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Cold plasma-activated hydrogen peroxide aerosols inactivate *Salmonella* Typhimurium and *Listeria innocua* on smooth surfaces and stem scars of tomatoes: Modeling effects of hydrogen peroxide concentration, treatment time and dwell time☆

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ABSTRACT

This study was conducted to evaluate the effects of hydrogen peroxide (H_2O_2) concentration, treatment time, and dwell time on the efficacy of cold plasma-activated hydrogen peroxide aerosols against bacteria on stem scar and smooth surfaces of tomatoes. Cherry tomatoes inoculated with cocktails of *Salmonella* Typhimurium and *Listeria innocua* on the smooth surface and stem scar area were treated for 10, 20 and 30 s followed by 15 and 30 min dwell times in a treatment chamber with cold plasma-activated aerosols generated from 0, 1, 2, 3, 4 and 6% and 7.8% H_2O_2. Results showed that the efficacy of the treatments was mostly influenced by the H_2O_2 concentration and treatment time. The reductions of the two bacteria were modeled as sigmoidal or exponential functions of H_2O_2. The models indicated that at H_2O_2 concentrations $\geq$4.2%, the treatments reduced populations of *L. innocua* to non-detectable levels (detection level: 0.70 log CFU/piece) achieving an average 5 log reduction on the smooth surface of tomatoes for all tested treatment times and dwell times. To achieve an average 5-log reduction of *Salmonella* populations on the smooth surface, H_2O_2 at concentrations $\geq$5.7% H_2O_2 was required. On the stem scar area of tomatoes, populations of *Listeria* and *Salmonella* were reduced by less than 2 logs. Our results demonstrated that the efficacy of cold plasma-activated hydrogen peroxide aerosol mainly depended on the concentration of H_2O_2, and type and location of inoculated bacteria.

1. Introduction

Fresh produce has continuously been found to be associated with outbreaks and recalls of foodborne illnesses in recent years (Carstens et al., 2019). Many chemical and physical intervention technologies have been investigated in attempt to find better and more effective ways to reduce populations of pathogens and to minimize the risk of pathogen contamination during growing, handling and processing of fresh produce (Gil et al., 2015; Hanning et al., 2009; Herdt and Feng, 2009; Murray et al., 2017; Sikin et al., 2013). One of the non-thermal physical treatments that has gained attention in recent years is cold plasma (Bourke et al., 2017; Gilmore et al., 2018; Mandal et al., 2018). Cold plasma is ionized gas comprised of reactive species and radicals (Niemira, 2012). It has been shown that production of reactive oxygen species (ROS) such as singlet oxygen and hydrogen peroxide is one of the major mechanisms in cold plasma-mediated inactivation of bacteria (Joshi et al., 2011). Studies have also demonstrated that cold plasma or plasma-activated water typically achieved 1–3 log reductions of pathogens on fresh produce items (Lacombe et al., 2015; Min et al., 2018; Min, Roh, Boyd, et al., 2016; Soni et al., 2021). For example, 1–2 log CFU/mL reductions of *Salmonella enterica*, Shiga toxin-producing *E. coli* or *L. monocytogenes* were observed on cherry tomatoes after 10 min cold plasma treatments (Timmons et al., 2018). On lettuce, cold plasma reduced the populations of *S.* Typhimurium and *L. monocytogenes* by 0.65 and 0.93 log CFU/g, respectively (Seong et al., 2017). Therefore, to achieve higher reductions of pathogens on fresh produce, combinations

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of cold plasma with chemical or other interventions are needed.

Among chemical sanitizers that have been evaluated in the last couple of decades, is hydrogen peroxide (H_2O_2). H_2O_2 has been shown to reduce populations of *E. coli* and *Salmonella* on apples, cantaloupe, and lettuce. (Lin et al., 2002; Sapers and Sites, 2003; Ukuku, 2004; Ukuku et al., 2016; Venkitanarayanan et al., 2002; Xie et al., 2008). However, the reductions were often in the range of 1–2 logs, unless combined with elevated treatment temperature or other sanitizers. To further improve the efficacy of cold plasma and H_2O_2, H_2O_2 aerosols were activated by passing through a cold plasma field, resulting in ionized H_2O_2 and production of more reactive species (Jiang et al., 2017). Results showed that cold plasma and hydrogen peroxide aerosol had significant synergistic effect in reducing populations of *Salmonella* and *Listeria* on lettuce, apples, cantaloupes, and spinach (Song and Fan, 2019). For example, H_2O_2 aerosols without passing through a cold plasma jet, reduced *Salmonella* population by 1.54–3.17 log CFU/piece, while the cold plasma-activated water aerosols achieved only 0.23–1.03 log CFU/piece reductions of *Salmonella* on surfaces of the four produce items. Similarly, H_2O_2 treatment alone and cold plasma-activated water achieved −0.01-0.40 and 1.57–3.77 log CFU/piece reductions of *L. innocua*, respectively. When H_2O_2 aerosols were combined with cold plasma, *L. innocua* was reduced by more than 5 log CFU/piece. When fresh produce items were treated with activated H_2O_2 in a treatment chamber, H_2O_2 aerosols was introduced for a short time (less than 1 min) following by a period (dwell time) of 30 min or less during which fresh produce was sealed in the chamber before being removed. The treatment time dictates the amount of H_2O_2 introduced into the chamber while produce items were exposed to aerosolized and residual H_2O_2 during both treatment and dwell times. In another study, the combination of cold plasma (10 min) and washing with low concentrations (0.05–0.2%) of H_2O_2 achieved more than 5 log reduction of *Salmonella* and *Listeria* biofilms (Govaert et al., 2019).

In our earlier studies (Jiang et al., 2017; Song and Fan, 2019), only one concentration of H_2O_2 (7.8%) was aerosolized followed by activation by cold plasma. Whether other concentrations of H_2O_2 are effective in reducing bacterial populations has not been evaluated. In addition, it is unclear whether the treatment time and dwell time influence the efficacy of ionized H_2O_2. Therefore, the objective of the present study was to evaluate the effect of H_2O_2 concentration, treatment time and dwell time on the efficacy of cold-plasma-activated hydrogen peroxide aerosols in inactivating *Salmonella* and *Listeria* on the smooth surface and stem scar area of tomatoes. The stem scar areas represent internalized bacteria which are known to protect microorganisms from inactivation (Gurtler et al., 2018). We intended to determine the conditions that could reduce populations of the bacteria by 2 and 5 log CFU/piece on the stem scar and smooth surface of tomatoes, respectively.

2. Materials and methods

2.1. Bacterial strains

Two strains of attenuated *Salmonella enterica* serovar Typhimurium (ATCC 53647 and 53,648) and three strains of *Listeria innocua* (ATCC 33090, ATCC 51742 and ATCC BAA680) were obtained from American Type Culture Collection (Manassas, VA, USA). Attenuated and non-pathogenic bacteria in place of human pathogens were used to minimize the risk of researchers being exposed to possible airborne bacteria during treatments. The *Salmonella* strains were made to resist 100 μg/mL nalidixic acid by successive transfers of the bacteria in Tryptic Soy Broth (TSB) (Difco, Sparks, MD, USA) containing increasing concentrations (0.5–100 μg/mL) of nalidixic acid (Yun et al., 2013). The *Salmonella* cultures were propagated individually in 9 mL TSB with 100 μg/mL nalidixic acid for 40 h at 37 °C. Each strain of *Listeria* was grown for 48 h at 37 °C. The bacteria were sub-cultured each time for use in the experiments. The cells were harvested by centrifugation at 4000 rpm for 10 min at 4 °C. The resulting pellets were then resuspended in 9 ml 0.1% peptone water (PW; Difco) and combined to produce two separate culture cocktails of *Salmonella* and *Listeria* with a cell density of ~10^9 CFU/ml.

2.2. Sample preparation and inoculation

Cherry tomatoes were purchased from a major supermarket chain through special orders in Philadelphia, PA, USA. and kept at 10 °C overnight prior to use. After removal from 10 °C refrigerator, the tomatoes were equilibrated to ambient temperature (~22 °C) for 2 h before being inoculated. Four tomatoes were used for each treatment per replicate for each bacterium. Each piece was spot inoculated with 10 μL of *Salmonella* and *L. innocua* by depositing multiple droplets on surface and 1 10 μL droplet onto stem scar with a micropipette. The inoculated samples were dried for 1 h (for tomato surfaces) or 2 h (for tomato stem scars) at ambient temperature (22 °C) in a laminar flow hood. The average populations of *L. innocua* on the stem scar and smooth surface of control fruit were 5.42 ± 0.17 and 5.46 ± 0.25 log CFU/piece, respectively, while the populations of *Salmonella* on the stem scar and smooth surface of control fruit were 6.11 ± 0.18 and 6.30 ± 0.41 log CFU/piece, respectively.

2.3. Treatments

The inoculated fruits were treated, at ambient temperature (22 °C), with cold plasma-activated H_2O_2 aerosols at concentrations of 0 (control), 1, 2, 3, 4, 6 and 7.8% with treatment times of 0, 10, 20 and 30 s, and 0, 15 and 30 min dwell times using the SteraMist Select Machine (TOMI Environmental Solutions Inc., Frederick, MD) as described earlier (Song et al., 2020). The highest concentration (7.8%) of H_2O_2 was used in previous studies as recommended by the manufacturer (Jiang et al., 2017; Song et al., 2020). Tomato fruits on a sterile test tube rack with the inoculated area facing up were placed inside a Pyrex glass treatment chamber (12 × 12 × 24 inch). H_2O_2 solutions of various concentrations were aerosolized with pressurized air and passed through a cold plasma jet which was generated between two pin electrodes with a distance and voltage between the two electrodes of 9 mm and 17 kV, respectively. The activated (ionized) aerosols were introduced into the treatment chamber, at a rate of 5.o ml/min, into which tomatoes were placed. The spray times (treatment time) were set as 10, 20 and 30 s followed by 15 and 30 min dwell times. Each treatment had a corresponding control to compensate for possible changes in bacterial population during the 15 or 30 (Song and Fan, 2021)min.

2.4. Enumeration of bacteria

After each treatment, the tomatoes were removed from the treatment chamber, and stem scars or smooth surfaces with the inoculated bacteria from 4 fruits were combined and transferred into 80 mL filtered stomacher bags. After the addition of 20 mL buffered peptone water (BPW; Difco) to each bag, the samples in the bags were pummeled with a mini blender (Mini Mix CC, Interscience Laboratories Inc., Wobum, MA, USA) for 2 min at a setting of 4. We did not use any reducing agent to neutralize H_2O_2 because the amount of H_2O_2 deposited on the surface of tomato was low and decreased rapidly. Even at 7.8%, the amount of H_2O_2 on the surface was less than 2 μg/g measured immediately after 30 min dwell time (Song and Fan, 2021). The homogenate (after passing through the build-in filtration) was serially diluted (if needed) and plated (0.1 mL or 1 mL) on tryptic soy agar with 200 μg/mL pyruvate acid 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 colonies on the plates were then counted.

2.5. Statistical analysis

For each chamber session, in which tomatoes were subjected to one of the six examined H_2O_2 treatment times (10, 20, and 30 s) and dwell times (15 and 30 min) combinations, untreated control tomatoes were included; and each of these experiments was repeated at least three times. The log CFU value measured on each plated H_2O_2 treated tomato sample was expressed as log CFU reduction, relative to untreated control tomatoes, by subtracting the average of the untreated control Log CFU values. When no colony was present on a plate, half of the limit of detection (0.70 log CFU/piece) was used to calculate the log reductions.

To examine the relationship between log CFU reduction and H_2O_2 concentration and estimate the lowest H_2O_2 concentrations required to achieve at least a 2-log average reduction on stem scar and a 5-log reduction on smooth surface or the lowest H_2O_2 concentrations at which the log CFU reduction were statistically different, in comparison to the untreated control, exponential (Ratkowsky, 1990) or sigmoidal (Schabenberger et al., 1999) regression models were fitted to the observed *Listeria* and the *Salmonella* log CFU reduction values as a function of H_2O_2 concentration ($1\% \leq \%H_2O_2 \leq 7.8\%$) for the 6 treatment time and dwell time combinations for bacteria on smooth surface and stem scar, resulting in 24 individual regression models.

The sigmoidal model is: Log reduction $= d + (a\text{-}d)/\{1 + \exp[\, b \bullet \log(\%H_2O_2/c)\,]\, \}$ where a is log reduction at 1% H_2O_2, d is log reduction at 7.8% H_2O_2, b is the rate of decline, and c is the H_2O_2 concentration ($1\% \leq \%H_2O_2 \leq 7.8\%$) at which 50% of reduction between initial (a) and terminal (d) asymptotes is achieved.

The exponential model is: Log reduction $= a + \exp(b \bullet \%H_2O_2)$ where a is log reduction at 1% and b is the rate of decline ($1\% \leq \%H_2O_2 \leq 7.8\%$).

Each regression model was used to estimate the lowest H_2O_2 concertation at which the average log reduction was statistically different, in comparison to the untreated control, by identifying the lowest H_2O_2 concentrations where the 95% confidence interval (produced by the model in the range of $1\% \leq \%H_2O_2 \leq 7.8\%$) first exceeded the standard error of the concurrently observed untreated control samples.

To estimate the lowest H_2O_2 concentrations at which a 5 log average reduction was achieved for *Salmonella* and *L. innocua* on smooth surface, it was necessary to utilize a resampling method (Efron and Tibshirani, 1994). The log reduction values at each observed H_2O_2 concentration were resampled, the regression model was fitted, and a unique estimate was obtained for H_2O_2 concentration at which 5-log average reduction was achieved. This resampling process was repeated 5 times and the resulting bootstrap estimates were statistically compared via ANOVA (SAS Institute Inc, 2020) and means comparisons among the six treatment time by dwell time combinations, using the adjust = sidak option in the SAS LSMEANS statement to maintain overall experiment wise $\alpha = 0.05$.

3. Results

3.1. Reductions of L. innocua on stem scar area of tomatoes

After treatments with cold plasma-activated H_2O_2, the reductions in populations of *L. innocua* on stem scar increased with increasing concentrations of H_2O_2 (Fig. 1). The curve of reductions as a function of H_2O_2 concentration was best fitted using an exponential model. Compared with the non-treated controls, the average log reductions of *Listeria* populations were statistically significant at H_2O_2 concentrations above 1% with treatment time of 30 s; however, the average log

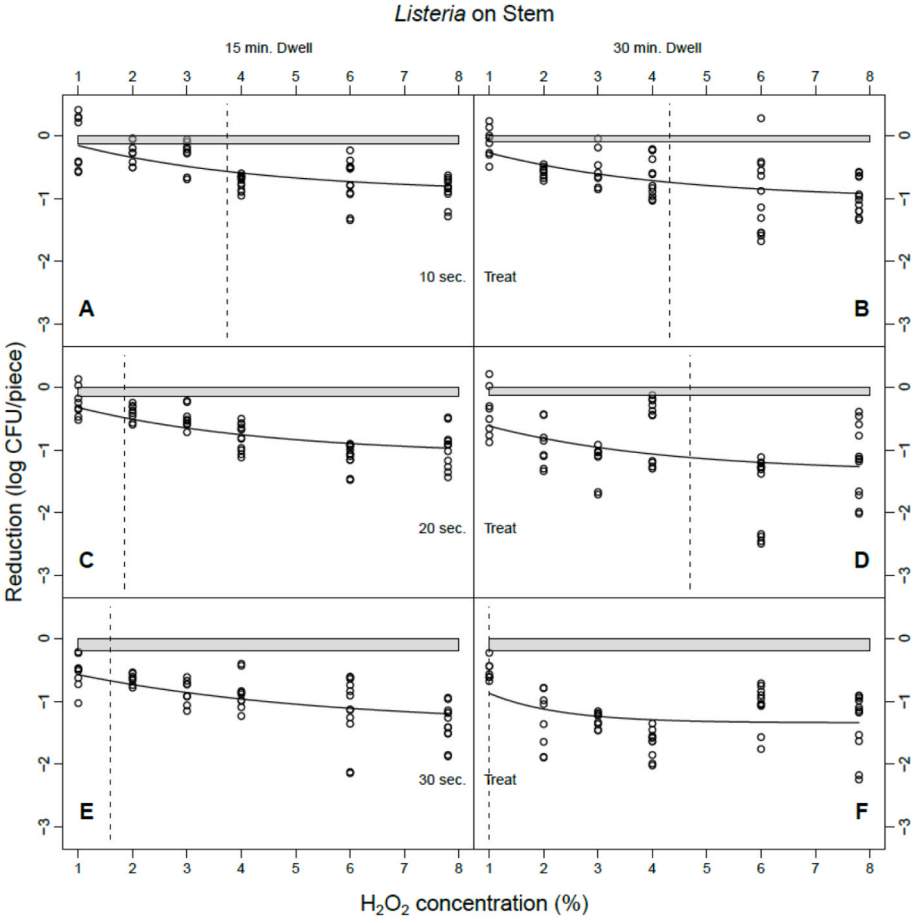

Fig. 1. Effects of H_2O_2 concentration, treatment time and dwell time on reductions of *Listeria innocua* inoculated on stem scar of tomato fruits. Tomato fruits were treated with cold plasma-activated aerosols generated from 1, 2, 3, 4, 6, and 7.8% H_2O_2 for 10 (A, B), 20 (C,D) and 30 (E,F) s followed by 15 (A, C, E) and 30 (B, D, F) min dwell time. Non-treated control (0% H_2O_2, 0 s treatment time, 0 min dwell time) was included for each combination treatment. The reductions of *Listeria* populations were calculated by subtracting populations of treated samples from non-treated samples which had an average population of 5.42 log CFU/piece. Thickness of horizontal shaded bar at top of graph represents maximum standard error of the non-treated samples at the observed H_2O_2 concentrations. Vertical broken lines on each graph indicates the lowest H_2O_2 concentrations at which the reduction was significant in comparison with the non-treated control.

reductions were less than 2 log CFU/piece even after treatment with 6 or 7.8% H_2O_2 for 30 s with 30 min dwell time. Reductions of *Listeria* populations on the stem scar area of tomatoes by 1% H_2O_2 were not significantly different for 10 s treatment time at both 15 and 20 min dwell times or for 20 s treatment time with 15 min dwell time, yet initial (1% H_2O_2) average log reductions were each significantly greater for treatment times 20 and 30 s with dwell time 30 min and for 30 s treatment time with 15 min dwell time (Table 1, parameter *a*). The greatest rates of decline in log reduction occurred for dwell time 30 min with treatment time 30 s, followed by treatment time 20 s and 10 s (Table 1, parameter *b*). Treatment time of 30 s (averages of both dwell times) achieved significantly higher average reductions of *Listeria* on stem scar area than the 10 s treatment at each H_2O_2 concentration (Fig. 1). Greater variability in *Listeria* reductions among the individual samples observed at dwell time of 30 min for treatment times of 10 and 20 s, indicated H_2O_2 concentrations greater than 4.3% and 4.7%, respectively, are necessary to ensure that 95% of individual samples exhibit log reductions significantly higher than the control (Fig. 1).

3.2. Reductions of Listeria on smooth surface of tomatoes

The *Listeria* reductions on smooth surface of tomatoes in relationship to H_2O_2 concentrations ($1\% \leq \%H_2O_2 \leq 7.8\%$) for all six treatment time and dwell time combinations was well characterized by the lower asymptote portion of sigmoidal models (Fig. 2). Activated H_2O_2 at concentrations of 4.2% or above reduced the average log reduction of the *Listeria* populations on the surface of tomatoes to non-detectable levels (detection limit, 0.70 log CFU/piece) for all tested treatment times and dwell times, achieving more than 5 log average reductions (Table 2). The following three treatment and dwell times achieved 5-log average reduction at the lowest H_2O_2 concentrations, not statistically different from one another: 1.75% for 20 s treatment time and 15 min dwell time, 2.21% for 20 s treatment time and 30 min dwell time, and 2.32% for 30 s treatment time and 15 min dwell time (Table 3). Even though the fitted regression models for all treatment and dwell times resulted in 5-log reductions on average (Table 2), just as many observed log reduction values (i.e., on individual samples) were less than 5-log as were greater than 5-log (Fig. 2). The reductions of less than 5 log reductions observed on some samples were mostly due to the low control inoculation levels of *Listeria* on the samples and the limit of detection (0.70 log CFU/piece). When the initial populations being lower than 5.35 log CFU/piece were reduced to non-detectable levels, the reductions would be less than 5 logs because the log reduction were calculated by subtracting the half (0.35 log CFU/piece) of detection limit from the corresponding control populations.

3.3. Reductions of Salmonella on stem scar area

The reductions of *Salmonella* populations as a function of H_2O_2 concentrations was well described using sigmoidal regression models (Fig. 3). There were no significant reductions of *Salmonella* on stem scar area of tomatoes at H_2O_2 concentrations lower than 2% regardless of treatment time or dwell time. Compared with the non-treated controls, H_2O_2 concentrations higher than 3% were required to achieve significant reductions of *Salmonella* on stem scar with 15 min dwell time. Noticeably greater variability in reduction of *Salmonella* populations among individual samples was observed for both dwell times with the 30 s treatment time. Activated H_2O_2 aerosols had limited effectiveness in reducing populations of *Salmonella* on stem scar area with maximum average reductions approximately 1-log CFU attained and maintained in the range of $4\% < H_2O_2 < 6\%$ for all cases, except treatment time of 30 s with dwell time of 30 min which further reduced the average bacterial populations to 1.65 log CFU/piece at 7.8% H_2O_2 (Table 4).

Table 1
Means comparisons for parameters *a* and *b* of the exponential regression models fitted to each of 5 bootstrap samples of observed *Listeria* log reduction on tomato stem scar. Parameters *a* and *b* indicate initial level (i.e. reductions by 1% H_2O_2) and rate of change in log reduction, respectively.

Treatment time (s)	Dwell time (min)	Exponential Model Parameter Estimates	
		A	b
10	15	−0.92 ± 0.06 w[a]	−0.30 ± 0.06 wx[a]
10	30	−0.94 ± 0.02 w	−0.40 ± 0.02 xy
20	15	−1.02 ± 0.01 w	−0.35 ± 0.01 x
20	30	−1.23 ± 0.02 x	−0.50 ± 0.02 y
30	15	−1.43 ± 0.02 z	−0.18 ± 0.01 w
30	30	−1.31 ± 0.01 y	−0.78 ± 0.01 z

[a] Parameter estimates (± standard error) followed by the same letter are not statistically different (α = 0.05).

3.4. Reductions of Salmonella on smooth surface

Salmonella populations on the smooth surface were significantly reduced from control by activated H_2O_2 treatments at all tested concentrations ($1\% \leq \%H_2O_2 \leq 7.8\%$) (Fig. 4). The changes in *Salmonella* reductions as a function of H_2O_2 concentration were fully represented by sigmoidal models for each of the observed treatment time and dwell time combinations. Initial reductions in the low range of H_2O_2 concentrations ($1\% \leq \%H_2O_2 \leq 3\%$), represented by model parameter *a*, were significantly greater for dwell time 30 min than for dwell time 15 min and significantly increased with increasing (10–20–30 s) treatment times (Table 5). The model parameter *b* represents the rate of decrease (greater reductions) in populations of *Salmonella* which were statistically significant and occurred for each treatment time and dwell time combination somewhere between 3% and 5% H_2O_2. *Salmonella* populations fell most quickly for treatment time of 30 s and dwell time of 30 min and for treatment time of 10 s and dwell time of 15 min. All 6 treatment time x dwell time combinations achieved the reductions of *Salmonella* population, represented by the model parameter *d*, to below the detection limit (Table 5). Treatment time of 30 s and dwell time of 30 min required the least H_2O_2 (3.24%) to reduce the *Salmonella* population on tomato surface to the undetectable level (Table 3); a significantly lower H_2O_2 concentration than required for the other 5 treatment time and dwell time combinations (Table 5). To consistently achieve more than 5 log average reductions of *Salmonella* on the surfaces with all tested dwell times and treatment times, H_2O_2 at concentrations of 5.72% or above were required (Table 3).

Compared with *Listeria*, *Salmonella* cells were more resistant to the treatments. For example, to achieve 5 log average reductions of *Salmonella* on smooth surface, H_2O_2 concentrations at or above 5.72% are required while a lower concentration (4.23%) of H_2O_2 were sufficient to achieve the 5 log average reductions of *Listeria* on the surface of tomatoes. In addition, there was a delay (a lag period) for the reduction of *Salmonella* as a function of H_2O_2 concentration while a sharp decline was obvious even at 1% H_2O_2 for *Listeria* on the surface.

Among the three factors evaluated at the tested ranges, H_2O_2 concentration was the most important factor affecting the efficacy of the activated H_2O_2 while dwell time was of least importance.

4. Discussion

Non-linear regression modeling of data estimated the conditions at which 5-log reductions of bacteria on the smooth surface can be obtained using activated H_2O_2. To achieve the 5-log reductions of *Listeria* and *Salmonella* populations on the smooth surface of tomatoes, 5.72% and 4.23% activated H_2O_2 are needed with treatment times of 10–30 s and dwell times of 15 and 30 min. However, the goal of achieving 2 log reductions of bacteria on the stem scar cannot be reached under the conditions tested in the present study. Increasing treatment time from 10 to 30 s or dwell time from 15 min to 30 min does not always yield higher reduction of *Listeria* or *Salmonella* on smooth surface or stem scar of tomatoes.

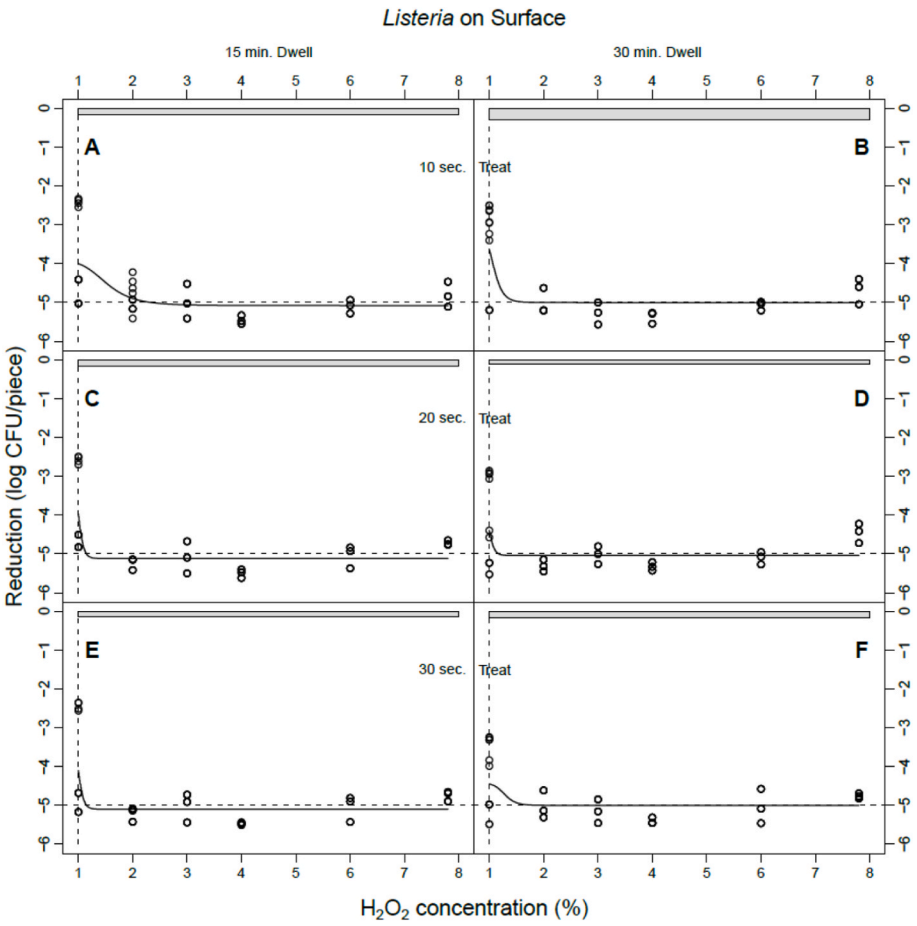

Fig. 2. Effects of H_2O_2 concentration, treatment time and dwell time on reductions of *Listeria innocua* inoculated on smooth surface of tomato fruits. Tomato fruits were treated with cold plasma-activated aerosols generated from 1, 2, 3, 4, 6, and 7.8% H_2O_2 for 10 (A, B), 20 (C,D) and 30 (E,F) s followed by 15 (A, C, E) and 30 (B, D, F) min dwell time. The reductions of *Listeria* populations were calculated by subtracting populations of treated samples from non-treated samples (0% H_2O_2, 0 s treatment time, 0 min dwell time) which has an average population of 5.46 log CFU/piece. Thickness of horizontal shaded bar at top of graph represents maximum standard error of the non-treated samples at the observed H_2O_2 concentrations. Horizontal broken line marks the targeted 5 log reductions compared with the non-treated controls. Vertical broken lines on each graph indicates the lowest H_2O_2 concentrations at which the reduction was significant in comparison with the non-treated control.

Table 2
Means comparisons for parameters: *a* (initial asymptote), *b* (rate of change in log reduction, *c* (%H_2O_2 where log reduction is halfway between the initial and lower asymptotes), and *d* (the lower asymptote) of the sigmoidal regression models fitted to each of 5 bootstrap samples of observed *Listeria* log reduction on tomato smooth surface.

Treatment time (s)	Dwell time (min)	Sigmoidal Model Parameter Estimates			
		A	B	c	D
10	15	−3.69 ± 0.15 z[a]	3.52 ± 0.25 y[a]	0.71 ± 0.03 z[a]	−5.07 ± 0.02 z[a]
10	30	−3.57 ± 0.17 z	7.69 ± 0.64 x	1.16 ± 0.06 z	−5.08 ± 0.02 z
20	15	−3.53 ± 0.16 z	1.24 ± 0.15 z	0.83 ± 0.32 z	−5.11 ± 0.01 z
20	30	−3.76 ± 0.12 z	1.58 ± 0.23 z	0.99 ± 0.17 z	−5.04 ± 0.01 z
30	15	−3.70 ± 0.12 z	1.64 ± 0.13 z	0.67 ± 0.25 z	−5.09 ± 0.02 z
30	30	−3.67 ± 0.21 z	2.15 ± 0.94 yz	0.67 ± 0.23 z	−5.03 ± 0.02 z

[a] Parameter estimates (± standard error) followed by the same letter are not statistically different (α = 0.05).

Table 3
Estimated concentrations of H_2O_2 required to achieve 5-log reduction of *Listeria* and *Salmonella* on the surface of tomatoes.

Treatment time (s)	Dwell time (min)	H_2O_2 concentration required (%)	
		Listeria	Salmonella
10	15	3.79 ± 0.33 a[a]	5.41 ± 0.06 c[a]
10	30	2.75 ± 0.29 b	5.44 ± 0.05 c
20	15	1.75 ± 0.24 c	5.72 ± 0.04 a
20	30	2.21 ± 0.63 bc	5.69 ± 0.12 ab
30	15	2.32 ± 0.37 bc	5.50 ± 0.07 bc
30	30	4.23 ± 0.17 a	3.24 ± 0.01 d

[a] Parameter estimates (± standard error) followed by the same letter are not statistically different (α = 0.05).

Our results demonstrated that *Salmonella* Typhimurium was more resistant to the cold plasma-activated H_2O_2 aerosols than *L. innocua*, i.e. the treatments achieved higher average reduction of *Listeria* than *Salmonella* on tomato surfaces at low H_2O_2 concentration range (Figs. 2 and 4), and it required lower concentrations of activated H_2O_2 to reduce the populations of *Listeria* by average 5 logs compared to *Salmonella* (Table 3) Earlier studies demonstrated that *Listeria* and *Salmonella* had similar resistance to aqueous or vaporous H_2O_2 treatments. For example, washing of cantaloupes with 3% H_2O_2 for 5 min at elevated temperatures reduced populations of *Salmonella* and L. *monocytogenes*, on rinds of cantaloupe by 2.9 and 2.8 log CFU/cm^2, respectively (Ukuku et al., 2016). When lettuce was treated with H_2O_2 vapor, the reduction levels of *S.* Typhimurium, and *L. monocytogenes* on lettuce were 3.12 and 2.95 log10 CFU/g, respectively. (Back et al., 2014).

It seems that the reduction of *Listeria* and *Salmonella* on the surface of tomatoes as a function of H_2O_2 concertation can be expressed as sigmoidal model with *Salmonella* reductions being more profound, suggesting that the populations of bacteria on the surface declined rapidly after certain threshold of H_2O_2 concentrations. Perhaps, at low concentrations, bacteria can survive the activated H_2O_2 treatments by utilizing their de-toxification enzymes such as superoxide dismutase which breaks down ROS, and catalase which reduces H_2O_2 to water (Hahn et al., 2021). At higher concentrations, H_2O_2 may overwhelm the bacteria defense systems, resulting in the inactivation of the bacteria.

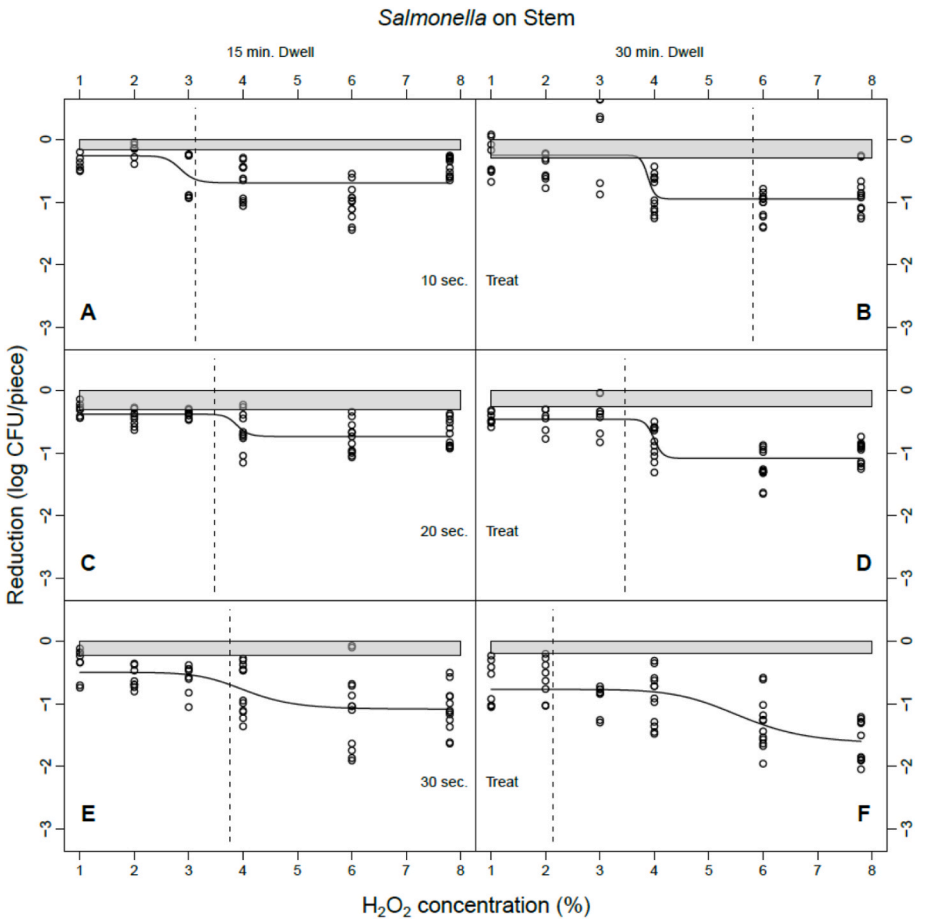

Fig. 3. Effects of H_2O_2 concentration, treatment time and dwell time on reductions of *Salmonella* inoculated on stem scar of tomato fruits. Tomato fruits were treated with cold plasma-activated aerosols generated from 1, 2, 3, 4, 6, and 7.8% H_2O_2 for 10 (A, B), 20 (C, D) and 30 (E,F) s followed by 15 (A, C, E) and 30 (B, D, F) min dwell time. The reductions of *Salmonella* populations were calculated by subtracting populations of treated samples from non-treated samples (0% H_2O_2, 0 s treatment time, 0 min dwell time) which had an average population of 6.11 log CFU/piece. Thickness of horizontal shaded bar at top of graph represents maximum standard error of the non-treated samples at the observed H_2O_2 concentrations. Vertical broken lines on each graph indicates the lowest H_2O_2 concentrations at which the reduction was significant in comparison with the non-treated control.

Table 4
Means comparisons for parameters: *a* (initial asymptote), *b* (rate of change in log reduction, *c* (%H_2O_2 where log reduction is halfway between the initial and lower asymptotes), and *d* (the lower asymptote) of the sigmoidal regression models fitted to each of 5 bootstrap samples of observed *Salmonella* log reduction on tomato stem scar.

Treatment time (s)	Dwell time (min)	Sigmoidal Model Parameter Estimates			
		A	*B*	*c*	*d*
10	15	−0.25 ± 0.01 x [a]	0.66 ± 0.03 z[a]	3.03 ± 0.191 z[a]	−0.90 ± 0.17 y[a]
10	30	−0.44 ± 0.04 y	5.21 ± 2.28 z	3.97 ± 0.004 z	−0.96 ± 0.03 y
20	15	−0.50 ± 0.02 y	0.60 ± 0.26 z	3.36 ± 0.158 z	−0.82 ± 0.17 y
20	30	−0.45 ± 0.01 y	36.27 ± 14.47 z	4.00 ± 0.002 z	−1.10 ± 0.03 y
30	15	−0.46 ± 0.02 y	4.89 ± 2.15 z	3.52 ± 0.312 z	−1.08 ± 0.03 y
30	30	−0.79 ± 0.01 z	3.07 ± 0.42 z	5.97 ± 0.005 y	−1.65 ± 0.01 z

[a] Parameter estimates (± standard error) followed by the same letter are not statistically different (α = 0.05).

Hydrogen peroxide and other ROS produced by the cold-plasma-activated H_2O_2 may damage DNA, and oxidize fatty acids in lipids, and amino acids in proteins (Farr and Kogoma, 1991).

The difference in the sensitivity to activated H_2O_2 between *Salmonella* and *Listeria* may be related to the detoxification ability of the bacteria. It has been shown that the catalase content of *L. monocytogenes* biofilms differs from the catalase content of *S.* Typhimurium biofilms (Govaert et al., 2019). In the present study, planktonic bacteria were used, and future studies may be conducted to evaluate if there is a difference in the detoxification systems such as catalase and superoxidase dismutase activities between the two bacteria.

The differences in the sensitivities of foodborne pathogens to chemical sanitizers may also be explained by differences in membrane structures between Gram-positive and Gram-negative bacteria. The cell walls of Gram-negative bacteria such as *Salmonella* may serve as a better chemical barrier than Gram-positive bacteria, because they consist of two membranes and a peptidoglycan layer (Rojas et al., 2018).

Most earlier studies on efficacy of cold plasma in inactivating human pathogens suggested that *Salmonella* and *L. monocytogenes* were similarly sensitive to cold plasma. For example, cold plasma treatment of strawberries for 300 s resulted in reduction of *Salmonella* and *L. monocytogenes* populations by 3.8 and 4.2 log10 CFU/sample, respectively (Ziuzina et al., 2014). Dielectric barrier atmosphere cold plasma treatment of lettuce for 5 min reduced *Salmonella*, and *L. monocytogenes* by 0.4 ± 0.3 and 1.0 ± 0.5 log CFU/g, respectively (Min, Roh, Niemira, et al., 2016). Average log CFU/mL reductions after treatment with dielectric barrier discharge cold plasma at 1 cm distance were 3.0 for *Salmonella*, and 2.6 for *L. monocytogenes* on glass coverslips (Timmons et al., 2018). Our earlier study indicated that cold plasma activated H_2O_2 at 7.8% reduced *S.* Typhimurium and *Listeria* populations by > 5.0 log CFU/piece on the smooth surface of tomatoes (Jiang et al., 2017).

The difference in the reductions of *Salmonella* and *Listeria* between our current study using cold plasma-activated H_2O_2 aerosol and earlier studies on cold plasma or H_2O_2 alone may reflect the differences in the inactivation mechanism among the treatments. In the present study, aerosolized H_2O_2 passed through a cold plasma arc which presumably activated H_2O_2 and produced free radicals (Fan and Song, 2020). It is

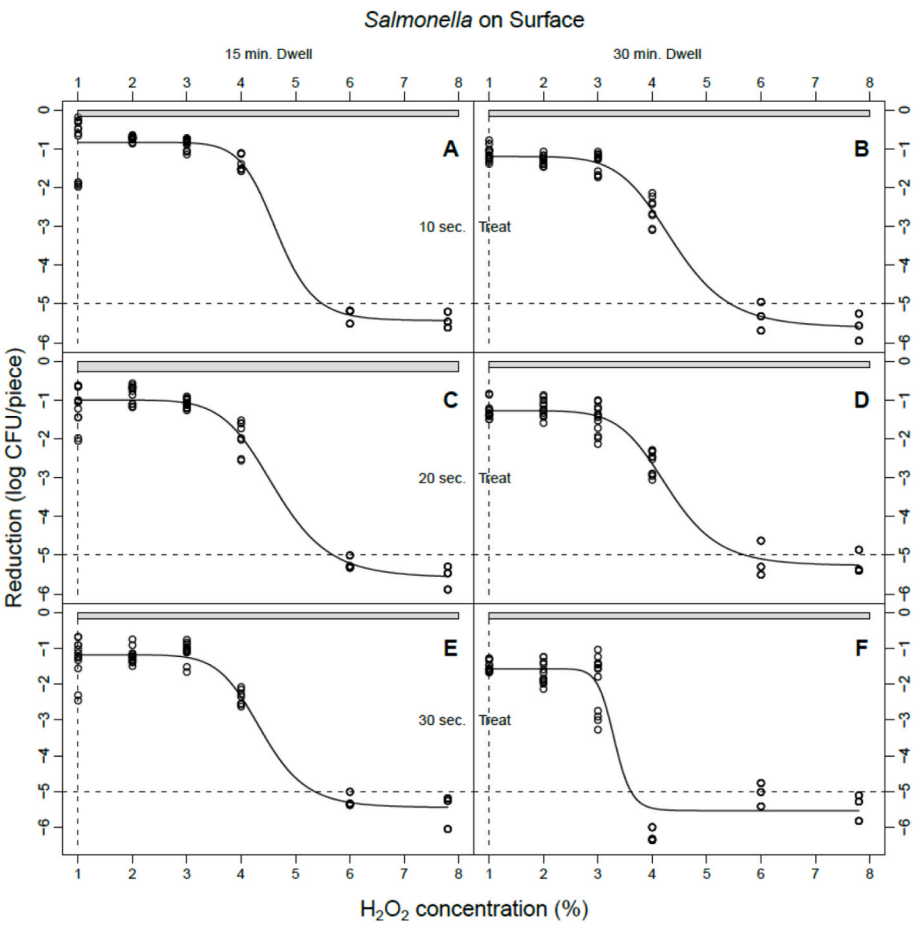

Fig. 4. Effects of H_2O_2 concentration, treatment time and dwell time on reductions of *Salmonella* inoculated on smooth surface of tomato fruits. Tomato fruits were treated with cold plasma-activated aerosols generated from 1, 2, 3, 4, 6, and 7.8% H_2O_2 for 10 (A, B), 20 (C,D) and 30 (E,F) s followed by 15 (A, C, E) and 30 (B, D, F) min dwell time. The reductions of *Salmonella* populations were calculated by subtracting populations of treated samples from non-treated samples (0% H_2O_2, 0 s treatment time, 0 min dwell time) which has an average population of 6.30 log CFU/piece. Thickness of horizontal shaded bar at top of graph represents maximum standard error of the non-treated samples at the observed H_2O_2 concentrations. Horizontal broken line marks 5 log reductions compared with the non-treated controls. Vertical broken lines on each graph indicates the lowest H_2O_2 concentrations at which the reduction was significant in comparison with the non-treated control.

Table 5
Means comparisons for parameters: *a* (initial asymptote), *b* (rate of change in log reduction, *c* (%H_2O_2 where log reduction is halfway between the initial and lower asymptotes), and *d* (the lower asymptote) of the sigmoidal regression models fitted to each of 5 bootstrap samples of observed *Salmonella* log reduction on tomato smooth surface.

Treatment time (s)	Dwell time (min)	Sigmoidal Model Parameter Estimates			
		A	*B*	*c*	*d*
10	15	−0.73 ± 0.01 v[a]	11.7 ± 0.07 x[a]	4.66 ± 0.05 x[a]	−5.41 ± 0.03 xy[a]
10	30	−1.21 ± 0.01 x	7.6 ± 0.35 z	4.30 ± 0.04 y	−5.46 ± 0.05 yz
20	15	−1.01 ± 0.02 w	9.5 ± 0.35 y	4.67 ± 0.06 x	−5.54 ± 0.04 yz
20	30	−1.31 ± 0.01 y	8.2 ± 0.22 yz	4.21 ± 0.02 y	−5.23 ± 0.03 w
30	15	−1.15 ± 0.03 x	8.5 ± 0.09 yz	4.20 ± 0.01 y	−5.30 ± 0.02 wx
30	30	−1.62 ± 0.03 z	39.2 ± 2.96 w	3.08 ± 0.01 z	−5.54 ± 0.02 z

[a] Parameter estimates (± standard error) followed by the same letter are not statistically different (α = 0.05).

possible that Gram-positive bacteria on tomatoes are more sensitive to the free radicals produced during the treatments. The strains we used in the present study are non-pathogenic surrogates. Further studies are needed to confirm the difference using pathogenic strains of *Salmonella* and *Listeria* and to study the exact mechanisms in the inactivation of bacteria by cold plasma-activated H_2O_2. The formation and nature of free radicals from H_2O_2 also need to be confirmed.

As expected, bacteria on smooth surfaces were much easier to be inactivated than on the stem scar area. It is known that surface roughness is an important factor for the antimicrobial efficacy of most intervention technologies including cold plasma treatment (Han et al., 2020). A targeted bacterial reduction of 2 log CFU/piece on stem scar was not achieved in any combination of the tested parameters. The extreme difficulty in reducing population of bacteria on the stem scar area of tomatoes suggests that the H_2O_2 aerosols cannot access the site where bacteria reside. The size of the holes in the stem scar area of tomatoes are normally in low μm ranges (Fan et al., 2018). Our results showed that droplets in the H_2O_2 aerosols distributed in two ranges: nanometers with average size of 40 nm and micrometer with average size of 3 μm (Jiang et al., 2017). The larger (μm) sized droplets may deposit on the holes of stem scar area and block the access of additional droplet into the crevices, similar to the effect of aqueous wash. Future studies may be conducted to reduce the sizes of droplets which may result in higher reduction of bacteria located inside the holes and valleys in the stem scar of tomatoes.

The stem scars provided a protective environment for bacteria against washing with aqueous sanitizers (Wei et al., 1995). The effectiveness of such protection is dependent on the characteristics of the stem scars. Our earlier results demonstrated that washing with combinations of three acids reduced populations of *S. enterica* on the stem scar area of grape tomatoes by 1.52–1.90 log CFU/fruit, compared with the non-treated control while the same acid treatments achieved 3.54 log CFU/fruit reduction of the pathogen on stem scar areas of large beefsteak tomatoes (Fan et al., 2018). The varying response to the acid washes between grape and large round tomatoes was related to the differences in surface characteristics of stem scar areas observed with scanning electron microscope. The stem scar area of small tomatoes had deeper crevices than that of large round tomatoes. It also seems that the

stem scar surface of the grape tomato was more complex with over-reaching structures, forming holes and valleys in micrometer sizes. *Salmonella* cells were often in the deep holes on the small tomatoes stem scar.

The most important factor affecting the efficacy of cold plasma-activated H_2O_2 aerosol against *Salmonella* and *Listeria* is H_2O_2 concentration. Treatment times in the range of 10–30 s had limited effects on the efficacy of activated H_2O_2 suggesting that, when maximum amounts of aerosolized H_2O_2 were rapidly established in the chamber, excessive amounts of H_2O_2 aerosols does not always lead to higher reductions of pathogens, and may lead to condensation of H_2O_2 on the surfaces of tomatoes, which negates the purpose of using aerosolized H_2O_2. Among the three factors evaluated, dwell time had the least effect on the efficacy of activated H_2O_2, indicating that most of the bactericidal effect of H_2O_2 aerosols occurred in the first 15 min. An earlier study showed that the amount of aerosolized H_2O_2 in the treatment chamber decreased rapidly, only small percentage of nano-sized droplets of aerosolized H_2O_2 was present, and all micrometer-sized droplets disappeared after 30 min (Jiang et al., 2017). In addition, H_2O_2 residues on the surface of tomato fruit decreased rapidly after treatment (Song and Fan, 2021), which may explain the limited effectiveness of dwell time.

The rate of reductions of *Listeria* populations on stem scar area as a function of H_2O_2 concentrations was higher for 30 min dwell time than for 15 min dwell time with 20 and 30 s treatment times (Table 1). In addition, the initial average reductions of *Listeria* (i.e. reductions at 1% H_2O_2) on stem scar were higher for 30 min dwell time than for 15 min dwell time with 20 and 30 s treatment times (Table 1), suggesting that longer dwell times are beneficial when higher amounts of activated H_2O_2 are introduced into the chamber. The initial reductions were sometimes influenced by treatment time. For example, the initial average reduction of *Salmonella* populations on the smooth surface increased with treatment time for both dwell times (Table 5), suggesting that, at low H_2O_2 concentrations, longer treatment times may be used to increase efficacy.

Our previous studies demonstrated that quality parameters such as color, texture, appearance and soluble solids of tomatoes were not affected by the treatments of activated H_2O_2 even at 7.8% (Jiang et al., 2017; Song et al., 2020). Sensory evaluation may be conducted in the future to evaluate if the treatments affect taste and flavor of treated fruit. In addition, the application of activated H_2O_2 was performed at ambient temperature (22 °C). Its effectiveness at lower temperatures may be different and needs further investigations.

5. Conclusion

In the present study, the effectiveness of cold plasma-activated H_2O_2 aerosols against *Salmonella* and *Listeria* on tomato smooth surface and stem scar areas of tomatoes was investigated as a function of H_2O_2 concentration, treatment time and dwell time. Results showed that the treatment time from 10 to 30 s or dwell time of 15 and 30 min exhibited limited effectiveness. H_2O_2 concentrations have more profound effects on the efficacy of activated H_2O_2 than treatment time and dwell time. *Salmonella* was less sensitive to the treatments than *L. innocua*. To achieve average 5 log reductions of *Salmonella* on the smooth surface of tomatoes, H_2O_2 at concentrations $\geq$5.72% was required for all treatment and dwell time combinations, while 4.23% H_2O_2 reduced the average *Listeria* populations by 5 logs on the smooth surfaces. The treatments achieved average reductions of *Salmonella* and *Listeria* populations by less than 2 log CFU/pieces of on the stem scar area of tomatoes even when 6 and 7.8% H_2O_2 were used. Future studies are needed to increase its efficacy against bacteria on stem scar area of tomatoes. In addition, the mechanism(s) for the difference in sensitivity of the two bacteria to the activated H_2O_2 need to be investigated.

5.1. Industrial relevance

Activated (ionized) hydrogen peroxide technology comprises applying cold plasma to H_2O_2 aerosols as it passes through a cold plasma arc to produce more reactive species. There are many factors that can influence the efficacy of the process against human pathogens, among which H_2O_2 concentration, treatment time and dwell time. The current study employs non-linear regression modeling to estimate the optimum settings for the three parameters with a goal of achieving 5-log and 2-log reductions of *Listeria* and *Salmonella* on the smooth surface and stem scar area, respectively, of tomatoes. The optimum conditions generated from the study would help the fresh produce industry to apply the novel technology to enhance microbial safety of tomatoes and other fresh produce items.

CRediT authorship contribution statement

Xuetong Fan: Conceptualization, Methodology, Writing – review & editingWriting- Reviewing and Editing. **Bryan T. Vinyard:** Statistical analysis, Visualization, Writing – review & editingWriting- Reviewing and Editing. **Yuanyuan Song:** Investigation, draft preparation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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