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Cold plasma-activated hydrogen peroxide aerosol on populations of *Salmonella* Typhimurium and *Listeria innocua* and quality changes of apple, tomato and cantaloupe during storage - A pilot scale study

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ABSTRACT

The occurrence of foodborne illness outbreaks associated with fresh fruits and vegetables continues to be a major concern. Effective technologies that can be applied commercially are urgently needed. A pilot scale study was carried out to investigate the efficacy of ionized hydrogen peroxide (iHP) treatment for the inactivation of *Salmonella* Typhimurium and *Listeria innocua* inoculated on apple and tomato smooth surfaces, tomato stem scars and cantaloupe rinds, and to evaluate changes in quality parameters during simulated shelf life study. iHP is the process of activating low concentration of aerosolized hydrogen peroxide with cold plasma. After treatments with 17.62 mL/m^3 iHP in a large chamber (4.27 × 2.44 × 2.14 m), the inoculated bacteria were significantly reduced to a level below the detection limit (0.70 log CFU/piece) on the smooth surfaces of apples and tomatoes placed as a single layer in crates. Reductions of more than 3 log CFU/piece and approximately 1 log CFU/piece were obtained for cantaloupe rinds and tomato stem scars, respectively. When apples were placed in crates with multiple layers, 22% of fruits exhibited detectable *Salmonella* and 11% of fruits had detectable *Listeria*. Treated fruits from the pilot scale study were stored at 17 °C for 14 days and analyzed for quality every week. Results showed that iHP did not significantly affect appearance, color, texture, pH, soluble solids content, ascorbic acid, and antioxidants of the fresh produce items. The study demonstrates that the technology can be applied to fresh fruits to enhance microbial safety while maintaining quality. Challenges remain as to how to facilitate iHP exposure to all fruit surfaces in commercial settings.

1. Introduction

Fresh fruits and vegetables are becoming more popular food choices for consumers compared to other food items because of their high nutritional values and health benefits. Increasing demand from consumers for produce that is safe to eat, has high quality and an extended shelf life is becoming more challenging to meet. Along with the increasing consumption, the occurrence of foodborne illness outbreaks associated with fresh fruits and vegetables has increased in recent years (Huang & Chen, 2011; Chapman, MacLaurin, & Powell, 2011; Back, Ha, & Kang, 2014; Bennett, Littrell, Hill, Mahovic, & Behravesh, 2015, 2018). During growing or harvesting, fruits and vegetables may be contaminated with soil, water, animals or humans, all of which may harbor human pathogens (Alexandre, Brandão, & Silva, 2012; Ukuku, 2004). Fruits and vegetables also require proper handling and storage to take full advantage of their benefits. Consumers are more conscious about the downsides of frequently-used sanitizers, such as chlorine, and are looking for safer high quality fresh produce.

There have been many processes applied to reduce or eliminate pathogens from food including traditional methods such as heat-based treatments (Ohlsson & Bengtsson, 2002), chemical sanitizers (Akbas & Ölmez, 2007; Casteel, Schmidt, & Sobsey, 2008; Rico, Martin-Diana, Barat, & Barry-Ryan, 2007) and recent technologies such as ozone (Fan, Sokorai, Engemann, Gurtler, & Liu, 2012), ultrasound (de São José & Vanetti, 2015; Tremarin, Brandão, & Silva, 2017), ultraviolet radiation (Bhat, 2016; Tremarin et al., 2017), and cold plasma (Gilmore et al., 2018; Sarangapani, Patange, Bourke, Keener, & Cullen, 2018; Schlüter et al., 2013). Postharvest treatments with hydrogen peroxide (H_2O_2) have been proposed as an alternative to current chemical treatments for extending the storage and shelf life of fruits and vegetables (Bayoumi, 2008; Bhagwat, 2006). H_2O_2 is a stable, partially reduced form of oxygen, and its rapid breakdown is characteristically mediated by enzyme action in fresh produce (Khandaker, Boyce, & Osman, 2012). It plays a role as a messenger molecule at low or normal concentrations

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(1–5 μmol g^{-1} fresh weight, FW) and orchestrates programmed cell death at high concentrations (above 7 μmol g^{-1} FW) (Cheeseman, 2006; Dat et al., 2000). Studies show that H_2O_2 treatment significantly reduced populations of *Salmonella* spp. and *Escherichia coli* O157:H7 on whole and fresh-cut melons (Ukuku, 2004; Ukuku, Bari, Kawamoto, & Isshiki, 2005). Also, Alexandre et al. (2012) found that 1% H_2O_2 did not damage the quality of red bell peppers, strawberries, and watercress while attaining microbial reduction; nevertheless, the effectiveness of H_2O_2 is rather limited.

Cold plasma is an emerging antimicrobial technology for sanitizing various food items. Plasma is composed of gas molecules, which have been dissociated by an energy input (Ramos, Miller, Brandão, Teixeira, & Silva, 2013). The primary biocidal means of cold plasma include UV light and reactive chemical products resulting from the cold plasma-activated ionization process (Niemira, 2012). Studies have demonstrated that under optimal conditions, a 15 min cold atmospheric gas plasma treatment resulted in 2.72, 1.76, and 0.94 log-reductions of *S.* Typhimurium viability on lettuce, strawberry, and potato, respectively (Fernandez, Noriega, & Thompson, 2013). Wang et al. (2012) applied a 2 min treatment of atmospheric-pressure cold air plasma to inactivate nearly 90% of the *Salmonella* (concentration of 10^6 CFU/mL) on carrot slices and 80% on cucumber and pear slices. Meanwhile, changes in physiochemical properties such as water content, color parameters, and nutritional content, as affected by the plasma were within acceptable ranges.

In previous studies (Jiang et al., 2017; Song and Fan, 2020), cold plasma-activated H_2O_2 aerosols (called ionized hydrogen peroxide, or iHP) were applied to tomato, cantaloupe, and leafy greens in a small treatment chamber. The treatment reduced populations of *E. coli* O157:H7, *S.* Typhimurium, and *L. innocua* by more than 5.0 log CFU/piece and also significantly reduced native microflora of tomatoes and spinach. However, the initial studies only tested the produce in a small chamber (30.48 × 30.48 × 60.96 cm) and did not determine the effects on quality attributes of fresh produce during post-treatment storage to demonstrate the feasibility of iHP on an industrial scale. Often, when applied on a large scale, the efficacy of an intervention technology is significantly compromised.

Therefore, the objective of this work was to carry out a scaled-up study of iHP treated apples, tomatoes, and cantaloupes. Inactivation of *Salmonella* Typhimurium and *Listeria innocua* were investigated as well as its effects on the appearance, color, texture, pH, soluble solids content, ascorbic acid, and antioxidants during 14-d storage at 17 °C.

2. Material and methods

2.1. Produce items

Beefsteak tomatoes and cantaloupes were obtained from a major supermarket chain via special orders (Philadelphia, PA, USA). The average weight of the tomatoes and cantaloupes were 240 g and 1856 g, respectively. Unwaxed Gala apples were harvested from a commercial orchard in Central Pennsylvania with an average weight of 145 g.

2.2. Microbial analysis

2.2.1. Bacterial strains and preparation of inocula

Two strains of non-pathogenic *Salmonella* Typhimurium (ATCC 53647 and 53648) and three strains of *Listeria innocua* (ATCC 33090, ATCC 51742, and ATCC BAA680) obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA) were used in this study. The *S.* Typhimurium strains were selected for resistance to nalidixic acid by successive, increasing-concentration transfers in Tryptic Soy Broth (TSB) (Difco, Sparks, MD, USA). Stock cultures of *Salmonella* and *Listeria* from a −80 °C freezer were maintained in 10 mL of TSB (supplemented with 100 g/mL nalidixic acid for *S.* Typhimurium) at 4 °C after incubating at 37 °C for 40 and 48 h, respectively, and sub-cultured each time.

Each strain of *S.* Typhimurium and *L. innocua* was grown in 9 mL of TSB (supplemented with 100 g/mL nalidixic acid for *S.* Typhimurium) and incubated at 37 °C, harvested by centrifugation at 4000 × *g* for 10 min at 4 °C, and suspended in 9 mL of 0.1% sterile peptone water (PW; Difco). The final pellets resuspended in PW attained a final concentration of ca. 10^8–10^9 CFU/mL. Subsequently, suspended pellets of two strains of *S.* Typhimurium and three strains of *L. innocua* were combined to obtain two separate culture cocktails.

2.2.2. Sample inoculation

For tomato and apple surfaces and cantaloupe rinds, spots (6) to be inoculated on half of each fruit were first marked with an indelible pen for easier bacteria recovery. Each spot (ca. 4 cm^2) was inoculated with 10 μL of *S.* Typhimurium or *L. innocua* by depositing 3 droplets onto the surface with a micropipette. For tomato stem scars, each stem scar was inoculated with 30 μL of bacteria. The inoculated samples were dried in a laminar flow cabinet for 1 h (for tomato surfaces, apple skins, and cantaloupe rinds) or 2 h (for tomato stem scars) at ambient temperature. Extra time was required to dry inocula on the stem scar area of tomatoes.

2.2.3. Treatments with iHP

For each replicate, 12 tomatoes (4 for surfaces and 8 for stem scars), 4 apples, and 4 cantaloupes that had been inoculated with the bacteria were placed in collapsible storage crates (ZJKK4835265C, Schiffmayer Plastics Corp., Algonquin, IL, USA) with an internal dimension of 45.0 × 32.5 × 24.0 cm, which was placed on stainless steel shelves in the chamber (4.27 × 2.44 × 2.14 m) (Sites, Walker, Burke, & Annous, 2008). The inoculated fruits were placed into crates with the inoculated area face up, and there was one layer of fruit in each crate with non-inoculated fruit as a filler. The chamber was sealed and samples in the chamber were treated with iHP for 16 min with a flow rate of 25 mL/min and an air pressure of 30 psi, resulting in a dose concentration of 0.5 mL/ft^3 of iHP in the chamber. The positions of applicator and crates are shown in Fig. 1(a). After 14 min dwell time, the exhaust fan in the chamber was turned on for a minimum of 1 h until the H_2O_2 concentration in the chamber was below 1 ppm. The changes of H_2O_2 concentration during the total 90 min are shown in Fig. 2. Samples were then removed from the chamber. Each experiment was repeated three times.

2.2.4. Effect of crate stacking, fruit location and air movement on the efficacy of iHP

To evaluate the effects of stacking crates and fruit position in the crates on the efficacy of iHP, apple fruits were spot inoculated with *S.* Typhimurium and *L. innocua* onto the lower cheek part of the fruit as described above. After drying for approximately 1 h in the laminar flow cabinet, the inoculated fruits were then placed into four crates with three layers of fruits per crate. Four pieces of fruit for each bacterium were randomly placed into each layer of fruit with un-inoculated fruits as fillers. Four crates of apples were stacked on top of each other with the bottom cart on perforated metal platform as shown in Fig. 1(b). The samples were then treated with iHP at the same conditions as described above. In addition, to study if air movement during treatment facilitates penetration of H_2O_2 droplets and consequently its efficacy, the fan contained within the refrigeration unit mounted on the ceiling of the chamber (Sites et al., 2008) was turned on (without activating the refrigeration), as a separate experiment, during the 16 min treatment time and 14 min dwell time. The fan blew in the same direction as the ionizer.

2.2.5. Enumeration

The tomato stem scars and surfaces with the inoculated bacteria were removed using a pair of sterile scissors, while the apple skins and cantaloupe rinds with the inoculated bacteria were cut with a sterile

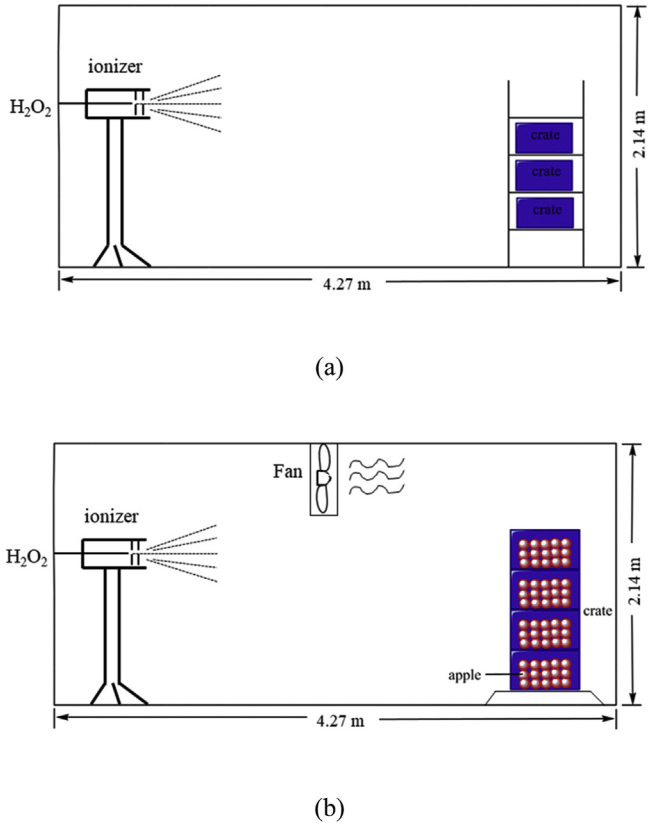

Fig. 1. Schematic view of iHP ionizer and position of crates in the treatment chamber. Fruits in crates were treated without (a) and with (b) using a circulation fan. The height of ionizer was approximately 1.5 m from the floor.

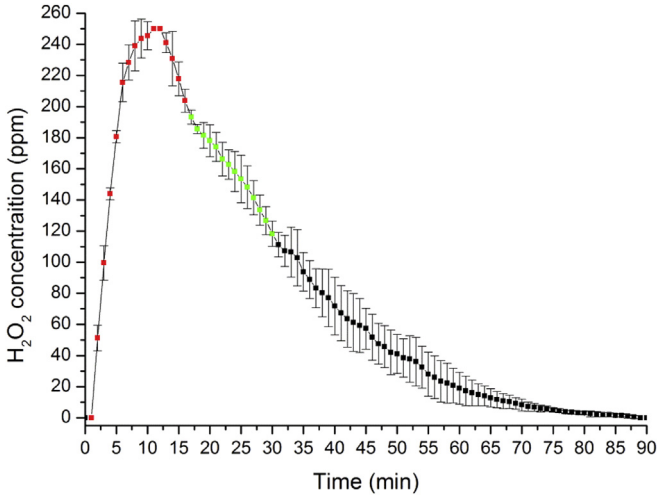

Fig. 2. Changes of hydrogen peroxide concentration in the chamber during the treatment and ventilation. The spray period was from 0 to 16 min followed by the dwell time of 17–30 min. After treatment, the chamber was ventilated (31–90 min).

knife. Composite samples were transferred into an 80 mL filtered stomacher bag. After the addition of 5 × Buffered Peptone Water (BPW; Difco) into the bag, the sample in the bag was homogenized using a mini blender (Mini Mix CC, Interscience Laboratories Inc., Wobum, MA, USA) for 2 min on a setting of 4. The homogenate (after built-in filtration) was serially diluted (if needed) and plated (0.1 or 1 mL) onto Tryptic Soy Agar with 200 g/mL sodium pyruvate and 100 μg/mL nalidixic acid (TSA-PN) and PALCAM Agars (Difco) for the enumeration of *S.* Typhimurium and *L. innocua*, respectively. The plates were incubated at 37 °C for 40 and 48 h, respectively, and then typical colonies were enumerated and reported as log CFU/piece of fruit. Prior to starting inoculation and treatment of fresh produce items, no detectable *Salmonella* or *Listeria* was found on apples, tomatoes, or cantaloupes using the TSA-PN or PALCAM, respectively.

2.3. Analysis of quality parameters

2.3.1. The treatment and storage

Uninoculated fruit were used for the quality study. The samples were divided into an untreated (control) group and a treated group. The treated group was placed on a shelf in the chamber (4.27 × 2.44 × 2.14 m) and treated with iHP as described in 2.2.3 while the non-treated samples were outside of the chamber in the same room. After the treatment, all samples were stored at 17 °C for 14 d to simulate conditions in supermarkets. Various quality parameters were assessed on 1, 7, and 14 d of storage. The appearance was evaluated first, followed by determination of other quality parameters. Every 10 tomatoes, 10 apples, and 8 cantaloupes were treated as a replicate. The experiments were repeated three times (3 replicates) conducted on different days.

2.3.2. Evaluation of appearance

Evaluation of appearance was performed by four experienced judges using a 9-point category scale as described by Fan, Gurtler, et al. (2018) and Fan, Sokorai, et al. (2018). The description for the scale was 9 = excellent quality, essentially free from defects, fresh appearing; 7 = good quality, minor defects, not objectionable; 5 = fair quality, slightly to moderately objectionable defects, lower limit of sale appeal; 3 = poor quality, excessive defects; 1 = extremely poor quality, unusable, moldy. Three judges independently performed subjective assessments.

2.3.3. Color measurements

Color change of samples was measured with an UltraScan VIS color spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA, USA) using a 1.3-cm measuring aperture (Jiang et al., 2017). Color parameters (CIE L*, a*, b*) were recorded on two opposite sides of each fruit. The spectrophotometer was calibrated using the standard black light trap and a white tile (L* 93.50, a*-0.89, and b * 1.01). D65/10° was the illuminant or viewing geometry.

2.3.4. Texture analysis

Texture was measured with a TA-XT2i Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA) (Jiang et al., 2017). A 3-mm diameter probe was used to penetrate the tomato and cantaloupe rinds and a 6-mm diameter probe was used to penetrate the apple fruit to 10 mm in depth at a speed of 10 mm/s. Two readings on the opposite side of each fruit were recorded. Firmness values were registered as the maximum force observed during compression of samples by using the Texture Expert software (version 1.22, Texture Technologies Corp., Scarsdate, NY, USA).

2.3.5. pH and soluble solids content

Juice was extracted from flesh of the apple, tomato, and edible parts of the cantaloupe by using a Champion juicer (MAR-48C, Plastaket Mfg. Co., Inc., Lodi, CA, USA). The pH was recorded with a pH meter (Orion 420A+, Thermo Fisher Scientific, Waltham, MA, USA). Soluble solids content was measured by a digital refractometer (AR200, Reichert Inc., Depew, NY, USA).

2.3.6. Ascorbic acid (AA) determination

Ascorbic acid (AA) was analyzed as described previously (Fan, Annous, Keskinen, & Mattheis, 2009) with minor modifications on sample pre-treatment. Tomatoes, apples (including skin and flesh), and

cantaloupes (without rinds) were cut to prepare 5 g of randomized samples, which were then homogenized with 15 mL of 5% (62.5 mM) metaphosphoric acid by using a mechanical and ultrasonic homogenizer (DPS-20, PRO Scientific Inc., Oxford, CT, USA) at a speed setting of 12,000 rpm for 2 min on ice, and then centrifuged at 13,639 × *g* for 10 min at 4 °C. Aliquots of supernatant filtered through a 0.45 μm membrane were analyzed using a 1260 Infinity HPLC system (Agilent Technologies, Palo Alto, CA, USA). AA was monitored at 245 nm and calculated from an external standard curve.

2.3.7. Antioxidants determination

(1) Ferric Reducing Antioxidant Power (FRAP) assay

The extraction was carried out as described by Fan, Toivonen, Rajkowski, and Sokorai (2003) with minor modifications. Briefly, 5 g of randomized samples were homogenized with 15 mL of 70% ethanol using a mechanical and ultrasonic homogenizer (DPS-20, PRO Scientific Inc., Oxford, CT, USA) at a speed setting of 12,000 rpm for 2 min on ice and then centrifuged at 13,639 × *g* for 10 min at 4 °C. The supernatant was analyzed to determine the antioxidant power by using the FRAP assay (Benzie & Strain, 1996).

(2) Oxygen Radical Absorbance Capacity (ORAC) assay

The sample extraction was the same as that of the FRAP assay. The supernatant was analyzed after approximate dilution with 75 mM phosphate buffer (pH 7.4). The ORAC assay was performed on a Synergy HT Multi- Detection Microplate Reader (BioTek Instruments, Inc., Winnooski, VT, USA). The procedure was based on the previous reports by Ou, Hampsch-Woodill, and Prior (2001 and 2002) with some modifications. Briefly, 8×10^{-5} mM sodium fluorescein and 153 mM 2, 2′-azobis (2-amidinopropane) dihydrochloride (AAPH) were prepared with 75 mM phosphate buffer and then set in dispenser #2 and dispenser #1, respectively. The 75 mM phosphate buffer was used as a blank and 6-hydroxy-2, 5, 7, 8-tetrametmethylchroman-2-carboxylic acid (Trolox) was used as a control standard with a range of 6.25–100 μM. The reagent wells were filled with Trolox standards, diluted samples, and blanks. Then the equipment stood for 180 s and incubated for 30 min after adding the fluorescein probe automatically at 37 °C. The analyzer was programmed to record the fluorescence readings every minute after the addition of AAPH. All fluorescent measurements are expressed relative to the initial reading. Samples and Trolox calibration solutions were always analyzed in triplicate in a reverse order in order to correct possible errors resulting from the signal drifting associated with the different positions on the 96 wells. The final values were calculated using the differences of areas under the fluorescein decay curves between the blank and a sample and were expressed as millimole Trolox equivalents (TE) per gram (mmol TE/g).

2.4. Statistical analysis

All experiments were repeated three times and each experiment was conducted on different days. When no colony was present on the plates, half of the detection limit (0.7 log CFU/piece) was used to calculate the log reduction. The results were expressed as mean ± standard deviation and data were analyzed by SPSS Statistics 22 software (IBM, Amok, USA) through one-way analysis of variance (ANOVA). $P < 0.05$ (Duncan Multiple Range test) was used to determine statistical significance.

Table 1
Effects of cold plasma-activated H_2O_2 aerosols in inactivating *Salmonella* Typhimurium inoculated on various fresh produce. Apple, cantaloupe and tomato in a single layer were treated with cold plasma-activated H_2O_2 aerosols.

Type of produce	Populations (log CFU/piece)		Reductions[a] (log CFU/piece)
	control	treated	
Apple	5.35 ± 0.79[b]	ND[c]	5.00 ± 0.91
Cantaloupe rind	5.27 ± 0.65[b]	1.69 ± 1.06[a]	3.58 ± 0.67
Tomato-surface	5.68 ± 0.89[b]	ND[c]	5.33 ± 1.03
Tomato-stem scar	6.24 ± 0.45[a]	5.85 ± 0.82[a]	0.63 ± 0.45

[a] Reductions were calculated by comparisons with the non-treated control using half of the detection limit (0.7 log CFU/piece).
[b] Means followed by the same letters in the same row for population are not significantly different (Duncan Multiple Range test, P = 0.05). Numbers are averages ± standard deviations (n = 3).
[c] ND: not detectable (Detection limit: 0.7 log CFU/piece).

3. Results and discussion

3.1. Efficacies of iHP treatment on populations of S. Typhimurium and L. innocua

The efficacies of iHP against *S.* Typhimurium and *L. innocua* on apples, cantaloupes, and tomatoes in the scale-up study are shown in Tables 1 and 2, respectively. Based on these results, it can be concluded that iHP was very effective in inactivating bacteria on the fresh produce surface when the inoculated surfaces faced up and there was only 1 layer of fruits in each container. The 17.62 mL/m^3 iHP treatment reduced the populations of inoculated *S.* Typhimurium and *L. innocua* on apple and tomato surfaces to a level below the detection limit (0.70 log CFU/piece). For the bacteria inoculated on cantaloupe rinds, the treatment obtained reductions of 3.58 and 3.72 log CFU/piece for *S.* Typhimurium and *L. innocua*, respectively. However, *S.* Typhimurium and *L. innocua* on stem scars of tomato were inactivated at the lowest rate with no more than 1 log CFU/piece of reduction being achieved. In our previous study conducted in the lab scale, 8 s treatment time (11.63 mL/m^3 of iHP concentration) was enough to reduce *S.* Typhimurium and *L. innocua* populations to a level below the detection limit on the smooth surfaces of apples and tomatoes (Song & Fan, 2020). The bacteria on the tomato stem scars was the most difficult to inactivate, and even after a 60 s treatment composed of 3 cycles of 20 s spray time plus 20 min dwell time (88.12 mL/m^3 of iHP concentration), the reductions were less than 3 log CFU/piece. It is obvious that the bacteria inoculated on the smooth surfaces such as apple and tomato surfaces were easier to reduce compared with that inoculated on the surfaces

Table 2
Effects of cold plasma-activated H_2O_2 aerosols in inactivating *Listeria innocua* on various fresh produce. Apple, cantaloupe and tomato in a single layer were treated with cold plasma-activated H_2O_2 aerosols. Reductions were calculated by comparisons with the non-treated control using half of the detection limit (0.7 log CFU/piece).

Type of produce	Populations (log CFU/piece)		Reductions[a] (log CFU/piece)
	control	treated	
Unwaxed apple	5.30 ± 0.25[b]	ND[c]	4.95 ± 0.28
Cantaloupe rind	5.59 ± 0.48[b]	1.87 ± 1.14[a]	3.72 ± 1.35
Tomato-surface	5.44 ± 0.21[b]	ND[c]	5.09 ± 0.23
Tomato-stem scar	6.23 ± 0.39[a]	5.37 ± 0.91[a]	0.86 ± 0.50

[a] Reductions were calculated by comparisons with the non-treated control using half of the detection limit (0.7 log CFU/piece).
[b] Means followed by the same letters in the same row for population are not significantly different (Duncan Multiple Range test, P = 0.05). Numbers are averages ± standard deviations (n = 3).
[c] ND: not detectable (Detection limit: 0.7 log CFU/piece).

Table 3
Effects of cold plasma-activated H_2O_2 aerosols in inactivating *Salmonella* Typhimurium on apples without a fan in the treatment chamber. Fruits in four stacked crates with three layers of fruit each crate were treated with cold plasma-activated H_2O_2 aerosols. The populations of *Salmonella* Typhimurium inoculated on non-treated samples of each replicate were 6.46, 6.40, 6.74 log CFU/piece, respectively.

Position of crate from bottom	Layer of fruit in each crate from bottom	Reductions[a] (log CFU/piece)		
		Replicate 1	Replicate 2	Replicate 3
1	1	6.11	6.05	6.39
1	2	6.11	6.05	6.39
1	3	6.11	6.05	6.39
2	1	6.11	6.05	6.39
2	2	6.11	6.05	2.82
2	3	2.26	6.05	3.35
3	1	6.11	6.05	6.39
3	2	6.11	6.05	3.94
3	3	6.11	6.05	6.39
4	1	6.11	6.05	6.39
4	2	6.11	6.05	6.06
4	3	1.46	5.57	3.09

[a] Reductions were calculated by comparisons with the non-treated control using half of the detection limit (0.7 log CFU/piece).

Table 4
Effects of plasma-activated H_2O_2 aerosols in inactivating *Listeria innocua* on apples without a fan in the treatment chamber. Fruits in four stacked crates with three layers of fruit each crate were treated with cold plasma-activated H_2O_2 aerosols. The populations of *Listeria innocua* inoculated on non-treated samples of each replicate were 5.74, 5.43, 5.40 log CFU/piece, respectively.

Position of crate from bottom	Layer of fruit in each crate from bottom	Reductions[a] (log CFU/piece)		
		Replicate 1	Replicate 2	Replicate 3
1	1	5.39	5.08	5.05
1	2	5.39	5.08	5.05
1	3	5.39	5.08	5.05
2	1	5.39	5.08	5.05
2	2	5.39	5.08	5.05
2	3	2.02	5.08	5.05
3	1	5.39	5.08	5.05
3	2	5.39	5.08	5.05
3	3	5.39	5.08	3.64
4	1	5.39	5.08	5.05
4	2	5.39	5.08	4.70
4	3	1.46	5.08	5.05

[a] Reductions were calculated by comparisons with the non-treated control using half of the detection limit (0.7 log CFU/piece).

with roughness and porosity, such as cantaloupe rinds and tomato stem scars. Earlier studies have demonstrated that sanitizers have difficulty in penetrating into porous surface of cantaloupe and tomato stem scars probably due to inoculum becoming internalized (Bowen, Fry, Richards, & Beauchat, 2006; Guo, Chen, Brackett, & Beuchat, 2002; Jiang et al., 2017). In addition, iHP is marketed as a topical antimicrobial treatment. In any case, this is the first report demonstrating cold plasma-activated hydrogen peroxide aerosol (iHP) was effective against bacteria on various fresh produce in a pilot scale study.

3.2. Effect of crate stacking, fruit location and air movement on the efficacy of iHP

Our results on a single layer of fruits treated with the inoculated area facing up during treatment showed that iHP reduced populations of the two bacteria on the smooth surfaces of fruits to undetectable levels. However, in actual commercial practice, fruit position in a container and contamination areas can not be predicated and fruits are often randomly positioned into containers. Therefore, in a subsequent experiment, we evaluated the effects of fruit location in the crate and crate stacking on the efficacy of iHP. Results shown in Tables 3 and 4 indicate that iHP reduced populations of both *Salmonella* and *Listeria* to undetectable levels on more than 75% of samples. The position of fruit in each crate did not affect the efficacy of iHP. Fruits in the bottom crate were free of detectable inoculated bacteria for all three replicate samples. However, iHP failed to achieve more than 5-log reductions of spot-inoculated bacteria on all fruits in the other three crates. Approximately, 22% of samples still exhibited detectable *Salmonella* and 11% of fruits had detectable *Listeria*.

In attempting to increase the chances of inactivating pathogens on all fruits, fans were used to circulate H_2O_2 micro-droplets in the chamber during treatment in subsequent experiments. Results indicate that bacteria on a similar percentage of fruit were decreased to undetectable levels (data not shown), suggesting that using air circulation did not increase the efficacy of the treatment. The iHP system relies on the small sizes (0.04–3 μm) of droplets to float and naturally migrate around the treatment space (Jiang et al., 2017). Adding a fan to increase air circulation may interrupt normal patterns of droplets resulting in formation of large droplets, which may be prone to settle or condense before being fully disbursed.

The failure of achieving > 5 log reduction on 100% fruits is probably due to the bacteria-inoculated areas of apples contacting with each other, and consequently preventing iHP accessing to some of the bacteria. In addition, we studied the efficacy of iHP in inactivating the bacteria dip-inoculated on apples, which were then treated in stacked crates. Results indicated that the reduction of *Salmonella* and *Listeria* were only 1.19 ± 0.37 and 1.98 ± 0.80 log CFU/piece, respectively. Dip-inoculated fruits have higher chances of bacteria being protected from aerosolized antimicrobials due to the fruit-to-fruit contact and bacteria residing in the protective site, such as stems. Therefore, other ways such as vibration of fruits during treatment or treating fruit on conveyer belt and roller, where fruits often rotate, may be investigated to increase the efficacy of inactivating the bacteria.

We found that *Salmonella* and *Listeria* populations were reduced to non-detectable levels on the smooth surfaces of tomatoes and apples as enumerated on selective media. It is possible that the media would not allow injured cells to grow. The injured cells could recover and multiply during storage. However, it is unlikely that the injured bacteria will grow on the surfaces of fresh produce items after treatments due to the presence of hydrogen peroxide residue on the surface of the three fruits we investigated. In the present study, we did not assess injury, survival, and possible growth of inoculated bacteria during post-treatment storage. The storage study would help to identify the fate of bacteria, i.e. whether populations of survival or injured *Salmonella* and *Listeria* would decrease or die down during storage.

In the present study, we used ATCC designated BSL-1 *Salmonella* and *Listeria* strains which are safer than pathogens to handle and use, particularly for situations of pilot scale studies. The strains have been used in several earlier studies and shows similar response to chemical and nonthermal treatments as corresponding pathogenic bacteria (Fan et al., 2012, 2018; Gurtler, Rivera, Zhang, & Geveke, 2010; Wang, Gurtler, Wang, & Fan, 2019; Yun et al., 2015). Human pathogens may be used in future studies to confirm the finding of the present study.

3.3. Effects of iHP treatment on quality during storage

The effects of iHP on the appearance, color, texture, pH, soluble solids content, ascorbic acid, and antioxidants of apples, tomatoes, and cantaloupes during 14-d storage are shown in Tables 5–7.

The first impression that a consumer has for the acceptance of a product is its appearance. As is shown in Table 5, there was no significant difference in the appearance of apples between iHP - treated samples and corresponding controls for apples during 14 days of shelf-life at 17 °C. They appeared fresh with acceptable quality after 14 days. However, both tomatoes and cantaloupes from the control and treated

Table 5
Appearance, firmness, pH and soluble solid content of apple, tomato, and cantaloupe after iHP treatment. After treatments, fruits were stored at 17 °C and assessed for appearance on day 1, 7 and 14 of storage.

Type of produce	Day 1		Day 7		Day 14	
	Control	Treated	Control	Treated	Control	Treated
Appearance (9–1)						
Apple	8.6 ± 0.7^{a}	8.6 ± 0.6^{a}	8.1 ± 1.3^{a}	8.2 ± 1.0^{a}	8.2 ± 1.0^{a}	8.5 ± 0.8^{a}
Tomato	8.5 ± 0.6^{a}	8.6 ± 0.5^{a}	4.9 ± 2.0^{b}	3.8 ± 2.4^{bc}	4.9 ± 2.3^{b}	5.4 ± 2.6^{b}
Cantaloupe	8.7 ± 0.6^{a}	8.6 ± 0.6^{a}	5.6 ± 1.7^{b}	3.9 ± 2.3^{b}	4.6 ± 2.5^{b}	5.8 ± 1.5^{b}
Firmness (kg)						
Apple	7.69 ± 1.00^{a}	7.40 ± 1.35^{a}	6.69 ± 1.82^{a}	6.98 ± 1.63^{a}	6.25 ± 1.47^{a}	7.00 ± 1.04^{a}
Tomato	0.75 ± 0.15^{a}	0.80 ± 0.13^{a}	0.65 ± 0.11^{a}	0.70 ± 0.14^{a}	0.57 ± 0.13^{a}	0.59 ± 0.11^{a}
Cantaloupe	6.76 ± 0.93^{a}	5.99 ± 0.76^{a}	6.84 ± 1.62^{a}	6.48 ± 1.26^{a}	5.67 ± 1.47^{a}	6.05 ± 1.60^{a}
pH						
Apple	3.56 ± 0.08^{bc}	3.52 ± 0.09^{c}	3.44 ± 0.06^{c}	3.49 ± 0.06^{c}	3.67 ± 0.07^{ab}	3.69 ± 0.05^{a}
Tomato	4.03 ± 0.17^{ab}	3.93 ± 0.05^{b}	4.04 ± 0.08^{ab}	3.93 ± 0.08^{b}	4.16 ± 0.15^{a}	4.08 ± 0.09^{ab}
Cantaloupe	6.05 ± 0.15^{a}	6.20 ± 0.13^{a}	6.01 ± 0.14^{a}	6.05 ± 0.10^{a}	6.07 ± 0.15^{a}	6.08 ± 0.12^{a}
Soluble solids (%)						
Apple	12.8 ± 0.5^{a}	13.2 ± 0.7^{a}	13.0 ± 0.7^{a}	13.3 ± 0.7^{a}	12.9 ± 0.7^{a}	13.6 ± 0.9^{a}
Tomato	4.7 ± 0.6^{a}	4.3 ± 0.2^{a}	4.7 ± 0.2^{a}	4.5 ± 0.1^{a}	4.6 ± 0.3^{a}	4.4 ± 0.3^{a}
Cantaloupe	8.6 ± 1.1^{b}	10.3 ± 1.1^{ab}	10.8 ± 1.0^{a}	10.1 ± 1.1^{ab}	10.0 ± 1.0^{ab}	10.2 ± 1.4^{ab}

Means followed by the same letters in the same row are not significantly different (Duncan Multiple Range test, P = 0.05). Numbers are averages ± standard deviations.

groups developed decay after 7 days and were not acceptable (below 5). The iHP did not reduce the decay rate of cantaloupe or tomatoes. Bayoumi (2008) reported that H_2O_2 treatment of peppers resulted in the best appearance at 5 and 15 mM concentrations based on the reduction of weight loss and rot rate. However, iHP did not improve the appearance of the fruits.

Color and texture are significant freshness-related characteristics, which play an essential role in consumers' choice of high-quality products. During storage, the b* values of apples and the a* values of tomatoes increased, indicating that apples turned yellower and tomatoes became redder (Table 6). However, the changes were not statistically significant and were not affected by the ionized hydrogen peroxide treatment. Besides, there was no consistent change for the color of cantaloupes. The firmness of all samples decreased during 14 days at 17 °C, although the decreases were not significant (Table 5). In general, treatment with iHP did not change the texture of the fruit. Some sanitizers have a strong oxidizing action and can cause deleterious changes on the color of fresh produce such as darkening or whitening (Vandekinderen et al., 2008). dos Reis Ponce et al. (2010) observed that 10% hydrogen peroxide reduced the L* value, contributing to a loss of brightness in strawberries. The sanitization treatments reduced the firmness of the strawberries (de São José & Vanetti, 2015). Overall, our results demonstrated that 7.8% hydrogen peroxide aerosol-activated by cold plasma did not affect the color or texture of cantaloupes, apples, or tomatoes.

The pH of apples, tomatoes, and cantaloupes did not exhibit obvious change during storage (Table 5). The soluble solids content of apples and tomatoes were relatively more stable compared with that of cantaloupe (Table 5). The iHP treatment contributed to an increase of soluble solids content from 8.6% to 10.3% after 1 day of storage. This was consistent with the results of Ozaki et al. (2009), which indicated that the application of H_2O_2 increased the soluble solids content in melon fruits.

As is shown in Table 7, iHP treatment had no significant effects on ascorbic acid content of apples, tomatoes, and cantaloupes and no consistent changes during storage was observed either. A declination of more than 50 μg/g of ascorbic acid content for untreated cantaloupe occurred during the first week. Studies have discovered that ascorbic acid content from the 0.1 and 0.2 M H_2O_2-treated fresh-cut tomatoes was 24% and 31% higher, respectively, than that of the control after 3 and 7 days storage at 17 °C, respectively (Kim, Fonseca, Kubota, & Choi, 2007). The increase in ascorbic acid content could be regenerated by two enzymes namely monodehydroascorbate reductase and dehydroascorbate reductase, as a response to H_2O_2-induced oxidative stress. (Lin et al., 2016; Nishikawa et al., 2003; Wolucka, Goossens, & Inzé, 2005).

The FRAP assay reflects only the antioxidant-reducing potential based on the ferric ion instead of the antioxidant preventive effect (Benzie & Strain, 1996). The ORAC assay directly measures the antioxidant activities of chain-breaking antioxidants against peroxyl

Table 6
Color of apple, tomato and cantaloupe after iHP treatment. After treatments, fruits were stored at 17 °C and measured for color parameters on day 1, 7 and 14 of storage.

Type of produce		Day 1		Day 7		Day 14	
		control	treated	control	treated	control	treated
Apple	L*	44.38 ± 9.38^{a}	45.48 ± 8.18^{a}	47.17 ± 8.67^{a}	46.06 ± 7.46^{a}	49.10 ± 7.97^{a}	49.00 ± 8.19^{a}
	a*	27.52 ± 4.58^{a}	27.66 ± 5.13^{a}	26.03 ± 6.76^{a}	27.79 ± 3.42^{a}	26.30 ± 5.23^{a}	26.13 ± 5.70^{a}
	b*	17.07 ± 5.33^{a}	17.61 ± 5.42^{a}	19.36 ± 6.03^{a}	18.64 ± 4.53^{a}	21.74 ± 6.19^{a}	22.15 ± 6.35^{a}
Tomato	L*	36.90 ± 1.04^{a}	36.68 ± 1.10^{a}	35.51 ± 0.75^{a}	35.56 ± 1.11^{a}	35.43 ± 1.10^{a}	35.37 ± 0.72^{a}
	a*	16.98 ± 2.36^{a}	16.68 ± 1.68^{a}	19.83 ± 1.90^{a}	19.21 ± 1.83^{a}	19.77 ± 1.93^{a}	19.91 ± 2.00^{a}
	b*	18.15 ± 2.23^{a}	17.42 ± 2.41^{a}	18.14 ± 1.90^{a}	17.23 ± 2.03^{a}	17.37 ± 1.99^{a}	17.47 ± 1.95^{a}
Cantaloupe	L*	66.87 ± 5.77^{a}	66.93 ± 5.29^{a}	66.27 ± 5.55^{a}	65.94 ± 5.86^{a}	66.36 ± 5.64^{a}	63.88 ± 6.13^{a}
	a*	1.49 ± 0.92^{a}	1.42 ± 0.98^{a}	1.81 ± 1.15^{a}	2.64 ± 1.17^{a}	2.84 ± 0.93^{a}	1.88 ± 1.49^{a}
	b*	18.12 ± 2.32^{a}	18.87 ± 2.67^{a}	19.65 ± 2.54^{a}	19.80 ± 2.06^{a}	20.07 ± 1.77^{a}	19.02 ± 2.59^{a}

Means followed by the same letters in the same row are not significantly different (Duncan Multiple Range test, P = 0.05). Numbers are averages ± standard deviations.

Table 7
Ascorbic acid contents, FRAC values and ORAC values of apple, tomato and cantaloupe after iHP treatment. After treatments, fruits were stored at 17 °C and analyzed for ascorbic acid on day 1, 7 and 14 of storage.

Type of produce	Day 1		Day 7		Day 14	
	control	treated	Control	treated	control	treated
	Ascorbic acid contents (μg/g)					
Apple	13.81 ± 8.16[a]	14.65 ± 6.07[a]	17.33 ± 4.56[a]	17.91 ± 3.19[a]	13.88 ± 2.38[a]	11.41 ± 3.71[a]
Tomato	115.24 ± 33.55[ab]	92.97 ± 12.44[b]	111.60 ± 26.08[ab]	96.18 ± 12.02[b]	141.14 ± 19.56[a]	119.23 ± 26.72[ab]
Cantaloupe	209.85 ± 35.62[a]	178.87 ± 24.68[ab]	131.89 ± 33.07[b]	120.04 ± 40.76[b]	125.33 ± 42.22[b]	123.97 ± 36.35[b]
	FRAP values (mmol/g)					
Apple	4.82 ± 0.74[a]	5.24 ± 0.78[a]	5.67 ± 0.68[a]	6.41 ± 0.74[a]	4.90 ± 1.01[a]	4.36 ± 1.50[a]
Tomato	2.49 ± 0.37[a]	2.27 ± 0.19[a]	2.71 ± 0.36[a]	2.49 ± 0.24[a]	2.46 ± 0.07[a]	2.55 ± 0.30[a]
Cantaloupe	2.01 ± 0.30[a]	2.41 ± 0.43[a]	2.57 ± 0.76[a]	2.15 ± 0.56[a]	2.07 ± 0.35[a]	2.10 ± 0.59[a]
ORAC values (mmol TE/g)						
Apple	17.45 ± 2.49[a]	17.23 ± 2.03[a]	15.95 ± 2.51[a]	16.41 ± 1.39[a]	13.78 ± 2.86[a]	14.63 ± 2.49[a]
Tomato	5.34 ± 0.62[a]	4.27 ± 0.15[a]	3.21 ± 0.28[a]	2.92 ± 0.90[a]	5.09 ± 2.87[a]	4.12 ± 0.60[a]
Cantaloupe	6.11 ± 1.39[a]	5.43 ± 1.38[a]	4.55 ± 0.95[a]	3.90 ± 1.89[a]	3.69 ± 1.00[a]	4.10 ± 0.81[a]

Means followed by the same letters in the same row are not significantly different (Duncan Multiple Range test, P = 0.05). Numbers are averages ± standard deviations.

radicals (Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002). Therefore, using only one result is inappropriate to claim total antioxidant activity. FRAP and ORAC values of apple, tomato, and cantaloupe during storage are shown in Table 7. There were no consistent changes for either FRAP or ORAC levels and the values were not affected by storage time or iHP treatment. It appears the values from FRAP and ORAC assessments mirror each other. Both showed that apples had higher antioxidant activities than tomatoes and cantaloupes.

4. Conclusion

In this study, iHP was applied to reduce populations of *Salmonella* Typhimurium and *Listeria innocua* inoculated on the surfaces of apples and tomatoes, tomato stem scars and cantaloupe rinds in a pilot scale. The results demonstrated that the treatment of 16 min spray followed by 14 min dwell time with a flow rate of 25 mL/min and an air pressure of 30 psi resulted in a reduction of ≥5 log CFU/piece for the populations of *S.* Typhimurium and *L. innocua* on apple and tomato surfaces. *S.* Typhimurium and *L. innocua* inoculated on cantaloupe rinds were reduced by 3.58 and 3.72 log CFU/piece, respectively. For the two bacteria on stem scars of tomatoes, the reductions obtained were less than 1 log CFU/piece. When inoculated apples were placed into crates with multiple layers, bacteria were not reduced to non-detectable levels on all treated fruits. Under the same conditions, iHP did not significantly affect the quality parameters of apples, tomatoes, and cantaloupes during 14-d storage at 17 °C. Overall, our results indicate that iHP may be applied in a large scale to enhance the microbial safety of fresh produce, although means to facilitate the exposure of all fruit surface to iHP deserve future evaluation.

CRediT authorship contribution statement

Yuanyuan Song: Methodology, Data curation, Formal analysis, Writing - original draft. **Bassam A. Annous:** Methodology, Writing - review & editing. **Xuetong Fan:** Conceptualization, Methodology, Writing - review & editing.

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