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Cold plasma-activated hydrogen peroxide aerosol inactivates *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria innocua* and maintains quality of grape tomato, spinach and cantaloupe☆

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ABSTRACT

The purpose of this study was to investigate the efficacy of aerosolized hydrogen peroxide in inactivating bacteria and maintaining quality of grape tomatoes, baby spinach leaves and cantaloupes. Stem scars and smooth surfaces of tomatoes, spinach leaves, and cantaloupe rinds, inoculated with *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria innocua*, were treated for 45 s followed by additional 30 min dwell time with hydrogen peroxide (7.8%) aerosols activated by atmospheric cold plasma. Non-inoculated samples were used to study the effects on quality and native microflora populations. Results showed that two ranges of hydrogen peroxide droplets with mean diameters of 40 nm and 3.0 μm were introduced into the treatment chamber. The aerosolized hydrogen peroxide treatment reduced *S.* Typhimurium populations by 5.0 log CFU/piece, and *E. coli* O157:H7 and *L. innocua* populations from initial levels of 2.9 and 6.3 log CFU/piece, respectively, to non-detectable levels (detection limit 0.6 log CFU/piece) on the smooth surface of tomatoes. However, on the stem scar area of tomatoes, the reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. innocua* were only 1.0, 1.3, and 1.3 log, respectively. On the cantaloupe rind, the treatment reduced populations of *E. coli* O157:H7, *S.* Typhimurium and *L. innocua* by 4.9, 1.3, and 3.0 log CFU/piece, respectively. Under the same conditions, reductions achieved on spinach leaves were 1.5, 4.2 and 4.0 log for *E. coli* O157:H7, *S.* Typhimurium and *L. innocua*, respectively. The treatments also significantly reduced native aerobic plate count, and yeasts and mold count of tomato fruits and spinach leaves. Furthermore, firmness and color of the samples were not significantly affected by the aerosolized hydrogen peroxide. Overall, our results showed that the efficacy of aerosolized hydrogen peroxide depended on type of inoculated bacteria, location of bacteria and type of produce items, and aerosolized hydrogen peroxide could potentially be used to sanitize fresh fruits and vegetables.

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1. Introduction

The increase in consumption of fresh fruits and vegetables has coincided with a rise in foodborne outbreaks associated with fresh produce in recent years in the United States. It is estimated that about 37.2 million people suffer from foodborne illnesses and about 2612 people die due to foodborne disease each year (Scallan et al., 2011). From 2004 to 2013, fresh produce was responsible for 19% of total solved outbreaks and 24% of total illnesses in the United States, more than any other single category of food (CSPI, Center for Science in Public Interest, 2015). Pathogenic microorganisms of most concern in fresh produce include *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* (Callejón et al., 2015). Therefore, controlling foodborne pathogens in fresh produce is extremely important to public health.

The traditional method of sanitation involves use of aqueous sanitizers among which chlorine-based sanitizers are most widely used as a postharvest treatment (Pao et al., 2009). However, many studies have demonstrated that washing with chlorine and other sanitizers only achieves 1–2 log reductions of pathogens (Davidson et al., 2013; Herdt and Feng, 2009). One of the major reasons for the limited effectiveness by the aqueous chemical sanitizers is the internalization of

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enteric pathogens, i.e. pathogenic bacteria enter plant tissues through both natural apertures (stomata, lenticels) and damaged tissue (wounds, cut surfaces) (Erickson, 2012). Aqueous chemical sanitizers used during postharvest processing of fresh and fresh-cut produce are unlikely to reach pathogens residing in the protected sites of plant tissue due to the low penetration capability of aqueous sanitizers (Shynkaryk et al., 2015). Another reason for the limited effectiveness of aqueous sanitizers may be due to the reactivity of sanitizers, such as chlorine, with organic materials released from fresh produce, making it difficult to maintain effective concentrations of the active chemical agents in wash water (Shen et al., 2012). In addition, sanitizers such as chlorine may react with certain organic compounds to form potentially carcinogenic byproducts (Fan and Sokorai, 2015; Richardson et al., 1998). Some studies reported that gaseous antimicrobials were more effective in inactivating pathogens (Han et al., 2001; Lee et al., 2004). However, gaseous sanitizers have limitations due to the sophisticated apparatus for gas generation and the shortage of applicable sanitizers.

Several other inactivation methods or sanitizers have been investigated to inactivate foodborne pathogens on fresh produce, including washing with other chemical sanitizers such as organic acids (Almasoud et al., 2015; Eswaranandam et al., 2004; Neal et al., 2012), ozone (Karaca and Velioglu, 2014; Ölmez, 2012), and electrolyzed water (Al-Holy and Rasco, 2015; Issa-Zacharia et al., 2011), and physical interventions such as ultraviolet (UV-C) light (Guan et al., 2012; Mukhopadhyay et al., 2014, 2015; Yun et al., 2013), gamma irradiation (Fan and Sokorai, 2008; Lee et al., 2006; Song et al., 2014), and high hydrostatic pressure (Huang et al., 2013, 2016; Wang et al., 2013). However, most of these techniques or sanitizers have limited ability to inactivate microorganisms or face obstacles for consumer acceptance. In addition, some treatments may be too intense and result in adverse effects on sensory properties and quality.

Nanotechnology has emerged as a promising alternative to the battle against foodborne diseases (Pyrgiotakis et al., 2016). Among them, a newly emerged technology is the Engineered Water Nanostructures that rely on electrosprayed water to inactivate bacteria on surface of various fresh fruits and vegetables (Pyrgiotakis et al., 2014, 2015, 2016). However, most of these methods are in developmental stage. So, new intervention technologies are needed to achieve more desirable inactivation of pathogens without comprising product quality.

Hydrogen peroxide (H_2O_2) is a well-studied sanitizer (Back et al., 2014; Chimbombi et al., 2013; Guan et al., 2013). It has both bacteriostatic and bactericidal activity (Ölmez and Kretzschmar, 2009; Parish et al., 2003). At concentrations between 1 and 5%, H_2O_2 is generally used to sanitize food contact surfaces and packaging materials in aseptic filling operations (Parish et al., 2003). One of the main advantages of using H_2O_2 as a disinfecting agent is that it produces no residue as it is decomposed into water and oxygen by catalase which is naturally found in plants. Therefore, H_2O_2 is a generally regarded as safe substance for certain applications. The antimicrobial efficiency of H_2O_2 as a wash sanitizer is generally low, being comparable to 100–200 ppm of chlorine treatment at concentrations of 4–5% (Ölmez and Kretzschmar, 2009). In addition to concerns on microbial food safety, fresh fruits and vegetables are susceptible to a variety of spoilage microorganisms (Abadias et al., 2008; Artés et al., 2009). It has been indicated that a significant extension of shelf life for fruits and vegetables can be achieved by H_2O_2 (Alexandre et al., 2012).

In the present study, we applied H_2O_2 as a form of activated aerosol by applying high electric field to a stream of aerosolized H_2O_2 using a specially designed aerosolizer. The strong electric field created by the device forms a discharge and produces a non-thermal plasma in which various active species such as hydroxyl radicals are produced. These radicals are expected to be extremely reactive. The objectives of this study were to characterize the size distribution of aerosolized H_2O_2 droplets, to evaluate the efficacy of activated and aerosolized H_2O_2 in inactivating *E. coli* O157:H7, *S.* Typhimurium and *L. innocua* on grape tomatoes, baby spinach leaves and cantaloupe rinds, and to examine the effects on native microflora and quality of the fresh produce items.

2. Materials and methods

2.1. Bacterial strains and preparation of inocula

To minimize the risk of possible bacteria becoming airborne during treatments, attenuated and non-pathogenic bacteria were used in the study. *E. coli* O157:H7 (ATCC 700728), *S.* Typhimurium (ATCC 53647 and 53648), and *L. innocua* (ATCC 33090) were obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA) and maintained as a part of the culture collection at the USDA Eastern Regional Research Center (Wyndmoor, PA, USA). The *S.* Typhimurium strains were selected for spontaneous mutants resistant to 100 ppm of nalidixic acid by successive transfers of the bacteria into tryptic soy broth (TSB) with increasing concentrations of nalidixic acid to a final concentration 100 μg/ml over 10 days. Prior to use, stock cultures from a −80 °C freezer were inoculated into 10 ml TSB (supplemented with 100 μg/ml nalidixic acid for *Salmonella*) and incubated at 37 °C for 24 h. Cultures were transferred twice at 24 h intervals prior to their use in the inoculum. Strains of *E. coli* O157:H7, *S.* Typhimurium and *L. innocua* were separately grown in 10 ml of TSB (Difco, Sparks, MD, USA) (with 100 μg/ml nalidixic acid for *Salmonella*) at 37 °C for 24 h, followed by centrifugation (4000 × *g* for 10 min at 4 °C) and washed three times with buffered peptone water (BPW; Difco). The final pellets were resuspended in sterile BPW, corresponding to approximately 8–9 log CFU/ml. *S.* Typhimurium strains were combined to obtain a cocktail for use in experiments.

2.2. Sample preparation and inoculation

Fresh and unblemished grape tomatoes, baby spinach leaves and cantaloupes were purchased from local markets (Philadelphia, PA, USA) and stored overnight at 10 °C. Fruits were removed from 10 °C and equilibrated to ambient temperature before being inoculated. Tomatoes, spinach and whole cantaloupes were sanitized with 200 ppm chlorine solutions for 2 min before being rinsed in sterilized deionized water and arranged in a single layer and air-dried for 1 h in a bio-hood at ambient temperature (22 °C). The chlorine pre-wash was used to reduce background microflora populations (Niemira and Cooke, 2010). Ten tomatoes, five spinach leaves and five pieces of cantaloupe rind were used for each treatment per replicate. Pieces (2 × 3 cm) of cantaloupe rinds with ~3 cm thickness of flesh were prepared from whole cantaloupes. The stem scar area and smooth surface of tomatoes, spinach leaves and cantaloupe rinds were inoculated with 50 μl (for cantaloupe and spinach) or 25 μl (for tomatoes) of *E. coli*, *Salmonella* and *Listeria* suspensions separately by depositing droplets with a micropipette at ambient temperature. Samples were dried in the bio-hood for 2 h at 22 °C with the fan running before being treated with aerosolized H_2O_2. Experiments were independently replicated in different times (weeks); New freshly grown inoculum, and different batch of produce items were used for each replicate.

2.3. Procedure for treatments

H_2O_2 (7.8%) (TOMI™ Environmental Solutions, Inc., MN, USA) was aerosolized into a treatment chamber (12 × 12 × 24 in.) containing the produce items using the SteraMist™ BIT™ Activated Ionized Hydrogen Peroxide (AIHP) system (TOMI™ Environmental Solutions, Inc.) (Fig. 1). Produce items were placed onto a sterile test tube rack with inoculated area facing up. The SteraMist™ Environment System (TOMI™ Environmental Solutions, Inc., MN, USA) not only aerosolizes the solution but also ionizes and activates aerosolized H_2O_2 as droplets pass a cold plasma field generated between two pin electrodes. The distance and voltage between the two electrodes were 9 mm and 17 kV,

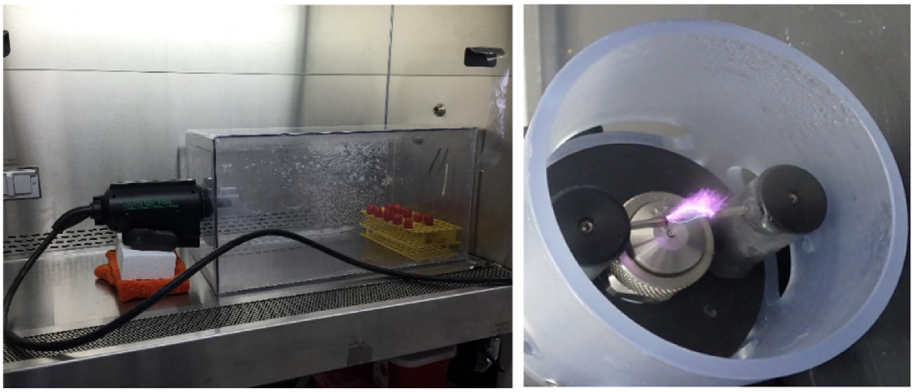

Fig. 1. Introduction of aerosolized H_2O_2 into a treatment chamber containing tomatoes (left) and close up of the aerosolization and activation delivering device (right).

respectively. The flow rate for H_2O_2 was 9.7 ml/min with an air pressure of 15 psi. After 45 s treatment, the chamber was sealed for 30 mins (dwell time) before removal of the fresh produce items. Experiments were repeated three times. The concentration (7.8%) of H_2O_2 and treatment time were chosen based on the manufacturer's recommendation and measurement of H_2O_2 in the chamber.

2.4. Characterization of aerosolized droplets

Multiple particle detection instruments were used to monitor and characterize size distribution and number concentration of H_2O_2 droplets in the treatment chamber as a function of time. Specifically, a scanning mobility particle sizer (SMPS Model 3080, TSI Inc., Shoreview, MN, USA) and an aerodynamic particle sizer (APS Model 3321, TSI Inc.) were connected to the treatment chamber through an access port in the back of the treatment chamber to measure droplet sizes ranging from 2.5 to 210 nm, and from 0.5 to 20 μm, respectively. In addition, H_2O_2, ozone, and environmental conditions (temperature and humidity) were monitored using a gas leak detector (Portasens II, Analytical Technology, Inc., Collegeville, PA, USA), an ozone monitor (model 202, 2B Technologies, Boulder, CO, USA), and Q-track (Model 8551, TSI Inc.), respectively.

2.5. Bacterial enumeration

After treatments, the tomato smooth skin and stem scar areas with the inocula were excised using a pair of surface-sterilized scissors. The smooth skins and stem scars from five fruits treated with the same sanitizers were combined (total weight: 1.0 ± 0.2 g for both smooth skin and stem scar) and five pieces of spinach leaves (2.4 ± 0.4 g) were placed into stomacher bags containing 20 ml of neutralizing buffer (Difco). Five pieces of rinds of cantaloupes (20.1 ± 6.8 g) after removal of flesh were transferred into sterile stomacher bags, containing 100 ml of neutralizing buffer (Difco). Stomacher bags were homogenized for 2 min at 260 rpm with a Stomacher (Interscience Laboratories Inc., Woburn, MA, USA). After homogenization, filtrates were serially diluted (if needed), and aliquots (100 μl or 1 ml depending on the treatments) were spread-plated onto selective media. Sorbitol MacConkey agar (SMAC), Tryptic Soy Agar (TSA) with 100 μg/ml nalidixic acid, and PALCAM Agars (Difco) were used as selective media for the enumeration of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. The plates were incubated at 37 °C for 24 h, and colonies were counted after incubation. When a sample did not yield any colonies on the plates, half the limit of detection (0.6 log CFU/piece) was used for calculation (Davidson et al., 2013). The populations of bacteria were expressed as log CFU per piece of fruit, cantaloupe or spinach leaf.

2.6. Native microflora

Whole tomatoes, spinach and rinds of cantaloupe without prior chlorine wash or bacteria inoculation were treated with aerosolized H_2O_2 as described earlier. The untreated (control) and treated samples were placed separately in a Stomacher bag with 20–100 ml of neutralizing buffer and pummeled at 260 rpm for 2 min using Stomacher (Interscience Laboratories Inc.). Decimal dilutions of the samples were made with 0.1% peptone (Difco) and aliquots (0.1 or 1 ml) were spread plated in duplicate onto TSA with incubation at 37 °C for 24 h for the enumeration of total aerobic plate count (APC), and onto Dichloran Rose Bengal Chlortetracycline (DRBC, Difco) agar with incubation at 25 °C for 5 days for enumeration of yeast and mold. DRBC plates were wrapped with aluminum foil to prevent dehydration. Experiments were conducted independently 8 times. Colonies were counted and reported as log CFU/piece.

2.7. Effects on quality

Grape tomatoes, spinach leaves and 2 × 3 cm pieces of cantaloupe (with flesh) were treated with aerosolized H_2O_2 as described earlier. The treated samples were placed into 8 oz. clamshell containers (for tomatoes and cantaloupe pieces) or perforated film bags (for spinach) and stored at 10 °C overnight before being measured for texture and color. Experiments were repeated 8 times.

2.7.1. Firmness measurements

Firmness was evaluated with a TA-XT2i Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA). A 3-mm diameter probe was used to penetrate tomato fruit and cantaloupe rind to a depth of 10 mm at a speed of 10 mm/s. Five fruits/pieces were used for firmness measurements, and there were a total of 40 measurements (eight replicates). For spinach, the five leaves were placed into a Kramer cell and texture was measured with the same speed setting as for tomatoes and cantaloupe. Maximum force was recorded using the Texture Expert software (version 1.22, Texture Technologies Crop.).

2.7.2. Analysis of color, appearance and off-odor

Surface color of samples was measured with a Hunter UltraScan®VIS colorimeter (Hunter Associates Lab, Reston, VA, USA) using a 1.3 cm measuring aperture. D65/10° was used as the illuminant-viewing geometry. The colorimeter was calibrated using the standard black and white plates. Two readings were made on each tomato fruit and on each piece of spinach leaf (top side) and cantaloupe rind. L*, a* and b*were recorded. In addition, the appearance and off-odor of the samples were assessed by three researchers (Fan and Sokorai, 2008).

2.8. Statistical analysis

Experiments were repeated at least three times while multiple pieces of samples were used for each replicate as subsamples or for pooling. Statistical analyses were conducted using SAS Version 9.4 (SAS Institute Inc., Cary, NC, USA). Treatment means and standard deviation were reported. The least significant difference test was used to test the effect of treatments with a significance level of $P = 0.05$.

3. Results and discussion

3.1. Size distribution of H_2O_2 droplets

Fig. 2 shows size distribution of droplets in the treatment chamber immediately after the introduction of aerosolized H_2O_2 and after additional 30 min dwell time. It appears that the aerosolizer introduced two size ranges of droplets into the chamber (Fig. 2A, B): one in nanometer range and the other in micrometer range. Nanosize droplets appeared to be polydisperse in size and followed a log normal distribution with a mean diameter of 40.3 nm, a mode (peak) of 33.4 nm and a standard deviation of 30.9 nm (geometric standard deviation 1.7). Total number of droplets in the nanosize range was 84,000 #/cc. For droplets in the micrometer range, mean diameter was 3.0 μm with a mode of 4.0 μm. About 80% of droplets were in the range of <5 μm with peaks in the range of 3.0–4.4 μm. Total number of droplets in micrometer range (0.5–20 μm) was 4390 #/cc. Due to the limitations by the utilized instrumentation there are no data available in the size range of 200–500 nm (Reischl, 2007).

After 30 min (end of treatment), about 8% nanosize droplets and virtually no micrometer-size droplets remained in the treatment chamber (Fig. 2C, D). Ozone was produced from the aerosolizing device. However, the ozone concentrations in the chamber were below 60 ppb during the entire treatment and dwell time. Humidity increased from 43% to ~90% after application of aerosolized H_2O_2 while H_2O_2 concertation exceeded 150 ppm.

Our results showed that both nano- and micro-size droplets were produced by the aerosolizer. During the post-generation time (dwell time), the number of droplets decreased rapidly with microsize droplets decreasing much faster than nanosize droplets. It is well known that size determines stability of droplets. The droplet size may be optimized by adjusting air/liquid flow. Future research may focus on producing more stable and higher concentrations of droplets, particularly in the nanosize range. Nano-water droplets developed by Pyrgiotakis et al. (2014) showed better stability as 50% of droplets remained in treatment chamber after 4 h. The stability has been attributed to the surface charge of droplets.

3.2. Effects of aerosolized hydrogen peroxide treatment on populations of E. coli O157:H7, S. Typhimurium, and L. innocua

The effects of aerosolized H_2O_2 on *E. coli* O157:H7 on tomatoes, spinach leaves and cantaloupe rind are presented in Table 1. After treated with aerosolized H_2O_2, the inoculated *E. coli* on the smooth surface of tomatoes were reduced to a level below detection limit (<0.6 log CFU/piece) while the bacterium was only reduced by 1.0 log CFU/piece on the stem scar area of tomatoes. On the surface of spinach leaves and cantaloupe rind, *E. coli* populations were reduced by 1.5 and 4.9 log CFU/piece, respectively.

The populations of *E. coli* O157:H7 on the non-treated (control) tomato fruit were less than those on non-treated spinach and cantaloupe samples. It appears that the inoculated *E. coli* O157:H7 cells on the surface of tomatoes (both smooth surface and stem scar area) were less stable during the drying period after inoculation compared with those on spinach leaves and cantaloupe rinds. After 2 h of drying in a biohood after inoculation, the populations of *E. coli* on tomato smooth surface were 3.4 and 2.2 log less than those on cantaloupe and spinach, respectively. Similarly, in field-inoculated lettuce, a rapid 2- to 3-log decrease in the population of the same strain of *E. coli* O157:H7 were observed during the first 2 h post-inoculation period (Moyne et al., 2011). The decline in bacterial populations slowed afterward. No difference in survival on the lettuce of the *E. coli* O157:H7 strain was observed in comparison with another strain (ATCC 43888) of *E. coli* O157:H7 (Moyne et al., 2011). Obviously, the environment conditions (temperature, humidity, etc.) for the field study were different from those used in the present study. Further study is needed to evaluate what contributes to the rapid decline in *E. coli* populations on tomato surface and whether the rapid decline is due to desiccation and strain-specific. Overall, our results showed that the aerosolized H_2O_2 was more effective in

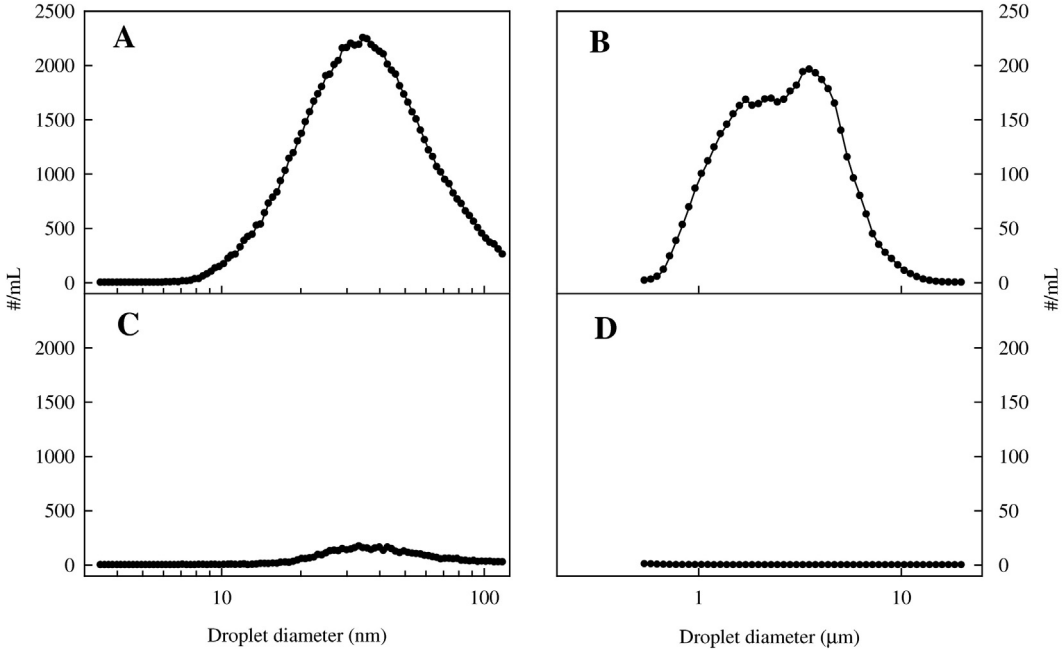

Fig. 2. Size distribution of H_2O_2 droplets in the treatment chamber measured immediately after application (A, B) and after 30 min (C, D) incubation.

Table 1
Effects of aerosolized H_2O_2 on populations (log CFU/piece) of *E. coli* O157:H7 inoculated on stem scar and smooth surface of tomatoes, and on spinach and cantaloupes.

Treatments	Tomato-smooth surface	Tomato-stem scar	Spinach	Cantaloupe
Control	2.9 ± 0.1[a]	3.6 ± 0.5a[b]	5.1 ± 1.0a	6.3 ± 0.6a
H_2O_2	ND[c]	2.6 ± 0.1b	3.6 ± 0.6b	1.4 ± 0.9b

[a] The numbers are means ± standard deviations ($n = 3$).
[b] Data in the same column followed by the same letter are not significantly different ($P > 0.05$).
[c] ND: not detectable (detection limit: 0.6 log CFU/piece).

reducing *E. coli* O157:H7 on smooth surface of tomato and surface of cantaloupes than on the stem scar area of tomatoes and spinach leaves.

The extent of *S.* Typhimurium reductions by aerosolized H_2O_2 depended on the types of produce (Table 2). The greatest reduction of *S.* Typhimurium was 5.0 log which was on the smooth surface of tomato. The same treatment achieved 4.2 log reduction of *S.* Typhimurium on spinach leaves. *S.* Typhimuriun cells on cantaloupe rind and the stem scar of tomato were more difficult to inactivate, with the same treatment achieving 1.3 log reductions. Therefore, aerosolized H_2O_2 treatment was more effective in reducing *S.* Typhimurium populations on the smooth surface of tomato or spinach leaves than on the stem scar area of tomatoes or cantaloupe rind.

The populations of *L. innocua* on the non-treated tomato's smooth surface and stem scar, spinach leaves and cantaloupe rind were 6.3, 6.2, 6.4 and 6.5 log CFU/piece, respectively (Table 3). Similar to the results on *Salmonella*, *L. innocua* cells on the smooth surface of tomato and spinach were easier to inactivate by the aerosolized H_2O_2, achieving approximately 6.0 and 4.0 log CFU/piece, respectively. *L. innocua* cells on cantaloupe and stem scar area of tomato were reduced by 3.0 and 1.3 log CFU/piece, respectively.

Our results indicated that *E. coli* O157:H7 showed greater resistance to the H_2O_2 treatment than *S.* Typhimurium on spinach, and yet lower resistance than *S.* Typhimurium on cantaloupe. It is difficult to explain the divergent results between the two bacteria on two produce items in response to the same treatment, considering the biological similarity between the two Gram-negative bacteria (*E. coli* and *Salmonella*). The disparate response may be due to distinct interactions among bacteria, surface characteristics of different produce items, and aerosolized H_2O_2. Clearly, more research is needed to study the reliability and stability of aerosolizing equipment against microorganisms using other strains of the bacteria.

It has been demonstrated that washing fresh produce with chlorine or other sanitizers has limited effectiveness in reducing pathogenic bacteria, as most treatments only achieve at the most 2 log reductions of pathogens associated with fresh produce (Beuchat et al., 2004; Gonzalez et al., 2004; Kondo et al., 2006; Shirron et al., 2009). For example, our earlier study (Fan et al., 2009) showed that most common sanitizers did not significantly reduce populations of *Salmonella* on the rind of cantaloupes, as even the best treatment (acidified sodium acid, 1000 ppm for 10 min) only achieved 1.7 log CFU/g of *Salmonella*. The results from this current study suggest that the H_2O_2 aerosolization technology may be used as an alternative to washes with common sanitizers.

There are a number of earlier studies involving the use of H_2O_2 as an aqueous wash. Sapers et al. (2000) showed that washing apples with 5% H_2O_2 for 2 min reduced *E. coli* population by about 2.8 log CFU/g. Washing dip-inoculated tomatoes with 5% H_2O_2 at 60 °C for 2 min reduced populations of *E. coli* and *Salmonella* by 2.6 log CFU/g (Sapers and Jones, 2006). Treatment with 5% H_2O_2 at 70 °C caused a 3.8-log CFU/cm^2 reduction of *Salmonella* on the rind of cantaloupes (Ukuku et al., 2004). Washing baby spinach with 2% H_2O_2 at 50 °C for 2 min reduced *E. coli* O157:H7 population by 2.2 log CFU/g (Huang and Chen, 2011). Our results showed that aerosolized H_2O_2 could achieve more than four log reduction of the inoculated bacteria, indicating that the technology can be more effective than the aqueous wash. Earlier studies indicated that the efficacy of aqueous H_2O_2 washes increased with increasing treatment temperature. Future research may be conducted to evaluate the effect of treatment temperature on the efficacy of aerosolized H_2O_2. High temperature may also affect the stability of H_2O_2 droplets in the treatment chamber.

Several earlier studies have demonstrated antimicrobial effects of aerosolized sanitizers on stainless steel surfaces and a few produce items. Choi et al. (2012) reported that treatments with unspecified aerosolized H_2O_2-based sanitizer reduced populations of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* on the stainless steel coupons to levels below the detection limit (1 log CFU/ml) after 60 min treatments. A 4.8 log reduction of viable *Bacillus anthracis* spore surrogates was achieved on wood and stainless steel surface by aerosolized peroxyacetic acid (Wood et al., 2013). Huang et al. (2012) found 2 min aerosolization treatments of aqueous 2.5–5% acetic acid and lactic acid or 3–5% H_2O_2 only reduced 0.3–1.2 log reduction of *E. coli* O157:H7 on spinach leaves. Our results showed that 45 s treatment plus 30 min dwell time of aerosolized H_2O_2 achieved 1.5 log reduction of *E. coli* O157:H7 and 4.0 log reductions of *Listeria* and *Salmonella* on spinach leaves. Park and Kang (2015) showed that treatments with 80 ppm aerosolized peroxyacetic acid for 20 min achieved 2.3, 1.9, and 0.8 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on spinach, respectively. Therefore, the aerosolization technology used in the present study may be similarly effective as the systems used in previous studies, although higher concentration of H_2O_2 was used in the present study.

The stem scar area of the tomato has been identified as the main harbor site for *Salmonella* (Guo et al., 2002). Due to the porous nature of the stem scar, as well as the inability of aqueous sanitizers to effectively penetrate these tissues, *Salmonella* in the area is much harder to inactivate than those on a smooth surface of tomatoes even when using gaseous antimicrobials (Fan et al., 2012). Our results also demonstrated that the three bacteria on the stem scar area of tomatoes were most difficult to inactivate with aerosolized H_2O_2.

The device we used not only aerosolizes H_2O_2, but also ionizes and activates H_2O_2 when the droplets pass through the atmospheric cold plasma field, producing highly reactive species such as hydroxyl radicals. However, hydroxyl radicals are not stable with an estimated half-life of approximately 10^{-9} s (Pryor, 1986). Even though we were unable

Table 2
Effects of aerosolized H_2O_2 on populations (log CFU/piece) of *S.* Typhimurium inoculated on stem scar and smooth surface of tomatoes, and on spinach and cantaloupes.

Treatments	Tomato-smooth surface	Tomato-stem scar	Spinach	Cantaloupe
Control	6.7 ± 0.1a[a,b]	7.1 ± 0.1a	6.7 ± 0.1a	6.9 ± 0.2a
H_2O_2	1.7 ± 1.3b	5.8 ± 0.6b	2.5 ± 1.1b	5.6 ± 0.5b

[a] The numbers are means ± standard deviations ($n = 3$).
[b] Data in the same column followed by the same letter are not significantly different ($P > 0.05$).

Table 3
Effects of aerosolized H_2O_2 on populations (log CFU/piece) of *L. innocua* inoculated on stem scar and smooth surface of tomatoes, and on spinach and cantaloupes.

Treatments	Tomato-smooth surface	Tomato-stem scar	Spinach	Cantaloupe
Control	6.3 ± 0.2[a]	6.2 ± 0.2a[b]	6.4 ± 0.1a	6.5 ± 0.2a
H_2O_2	ND[c]	4.9 ± 0.4b	2.4 ± 1.9b	3.5 ± 0.2b

[a] The numbers are means ± standard deviations ($n = 3$).
[b] Data in the same column followed by the same letter are not significantly different ($P > 0.05$).
[c] ND: not detectable (Detection limit: 0.6 log CFU/piece).

to confirm the production of reactive species due to lack of appropriate analytical instruments, the free radicals would mostly disappear before reaching the produce items. Further research is needed to evaluate the impact of cold plasma, and whether and how the charge and production of hydroxyl radicals contribute to the efficacy of the aerosolized H_2O_2. It should also be pointed out that the fresh produce samples were treated in a small chamber while the aerosolizing device is designed to treat large rooms. Therefore, the treatment conditions were less ideal for the technology.

In the present study, 1–2 strains of biosafety level 1 bacteria as designated by ATCC were used to inoculate fresh produce items. Future studies may be conducted to investigate the efficacy of the aerosolization technology against human pathogens associated with produce outbreaks and to use multi-strain cocktails to minimize the possibilities of strain-dependent response.

3.3. Effect of aerosolized hydrogen peroxide treatment on the native microflora of tomato, spinach and cantaloupe

The effect of aerosolized H_2O_2 on APC count, and yeast and mold count on grape tomatoes, spinach leaves and cantaloupe rinds were evaluated in the present study. The APC and yeast and mold counts of untreated tomato fruits were 5.9 ± 0.5 and 6.1 ± 1.4 log CFU/piece, respectively (Table 4). The findings are in agreement with earlier reports on native microbial populations of tomato (Mahmoud, 2010; Mukhopadhyay et al., 2015). The initial APC and yeast and mold counts on untreated cantaloupe were 5.2 ± 0.5 and 5.5 ± 0.7 log CFU/pieces, respectively. Our previous study (Fan et al., 2009) found APC and yeast and mold counts on the rinds of the whole cantaloupes to be 4.8 and 4.0 log CFU/cm^2, respectively while Johnston et al. (2005) showed that APC for cantaloupe were in the range of 6.4 to 7.0 log CFU/g. The initial APC and yeast and mold counts on spinach samples were 6.0 ± 0.7 and 6.4 ± 0.7 log CFU/leaf, respectively. Rahman et al. (2008) reported that the populations of total aerobic bacteria on the spinach leaves were 5.6 log CFU/g while Poimenidou et al. (2016) found 7.4 log population of mesophilic microorganisms on spinach leaves.

The aerosolized H_2O_2 treatment achieved small but statistically significant ($P < 0.05$) reductions (0.5, and 1.3 log, respectively) in APC of tomatoes and spinach leaves. The yeast and mold count of tomato and spinach were also significantly ($P < 0.05$) reduced by the aerosolized H_2O_2 with 3.9 and 2.2 log reductions, respectively. It seems that the treatment was more effective in reducing yeast and mold count than APC. The reductions of APC and yeast and mold count on cantaloupe rind were not significant ($P > 0.05$).

Our results suggest that native microorganisms on the surface of fresh produce (particularly on cantaloupe) are harder to inactivate compared with the inoculated bacteria. Our earlier study (Fan et al., 2009) showed that chlorine (200 ppm) or peroxyacetic acid (80 ppm) did not result in any significant reductions in APC on the rind of cantaloupe, and only marginally reduced yeast and mold count. Ukuku (2006) also demonstrated that 200 ppm chlorine wash was ineffective in reducing the total bacterial count from cantaloupe rind. The ineffectiveness of aqueous sanitizers is probably due to the presence of microorganisms in biofilms. Biofilms are assemblages of microorganisms in which cells are attached to a surface and to each other, and are embedded in a self-produced matrix of extracellular polymeric substances (Costerton et al., 1999). Biofilms are likely formed in the protective sites such as the cut surface of spinach, the stem scar of tomatoes and, most noticeably, the netting surface of cantaloupes. The surface of the cantaloupe, with its meshwork of lenticellar netting, provides a large number of attachment sites for microorganisms to grow and form biofilms. Cells in the biofilm are more resistant to chemical sanitizers by providing a physical barrier against the diffusion of antimicrobial agents. Our results showed that aerosolized H_2O_2 was not as effective against native microflora as against the inoculated bacteria. It is possible that the effectiveness of aerosolized H_2O_2 may be less effective against pathogens in biofilms or when embedded in native microflora.

In this study, we washed fresh produce with chlorine to reduce the populations of native microflora, prior to inoculation. Use of chlorine prior to inoculation may alter the ecology and profile of the native microflora and surface chemistry of the produce items, even though the produce items were rinsed with sterilized deionized water after chlorine wash. There is a possibility that those changes can affect the attachment of inoculated bacteria and the effectiveness of aerosolized H_2O_2. Future research may be conducted to study the interaction between inoculated bacteria and background microflora in response to the aerosolized H_2O_2 treatment.

3.4. Quality of tomato, spinach leaves and cantaloupe

Texture and color of samples were measured after 1 day storage at 10 °C. There were no significant differences in texture of tomato, cantaloupe and spinach between the treated and non-treated samples (Table 5).

Color was expressed in terms of L*, a* and b* values, where L* values indicate luminosity (level of light or darkness); a* indicates chromaticity on a green (negative number) to red (positive number), and b* values indicate chromaticity on a blue (negative number) to yellow

Table 4
Total aerobic plate and yeast and mold counts (Log CFU/piece) on tomato, spinach and cantaloupe treated with and without aerosolized H_2O_2.

Treatment	Total plate count			Yeast and mold		
	Tomato	Spinach	Cantaloupe	Tomato	Spinach	Cantaloupe
Control	5.9 ± 0.5a[a,b]	6.0 ± 0.7a	5.2 ± 0.5a	6.1 ± 1.4a	6.4 ± 0.7a	5.5 ± 0.7a
H_2O_2	5.4 ± 0.5b	4.7 ± 1.3b	4.6 ± 1.2a	2.2 ± 1.5b	4.2 ± 2.0b	4.7 ± 0.9a

[a] Data are expressed as means ± standard deviations ($n = 8$).
[b] Data in the same column followed by the same letter are not significantly different ($P > 0.05$).

Table 5
Firmness (kg) of tomato, spinach and cantaloupe treated with and without aerosolized H_2O_2. Firmness was measured 1 day after treatment.

Treatments	Tomato	Spinach	Cantaloupe
Control	1.26 ± 0.12a[a,b]	9.52 ± 2.19a	5.90 ± 1.77a
H_2O_2	1.17 ± 0.13a	9.87 ± 1.94a	6.51 ± 0.48a

[a] Data expressed as means ± standard deviations ($n = 8$).
[b] Data in the same column followed by the same letter are not significantly different ($P > 0.05$).

Table 6
Color parameters of tomato, spinach and cantaloupe after being treated with and without aerosolized H_2O_2. Color was measured 1 day after treatment.

Treatments	L*			a*			b*		
	Tomato	Spinach	Cantaloupe	Tomato	Spinach	Cantaloupe	Tomato	Spinach	Cantaloupe
Control	33.29 ± 0.75a[a,b]	34.66 ± 1.58a	64.32 ± 1.26a	23.30 ± 1.79a	−8.37 ± 0.24a	3.12 ± 0.39a	20.28 ± 0.94a	17.81 ± 0.76a	28.89 ± 0.91a
H_2O_2	32.41 ± 0.64b	33.71 ± 1.47a	64.16 ± 1.55a	22.88 ± 0.67a	−8.13 ± 0.25a	2.54 ± 0.66a	19.77 ± 0.94a	17.70 ± 0.75a	28.80 ± 1.19a

[a] Data expressed as means ± standard deviations ($n = 8$).
[b] Data in the same column followed by the same letter are not significantly different ($P > 0.05$).

(positive number). L* values of tomatoes were reduced by the aerosolized treatment, indicating the darkening and less yellowing of tomato skin (Table 6). However, no visual changes were noticed. The a* values, an indication of tomato redness, were not affected by the treatment. The treatment did not significantly affect any color parameters for spinach or cantaloupe rind (Table 6). Furthermore, compared with the control, the aerosolized H_2O_2 did not affect the appearance or odor of the samples assessed 1 day after treatment (data not shown). In addition, the soluble solid contents of cantaloupe or tomatoes were not significantly influenced by the treatment either (data not shown). Therefore, the treatment did not have a significant impact on quality of the three produce items. In the present study, we evaluated the native microflora and quality after 1 day of storage. Further research is needed to evaluate the changes during longer storage time, and efficacy of the technology on larger scales using whole cantaloupes and bulk amount of fresh produce items.

4. Conclusions

In this study, H_2O_2 was applied as a cold plasma-activated aerosol to reduce populations of *E. coli* O157:H7, *S.* Typhimurium and *L. innocua* on tomato, spinach and cantaloupe rind. Our results revealed that populations of *E. coli* O157:H7, *S.* Typhimurium and *L. innocua* inoculated on the smooth skin surface and stem scar area of tomato, spinach and cantaloupe could be significantly reduced by a 45 s aerosolized H_2O_2 treatment plus 30 min dwell time. The treatment resulted in >5 log CFU/piece reduction of *S.* Typhimurium and *L. innocua* and reduced *E. coli* to nondetectable levels on the tomato's smooth surfaces. For the three bacteria on the stem scar areas of tomatoes, the reductions were 1.0–1.3 log CFU/piece. Under the same conditions, reductions achieved on the surface of spinach leaves were 4.2 and 4.0 log CFU/leaf for *Salmonella* and *L. innocua*, respectively. On cantaloupe, the reductions were 4.9, 1.3, and 3.0 log CFU/piece for *E. coli* O157:H7, *S.* Typhimurium and *L. innocua*, respectively. The treatment also significantly reduced populations of native microorganisms on tomato and spinach leaves. Color and texture of the produce items were not significantly affected by the aerosolized H_2O_2. Overall, our results demonstrate that the aerosolized technology can be used to enhance microbial safety of fresh fruits and vegetables, although additional research is needed to optimize the technology.

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