only be safely preformed in a highly controlled setting that circulates generated gas to provide an even distribution of treatment while simultaneously preventing any human exposure. This indicates that a storage setting, either in a warehouse or in a large vehicle during transportation, is the ideal scenario where gaseous treatment intervention can be implemented. Many gas treatments are commonly being used in the produce industry but are often limited to specific situations or food commodity applications. These include, but are not limited to, hydrogen phosphide (PH_3) fumigation of grain stacks and silos (Aulicky, Stejskal, Frydova, & Athanassiou, 2015), propylene oxide (CH_3CHCH_2O) pasteurization of tree nuts (Pan, Bingol, Brandl, & McHugh, 2012), and sulfur dioxide (SO_2) fungicidal treatment of grapes (Carter, Chapman, Gabler, & Brandl, 2015). However, some of these gases are extremely toxic to humans (e.g. PH_3 (Wilson, Lovejoy, Jaeger, & Landrigan, 1980)) and can form unwanted byproducts (e.g. sulfites after SO_2 treatment (Timbo, Koehler, Wolyniak, & Klontz, 2004)). Comparatively, gaseous chlorine dioxide (ClO_2) and ozone (O_3) are relatively safe to generate and handle and cause minimal byproduct formation. These factors, along with their reportedly powerful antimicrobial properties, have made gaseous ClO_2 and O_3 treatment technologies promising candidates for post-harvest produce treatment applications (Tzortzakis, 2016, pp. 175–207; Wu, 2016, pp. 209–218).

Previous studies have established that gaseous treatment of produce works as an effective antimicrobial agent in small lab-scale settings (Akbas & Ozdemir, 2008; Selma, Ibáñez, Cantwell, & Suslow, 2008; Sy et al. 2005a, 2005b; Wu & Rioux, 2010). However, studies focused on actual utilization of gaseous treatment to reduce foodborne pathogenic bacteria in an industrially realistic scenario have not been done before. Therefore, the objectives of this study were to (1) determine the effectiveness of gaseous treatments of ClO_2 and O_3 against shiga toxin-producing *Escherichia coli* (STEC), serovars of *Salmonella enterica*, and *Listeria monocytogenes*, on 2kg stacks of baby-cut carrots, lowbush blueberries, and beefsteak tomatoes in a simulated storage setting and (2) determine if gaseous treatment left inappropriate levels of treatment residue or caused notifiable alteration of visual quality. Unlike most previous work with gaseous treatment, which used small mass-loads of produce, this study used 2 kg of each produce model arranged in a pallet design during treatment to better represent industrial storage scenarios. The findings of this study not only help to clarify numerous discrepancies in the literature regarding the antibacterial effectiveness of the gaseous forms of both chlorine dioxide and ozone treatments, but provide information needed for convenient implementation of gaseous intervention in already existing processing infrastructures of the produce industry.

2. Materials and methods

2.1. Bacterial strain preparation

Three strains of *E. coli* O157:H7 (ATCC 35150, 700599, and NCTC 129000), three non-O157 strains (O26:H11 SJ2; O45:H2 05–6545; O103:H11 SJ12), five *Salmonella* serovars (ATCC Typhimurium 14028; Heidelberg 45955; Enteritidis PT30; Montevideo 51, and Newport H1073), and five *L. monocytogenes* strains (ATCC 19115, 19111, 49594, 54, and 7644) were obtained from the University of Maine-Pathogenic Microbiology Laboratory, Orono, ME and USDA-Agricultural Research Services (ARS) Centers (Produce Safety and Microbiology Unit, Albany, CA and Wyndmoor, PA). Culture collections were maintained on tryptic soy agar (TSA) at 4 °C throughout the study and outbreak strains were selected if available. Prior to produce inoculation, individual strains of each species were grown overnight at 37 °C in 50 ml of tryptic soy broth (TSB).

2.2. Produce model inoculation

Upon arrival, carrots, blueberries, or tomatoes were visually examined and all materials that were significantly damaged or had signs of mold growth were removed. Additionally, blueberries and tomatoes were gently washed to remove any debris or juice that could be present on the fruit surface. All tomatoes and blueberries were shipped directly after harvest with no post-harvest processing while baby-cut carrots arrived already processed and packaged by the distributer. Preliminary work was done to ensure that background flora did not interfere with pathogen inoculation and recovery using selective media.

Overnight cultures were centrifuged for 15 min at 5000x*g* and had supernatant media removed. Bacterial pellets were then washed twice and resuspended in 0.1% peptone water. The individual strains from each species were combined to make an intraspecific cocktail and 200 g of baby-cut carrots, tomatoes, or blueberries were dipped in the cocktail and gently shaken by hand for 2 min. The produce models were then allowed to dry in a biological safety cabinet for 2 h to allow for bacterial attachment. This inoculation procedure, through preliminary work, was determined to consistently result in a pathogen concentration on the produce models to be > 6.5 log CFU/g.

2.3. Fumigation pail setup

Treatments were performed in five-gallon buckets with a laptop cooling fan (All Electronics; Van Nuys, CA) attached internally to the lid to circulate gas during treatment Individual produce models (2 kg) were arranged in polypropylene clamshells to form two layers of 1 kg each (Fig. 1). Before treatment, one of the clamshells in each bucket was randomly selected to contain produce inoculated with bacteria using a random number generator (https://www.random.org). During all treatments, only the randomly selected clamshell contained produce inoculated with pathogens while the others served as mass-holders. Preliminary work was performed to ensure that there was a consistent distribution of bacterial reduction between each clam shell using this setup. Both the ClO2 and O3 trials consisted of either a 2.5 or 5.0 h exposure time at room temperature (23 °C) in a chemical fume hood and a control without any gaseous treatment was included with every replicate.

2.4. Chlorine dioxide treatment

Similar to the setup used by Wu and Rioux (2010), gaseous ClO_2 was generated using a two-part, fast-activation dry media system (ICA Trinova, LLC., Forest Park, GA). Using this method, pure ClO_2 is released throughout the treatment time in a predictable fashion and can be measured through iodometric titration of gas samples collected within the chambers using a syringe (Harp, 2002). Additionally, gaseous ClO_2 is released gradually overtime resulting in a slight increase in the amount of gas generated between the 2.5 and 5.0 h exposures. For example, using 7.5, 15, and 30 g of each ClO_2 precursor generated 0.03, 0.06, and 0.12 mg ClO_2/g produce during a 2.5 h exposure and 0.04, 0.07, and 0.15 mg ClO_2/g after 5.0 h. To begin treatment, two sachets each containing half of the total ClO_2 precursors were placed in the center of each layer and then the chambers were sealed. Treatment conditions were designed based on the maximum bacterial reduction with minimal visual damage that could be achieved.

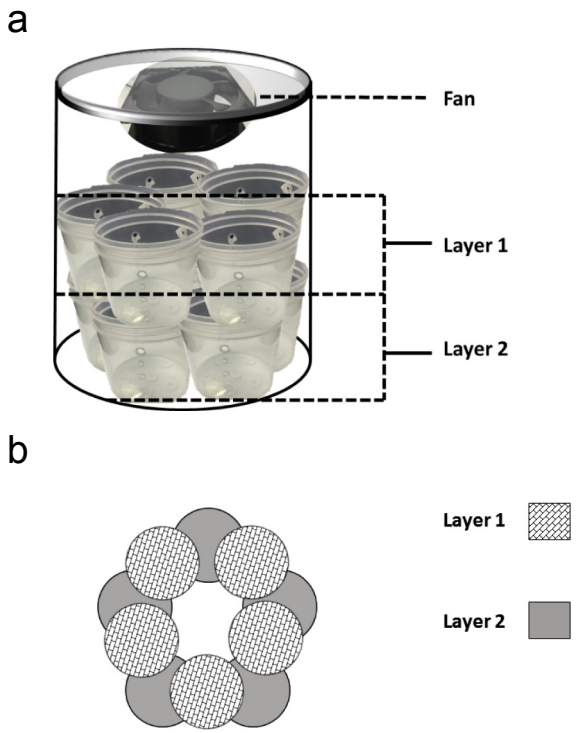

Fig. 1. Schematic presentation of bucket layout during gaseous treatment. (a) Side profile of the in-bucket treatment layout and (b) Top view of the clam-shell arrangement within the buckets during treatment.

2.5. Ozone treatment

Gaseous O_3 was generated through corona discharge of a dry oxygen gas feed using a LG-7 ozone generator (Del-Ozone, San Luis Obsipo, CA). Ozonated oxygen was then fed through air tight hosing into the treatment chambers at a flow rate of 2 l/min for 2 min, allowing for 4 L of gas to enter the chambers. Produce mass-loads were exposed to either 428 or 856 mg/m^3 ozone (200 ppm and 400 ppm if in aqueous form) for 2.5 or 5.0 h and concentrations of the ozonated-oxygen feed were measured using a 49*i* ozone analyzer (Thermo Fisher Scientific). To make similar working units as used for ClO_2, further unit conversion changed the treatment doses to 0.86 and 1.71 μg O_3/g of produce. Treatment conditions were designed based on the maximum bacterial reduction with minimal visual damage that would be achieved.

2.6. Bacterial recovery and enumeration

After treatment, 50 g of blueberries, carrots, or one whole tomato that was previously inoculated was removed and gently washed for 2 min with a 1:1 g:ml ratio of 0.1% peptone water. The rinse was then serially diluted and plated on MacConkey Sorbitol Agar supplemented with cefixime and tellurite (CT-SMAC), bismuth sulfite agar (BSA), or PALCOM agar for STEC, *Salmonella*, or *L. monocytogenes* respectively. A thin TSA layer was added to each plate prior to plating (Thin Agar Layer method) to aid in the recovery of injured bacterial cells following the protocol outlined in Wu, Qiu, Bushway, and Harper (2008). Reduction of viable cell counts were calculated as the log fold-change of bacterial population (log CFU/g) recovered from treated produce comparing to that recovered from the untreated control.

2.7. Measurement of treatment residue

Following gas treatment, one whole tomato or 50 g of carrots/blueberries were washed with 0.1% peptone water in a 1:1 ml/g ratio. Chlorine dioxide residue in the rinse was measured following the Hach Company© protocol. In short, 5 ml of rinse water was injected in a 10% potassium iodine solution with starch indicator, acidified with sulfuric acid, and titrated to a colorless endpoint with sodium thiosulfate. Chlorine dioxide present in the solution could then be calculated by using the amount of sodium thiosulfate required for titration to a colorless endpoint (Harp, 2002). A DO3 dissolved ozone monitor (Eco Sensors) was used to measure residual ozone present in the rinse via gaseous diffusion in a cylinder. Residues from both gases were measured 0 h and 24 h post-treatment.

2.8. Visual quality check

Produce models were visually examined before and after treatment. Signs of bleaching or whitening due to treatment were recorded and verified by a second person when observed.

2.9. Statistical analysis

Experiments were performed three times separately and statistical analysis was performed using JMP (ver. 12) software with $\alpha = 0.05$. One-way ANOVAS, coupled with Tukey's HSD post-hoc tests, were used to determine if any treatment had a statistically relevant ($P < 0.05$) effect on microbial reduction compared to the others. Treatment residual levels at 0 h and 24 h were compared to each other using a paired *t*-test.

3. Results

3.1. Gaseous antimicrobial treatment of baby-cut carrots

The six strains of STEC were the most sensitive among the three species bacterial pathogens to ClO_2 treatment of carrots, with mean population reductions down to undetectable levels (7.4 and 7.7 log reductions, detection limit < 1 log CFU/g) after both the 0.04 and 0.07 mg ClO_2/g carrot treatments for 5.0 h. Due to this sensitivity, lower ClO_2 concentrations were used for STEC treatment on carrots (0.03 mg ClO_2/g carrot and 0.04 mg ClO_2/g carrot) than the other two pathogens. Exposure to 0.15 mg ClO_2/g for 5.0 h resulted in log reductions of 4.9 and 5.5 for *Salmonella* and *L. monocytogenes*, respectively (Table 1). Ozone was not as effective at reducing bacteria on the carrots with a maximum reduction of 1.2, 0.5, and 0.8 log observed for STEC, *Salmonella,* and *L. monocytogenes* after treatment of 1.71 μg O_3/g carrot for 5.0 h. This ozone treatment parameter also resulted in noticeable bleaching of the carrots (Table 2). For all three bacteria, increasing just the exposure time or the gas concentration did not always result in a statistically relevant ($P < 0.05$) increase in microbial reduction. However, except in the case of ozone treatment of *Salmonella* on carrots, simultaneously increasing both the gas concentration and exposure time of treatment resulted in a significant increase in microbial inactivation.

3.2. Gaseous antimicrobial treatment of lowbush blueberries

Chlorine dioxide treatment of blueberries was not as effective at pathogen reduction compared to the other two produce models. STEC was the most sensitive to ClO_2 exposure, with a maximum log reduction of 3.7 observed after exposure to 0.15 mg ClO_2/g blueberry for 5.0 h. This same treatment scenario also resulted in log reductions of 2.7 and 2.1 for *Salmonella* and *L. monocytogenes*,

Table 1
Microbial reduction by gaseous chlorine dioxide (ClO_2) treatment of baby-cut carrots, lowbush blueberries, and beefsteak tomatoes at different concentrations and exposure times. Reduction data are represented as means ± standard deviation and consist of three replicates compared to the pathogen population (log CFU/g) recovered from untreated controls. Population reductions with different letters for the same species and produce model indicates a statistically significant difference ($P < 0.05$).

Produce Model	Species	ClO_2 gas generated (mg ClO_2/g produce)	Exposure time (h)	Log bacterial reduction
Baby-cut carrots	STEC	0.03	2.5	4.2 ± 0.4^{A}
		0.04	5.0	$7.4 \pm 0.0^{B*}$
		0.06	2.5	7.0 ± 0.6^{B}
		0.07	5.0	$7.7 \pm 0.0^{B*}$
	Salmonella	0.06	2.5	4.2 ± 0.6^{A}
		0.07	5.0	4.8 ± 0.1^{B}
		0.12	2.5	5.1 ± 0.3^{B}
		0.15	5.0	4.9 ± 0.0^{B}
	L. monocytogenes	0.06	2.5	2.0 ± 0.2^{A}
		0.07	5.0	2.5 ± 0.2^{A}
		0.12	2.5	5.0 ± 0.1^{B}
		0.15	5.0	5.5 ± 0.2^{B}
Lowbush blueberries	STEC	0.06	2.5	2.8 ± 0.4^{A}
		0.07	5.0	2.9 ± 0.2^{A}
		0.12	2.5	3.6 ± 0.0^{B}
		0.15	5.0	3.7 ± 0.1^{B}
	Salmonella	0.06	2.5	0.9 ± 0.1^{A}
		0.07	5.0	0.9 ± 0.3^{AB}
		0.12	2.5	1.6 ± 0.2^{B}
		0.15	5.0	2.7 ± 0.4^{C}
	L. monocytogenes	0.06	2.5	0.9 ± 0.0^{A}
		0.07	5.0	1.0 ± 0.1^{A}
		0.12	2.5	2.1 ± 0.3^{A}
		0.15	5.0	2.1 ± 0.2^{A}
Beefsteak tomatoes	STEC	0.06	2.5	3.4 ± 1.9^{A}
		0.07	5.0	$7.8 \pm 0.0^{B*}$
		0.12	2.5	$5.6 \pm 1.9^{ABC\#}$
		0.15	5.0	$7.5 \pm 0.0^{BC\#*}$
	Salmonella	0.06	2.5	5.7 ± 0.7^{A}
		0.07	5.0	$7.0 \pm 0.0^{A*}$
		0.12	2.5	$5.0 \pm 1.6^{A\#}$
		0.15	5.0	$7.2 \pm 0.0^{A\#*}$
	L. monocytogenes	0.06	2.5	5.7 ± 1.0^{A}
		0.07	5.0	$7.0 \pm 0.0^{A*}$
		0.12	2.5	$6.0 \pm 1.3^{A\#}$
		0.15	5.0	$7.1 \pm 0.0^{A\#*}$

*Reduction to below the detection limit (<1 log CFU/g).
Treatment resulted in noticeable bleaching of tomato epidermis.

respectively (Table 1). Ozone treatment resulted in a maximum log reduction of 1.8 after 1.71 μg O_3/g blueberry exposure of *L. monocytogenes* for 5.0 h. STEC and *Salmonella* on the berries reduced by ≤ 1.0 log after all O_3 treatment scenarios (Table 2). For STEC and *Salmonella,* increasing the ClO_2 dose resulted in a significantly higher ($P < 0.05$) reduction in bacteria while increasing both the dose and exposure time of O_3 increased reduction of all three species.

3.3. Gaseous antimicrobial treatment of beefsteak tomatoes

A 5.0 h chlorine dioxide exposure resulted in reductions (≥7 log) below the detectible limit of plating (<1 log CFU/g) for all three species for both the 0.07 and 0.15 mg ClO_2/g tomato treatments (Table 1). However, the higher treatment of 0.15 mg ClO_2/g tomato resulted in noticeable bleaching of the tomato epidermis. Ozone treatment of tomatoes also resulted in bleaching after exposure to 1.71 μg O_3/g tomato. This concentration only had a maximum log reduction of 1.6 observed from STEC after 5.0 h exposure followed by 1.1 log for both *L. monocytogenes* and *Salmonella* (Table 2). For both *Salmonella* and L. *monocytogenes,* increasing the ClO_2 dose or treatment time did not result in a significant ($P < 0.05$) change in reduction while efficacy of ozone treatment, comparatively, was statistically more effective after increase both the exposure time and concentration for STEC and *L. monocytogenes.*

3.4. Gas residue measured post-treatment

Levels of residual ClO_2 in rinse water at 0 and 24 h post treatment are listed in Table 3. The carrots had the highest ClO_2 levels post-treatment with a maximum of 29.2 mg/l measured immediately after 0.12 mg ClO_2/g produce treatment for 2.5 h. However, all ClO_2 residue decreased to below the U.S. Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) acceptable limit of 3 mg/l after 24 h. There was no ozone residue measured after any trial for all three produce models.

4. Discussion

This study demonstrated that close-circulation gaseous ClO_2 treatment of produce could be highly effective at reducing foodborne bacterial pathogens when implemented correctly in an industrial storage setting. Chlorine dioxide exposure reduced populations of viable STEC, *Salmonella*, and *Listeria* on tomatoes and STEC on carrots below the detection limit (<1 log CFU/g) using concentrations that did not bleach the plant tissues. Comparatively, ozone was not capable of reducing present pathogens more than 2 log and treatments with the 1.71 μg O_3/g of produce resulted in bleaching of both carrot and tomato tissue, indicating that it is not an effective antibacterial treatment for these produce commodities using this treatment design.

Given that there is no real “standardized” treatment setup, and

Table 2
Microbial reduction by gaseous ozone (O_3) treatment of baby-cut carrots, lowbush blueberries, and beefsteak tomatoes at different concentrations and exposure times. Reduction data are represented as means ± standard deviation and consist of three replicates compared to the pathogen population (log CFU/g) recovered from untreated controls. Population reductions with different letters for the same species and produce model indicates a statistically significant difference ($P < 0.05$).

Produce Model	Species	ClO_2 gas generated (mg ClO_2/g produce)	Exposure time (h)	Log bacterial reduction
Baby-cut carrots	STEC	0.86	2.5	0.5 ± 0.1^{A}
		0.86	5.0	0.7 ± 0.3^{AB}
		1.71	2.5	$0.7 \pm 0.1^{AB\#}$
		1.71	5.0	$1.2 \pm 0.1^{B\#}$
	Salmonella	0.86	2.5	0.3 ± 0.1^{A}
		0.86	5.0	0.4 ± 0.1^{A}
		1.71	2.5	$0.4 \pm 0.1^{A\#}$
		1.71	5.0	$0.5 \pm 0.1^{A\#}$
	L. monocytogenes	0.86	2.5	0.3 ± 0.1^{A}
		0.86	5.0	0.4 ± 0.0^{A}
		1.71	2.5	$0.4 \pm 0.1^{A\#}$
		1.71	5.0	$0.8 \pm 0.1^{B\#}$
Lowbush blueberries	STEC	0.86	2.5	0.2 ± 0.1^{A}
		0.86	5.0	0.2 ± 0.1^{A}
		1.71	2.5	0.3 ± 0.0^{AB}
		1.71	5.0	0.5 ± 0.1^{B}
	Salmonella	0.86	2.5	0.5 ± 0.0^{A}
		0.86	5.0	0.8 ± 0.2^{B}
		1.71	2.5	0.7 ± 0.2^{AC}
		1.71	5.0	1.0 ± 0.1^{C}
	L. monocytogenes	0.86	2.5	0.3 ± 0.2^{A}
		0.86	5.0	0.6 ± 0.2^{AB}
		1.71	2.5	1.2 ± 0.5^{BC}
		1.71	5.0	1.8 ± 0.2^{C}
Beefsteak tomatoes	STEC	0.86	2.5	0.7 ± 0.3^{A}
		0.86	5.0	1.0 ± 0.3^{AB}
		1.71	2.5	$1.3 \pm 0.3^{AB\#}$
		1.71	5.0	$1.6 \pm 0.3^{B\#}$
	Salmonella	0.86	2.5	0.6 ± 0.3^{A}
		0.86	5.0	0.7 ± 0.3^{A}
		1.71	2.5	$0.9 \pm 0.4^{A\#}$
		1.71	5.0	$1.1 \pm 0.3^{A\#}$
	L. monocytogenes	0.86	2.5	0.4 ± 0.1^{A}
		0.86	5.0	0.5 ± 0.2^{AB}
		1.71	2.5	$0.6 \pm 0.1^{AB\#}$
		1.71	5.0	$1.1 \pm 0.5^{B\#}$

#treatment resulted in noticeable bleaching.

Table 3
Residual ClO_2 measured by titration on produce models post gaseous treatment. All residue data are represented as means ± standard deviation of three replicates. Statistically different reductions ($P < 0.05$) in residue between 0 h and 24 h are indicated by different letters.

Produce model	Exposure time (h)	Treatment concentration (mg ClO_2/g produce)	Measured residue 0 h post treatment (mg/l)	Measured residue 24 h post treatment (mg/l)
Carrots	2.5	0.03	5.6 ± 2.0^{a}	0.5 ± 0.2^{b}
		0.06	18.0 ± 2.0^{a}	0.7 ± 0.3^{b}
		0.12	29.2 ± 9.8^{a}	0.6 ± 0.4^{b}
	5.0	0.04	5.4 ± 1.3^{a}	0.9 ± 0.7^{b}
		0.07	7.9 ± 2.0^{a}	0.7 ± 0.3^{b}
		0.15	15.8 ± 2.0^{a}	0.9 ± 1.0^{b}
Blueberries	2.5	0.06	$0.0 \pm 0.0^{*}$	$0.0 \pm 0.0^{*}$
		0.12	0.2 ± 0.2^{a}	$0.0 \pm 0.0^{b*}$
	5.0	0.07	$0.0 \pm 0.0^{*}$	$0.0 \pm 0.0^{*}$
		0.15	$0.0 \pm 0.0^{*}$	$0.0 \pm 0.0^{*}$
Tomatoes	2.5	0.06	1.0 ± 0.9^{a}	$0.0 \pm 0.0^{a*}$
		0.12	2.3 ± 1.4^{a}	0.6 ± 1.0^{a}
	5.0	0.07	0.1 ± 0.2^{a}	$0.0 \pm 0.0^{a*}$
		0.15	0.2 ± 0.3^{a}	0.0 ± 0.0^{b}

*residue level below titration detection limit (<0.01 mg/l).

there is little consistency between treatment parameters implemented in previous studies, direct comparison between the results presented here and those in published literature is difficult. For example, Singh, Singh, Bhunia, and Stroshine (2002) previously investigated gaseous ClO_2 and O_3 treatment of carrots (10 g samples) and found a 3 log STEC reduction with 1 mg/l gaseous ClO_2 exposure for 15 min and a 1.81 log reduction after 7.6 mg/l O_3 treatment. A 10-L container was used for all their gaseous trials, meaning there was a maximum of 10 mg of ClO_2 in the chamber. Given that only 10 g of carrot (~1 stick) was treated, this gives a treatment concentration of 1 mg ClO_2/g carrot; which is more than 14 times the concentration used to treat STEC in the present study. Similarly, gaseous ClO_2 or O_3 treatment has been previously studied with both blueberries and tomatoes, albeit using lower produce

masses. For example, gaseous ClO_2 treatment of blueberries to reduce *Salmonella*, yeasts, and molds was previously reported by Sy et al. (2005a). *Salmonella* inoculated on the berries (20 g treatment size) had a maximum reduction of 3.7 log after exposure to 8.0 mg/l ClO_2. Although they reported higher *Salmonella* reductions than this present study does, the berry mass-load they used was also 100-fold less, making direct comparison difficult. Bialka and Demirci (2007) previously examined efficacy of blueberry (30 g) treatment with 5% O_3 to reduce *E. coli* O157:H7 and *Salmonella* Typhimurium. Continuous O_3 treatment for 64 min resulted in a maximum reduction of *E. coli* and *Salmonella* by 2.2 log and 1.0 log, respectively. Regarding tomatoes, Netramai, Kijchavengkul, Sakulchuthathip, and Rubino (2016) treated grape tomatoes (sample size = 4 tomatoes) with surface-inoculated *S.* Typhimurium and were able to achieve 7.36 log reductions after 50 min exposure of 0.85 mg ClO_2 and Daş, Gürakan, and Bayındırlı (2006) reported that 20 mg/l gaseous O_3 treatment of tomatoes (25 g) completed reduced viable *Salmonella* cells (>7 log CFU/tomato) after 15 min. However, significant color change of the tomatoes after O_3 treatment was also reported.

The difference in treatment efficacy, noticeably ClO_2, observed among the three produce models here can be explained by the intrinsic factors of the carrots, blueberries, and tomatoes themselves. Previously, Park and Kang (2017) demonstrated that the hydrophobicity of the treatment surface was an important factor in the antimicrobial efficacy of ClO_2. The carrot sticks used in this study were already skinned, exposing the water filled tissues. Carrots have an average moisture content of 86–89% and contain high levels of unsaturated carotenes (e.g. β-carotene) and antioxidants (Sharma, Karki, Thakur, & Attri, 2012). Taking all of this into consideration, carrot tissues are great environments for both strong oxidizers like O_3 and selective oxidizers like ClO_2 to react. Given that O_3 is a much more powerful oxidizer than ClO_2, it is likely that the carrot tissue itself was a contributing factor that limited efficacy of O_3 treatment. Ozone will readily react with any available antioxidants, unsaturated aromatics, and unsaturated fatty acids (Khadre, Yousef, & Kim, 2001). Meanwhile, ClO_2 usually will only react with sulfur containing organic molecules, amines, and a handful of other substances (Sharma & Sohn, 2012). The smooth surface of the skinned carrot sticks in combination with high water content of the plant tissue creates an environmental that is highly suitable for ClO_2 treatment of produce (Singh et al., 2002) while O_3 reacts too quickly with the exposed plant tissue to be useful for the same application (Khadre et al., 2001). In fact, the bleaching observed on the carrot tissue following O_3 treatment indicates that either the gas or ROS byproducts are reacting with present β-carotene causing discoloration. Chlorine dioxide is also stable in water, which could explain the relatively high levels of residual initially measured on the carrots post-treatment that was not seen on the tomatoes or blueberries, which both have hydrophobic waxy cuticles (Lara, Belge, & Goulao, 2015). Additionally, the large difference in total surface area between 2 kg of blueberries and beefsteak tomatoes could be a cause for the major difference in observed treatment efficacy.

It is likely that low-dose gas treatments using longer exposures are more effective treatment scenarios, given the observed tendency for both ClO_2 and O_3 to bleach plant tissues at higher concentrations in this study. These results are indicative of storage settings or other "waiting" periods to be ideal scenarios for gaseous intervention. For example, tomatoes are commonly treated with ethylene to stimulate ripening and both ClO_2 and O_3 can break down ethylene (Guo et al., 2014; Tzortzakis et al., 2011), indicating that an intervention with either gas after ethylene exposure can help slow down further ripening and increase shelf life. Gaseous treatment of berries and carrots could be implemented during transport from the field to the processing plant, shipping, cold or even frozen storage. Although results of this study provide a promising outlook to successful intervention into already existing steps in produce infrastructures, variables that can heavily influence antimicrobial activity (e.g. treatment concentration) could prove to be problematic when scaled up. On top of intrinsic factors specific for each produce commodity, antimicrobial efficacy of gaseous treatments also depends on numerous extrinsic factors in the treatment environment. For example, higher levels of relative humidity (>70%) increase the effectiveness of both ClO_2 and O_3 treatment as both gases react more efficiently in the presence of water (Park & Kang, 2015; Tzortzakis 2017). To this same effect, a higher moisture content of the treated produce commodity will subsequently increase rate of gas consumption within the treatment chamber. At lower temperatures gas diffusion is slower, meaning that longer exposure times would be needed to ensure that treatments remain effective during refrigeration or freezing; further indicating that storage or other extended periods where produce is stationary is the idea setting to implement gaseous intervention. Previously, Netramai et al. (2016) treated grape tomatoes (sample size = 4 tomatoes) with surface-inoculated *S.* Typhimurium and were able to achieve 7.36 log reductions after 50 min exposure of 0.85 mg ClO_2. Changing the treatment temperature from 25 °C to 4 °C decreased the reduction to 3.95 log. Given that gaseous antimicrobial treatments have been well established to be a promising complement to chlorine washes, further pilot-scale studies using industrially realistically produce masses and storage settings are needed to evaluate if industrial gaseous intervention is effective and economically feasible.

5. Conclusions

The results of this study show that a batch-treatment of tomatoes and baby-cut carrots with ClO_2 in a closed-chamber system was capable of >7 log reduction of bacterial pathogens while leaving minimal residue levels. Lower levels of microbial reduction observed after equivalent treatments of blueberries could be a function of increased surface area. Gaseous O_3 using the same treatment system did not reduce significant pathogen levels to be considered a suitable treatment option for a closed-circulation treatment of the produce models used in this study.

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