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Research Note

Hydrogen Peroxide Residue on Tomato, Apple, Cantaloupe, and Romaine Lettuce after Treatments with Cold Plasma–Activated Hydrogen Peroxide Aerosols

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ABSTRACT

Hydrogen peroxide (H_2O_2) has long been studied as an aqueous sanitizer to enhance microbial safety of fresh produce. Recently, we demonstrated that cold plasma–activated H_2O_2 aerosols, hereafter called ionized hydrogen peroxide (iHP), reduced populations of *Salmonella, Listeria,* and *Escherichia coli* by up to 5.5 log on surfaces of various produce items. However, the amount and fate of H_2O_2 residue left on fresh produce after treatments have not been evaluated. In the present study, H_2O_2 residue levels on apples, tomatoes, cantaloupe, and romaine lettuce were analyzed after treatments with 7.8% iHP at conditions that had been optimized and tailored for *Salmonella* reductions and each produce item. Results showed that higher residue levels were found on lettuce than on cantaloupe, tomatoes, and apples immediately after treatments. During storage at 10 and 22°C, H_2O_2 levels decreased rapidly and fell below 1 mg/kg within 1 day after treatments for all fresh produce items. Furthermore, the decrease was faster at 22°C than at 10°C. Most importantly, the levels of H_2O_2 residue on the fresh produce items were lower than those after wash with 1% H_2O_2 for 1 min. Overall, our results demonstrated that levels of H_2O_2 residue on fresh produce surfaces decomposed rapidly after treatment with iHP and did not appear to pose a safety concern after 1 day of storage.

HIGHLIGHTS

- Apples, tomatoes, lettuce, and cantaloupe were treated with cold plasma–activated H_2O_2.
- The highest level of H_2O_2 residue was detected on cut lettuce.
- H_2O_2 levels decreased rapidly after treatment and depended on temperature.
- Levels of H_2O_2 residue after iHP treatment were lower those after washing with 1% H_2O_2.

Key words: Aerosol; Antimicrobial; Cold plasma; Food safety; Hydrogen peroxide; Ionized hydrogen peroxide

Contamination of fresh produce with human pathogens and consequent outbreaks and recalls continue to be major concerns in the United States and around the world *(2, 15, 16)*. Effective intervention technologies and treatments are needed to mitigate the problem at all points from field to table. Currently, the fresh produce industry relies on continuous use of aqueous sanitizers, such as chlorine to minimize cross-contamination and reduce populations of pathogens during washing of produce *(7)*. Washing fresh produce with aqueous sanitizers is not effective, frequently achieving reductions of less than 2 log CFU/g of human pathogens on fresh produce *(5)*.

Hydrogen peroxide (H_2O_2), as a strong oxidizing agent, has antimicrobial properties that lead to toxic effects on microorganisms. Oxidation of proteins, lipids, and DNA and membrane damage by H_2O_2 are believed to be the major inactivation mechanisms, although mechanisms of its action such as a biocide require further investigation *(9, 11)*. The application of H_2O_2 on foods has advantages over many other sanitizers, such as chlorine, because it produces no residues as it breaks down to harmless by-products (water and oxygen) once decomposed. In living cells of plants and animals, an endogenous enzyme, catalase, makes the reaction occur rapidly. H_2O_2 as an aqueous sanitizer has previously been investigated to determine its ability to inactivate human pathogens and spoilage microorganisms and to degrade pesticide residues on fresh and fresh-cut produce *(3, 4, 6, 24)*. Washing cantaloupe in a 2.5 and 5% concentration of H_2O_2 in a bath for 5 min resulted in a 3-log reduction of *Salmonella* spp. on melon surfaces *(19)*. Similarly, Sapers and Sites *(14)* found that washing apples with 1% H_2O_2 at 20 and 40°C reduced *Escherichia coli* by up to 3 log. To increase the efficacy of H_2O_2 against human pathogens, H_2O_2 has been studied in combination with many other antimicrobials, such as organic acids, at elevated temperatures *(8, 20, 21),* and when applied as a vapor *(12)*.

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Recently, we have investigated an application of novel technology involving activation or ionization of H_2O_2 aerosols by nonthermal cold plasma *(10, 18)*. For the technology, nano- and micrometer droplets of H_2O_2 generated from a 7.8% H_2O_2 solution by an atomizer passed through a cold plasma arc and became activated or ionized to create a fog or mist called ionized hydrogen peroxide (iHP). The cold plasma activation, as an advanced oxidation process, generates other oxidizing species such as hydroxyl radicals. In previous studies, iHP was used to treat fresh produce including cantaloupe, apples, tomatoes, lettuce, and spinach. Results showed that populations of *Salmonella* spp. were reduced by up to 5.5 log after less than 1 min of treatment followed by a 30-min dwell time. However, the presence of H_2O_2 residue on the produce items after treatment has not been evaluated. Although H_2O_2 is generally regarded as safe and allowed for use in some foods such as milk *(23),* the presence of H_2O_2 on food and in the environment may be harmful to humans depending on the concentration of H_2O_2. H_2O_2 exposure can cause irritation of the eyes, throat, respiratory airway, and skin *(22)*. Drinking concentrated liquid can cause severe gastrointestinal effects and even death *(25)*. The maximum exposure level of H_2O_2 permitted in the environment is 1 μL/L for an 8-h workday *(22)*. Therefore, it is important to determine the levels of H_2O_2 in the fresh produce items treated by iHP at various time and temperature points. In an earlier study *(1),* lettuce was treated with vaporized 10% H_2O_2 for 10 min, and the concentration of H_2O_2 residue on the lettuce was found to be more than 1,000 mg/kg.

The objective of the present study was to determine the H_2O_2 residue on tomatoes, apples, cantaloupe, and lettuce after treatments with iHP as affected by treatment time and storage temperature at conditions that achieved maximum reduction of *Salmonella* based on earlier studies.

MATERIALS AND METHODS

Sources of fresh produce. Cantaloupe, cherry tomatoes, and romaine lettuce were purchased through a major supermarket chain in Philadelphia, PA, via special orders to guarantee the freshness of the produce. Gala apples were harvested from an orchard in Central Pennsylvania and stored at 4°C. All produce items were brought in equilibrium to ambient temperature before being treated. Romaine lettuce was cut into pieces. The average weight of each piece of lettuce was 14.8 ± 5.4 g. Cantaloupe, apples, and cherry tomatoes were used without processing. Apples, cantaloupe, and cherry tomatoes weighed 185.4 ± 15.0, 1,667.9 ± 116.7, and 8.0 ± 1.1 g, respectively.

iHP treatments. Produce items were placed into a treatment chamber with a dimension of 12 by 12 by 24 in. A solution of 7.8% H_2O_2 (TOMI Environmental Solutions, Frederick, MD) was aerosolized into a treatment chamber using the SteraMist Select Machine (TOMI Environmental Solutions), which generated two groups of droplets with mean diameters of 40 nm and 3.0 μm *(10)*. pH, conductivity, and density of the 7.8% H_2O_2 were pH 3.5, 8.6 μs/cm, and 1.02 g/mL, respectively. The aerosolized H_2O_2 was activated by cold plasma generated between two pin electrodes in the TOMI SteraMist Select applicator to create iHP, which was introduced into the chamber *(10)* that contained the produce items. The flow rate for H_2O_2 used in each trial was 5.0 mL/min with an air pressure of 7 lb/in^2.

For apples and tomatoes, the treatment time (spray time) was 8 s followed by a 30-min dwell time. In addition, tomatoes were treated with three cycles of a 20-s treatment time, plus a 30-min dwell time. For romaine lettuce and cantaloupe, the treatment time was 30 s followed by a 30-min dwell time. The treatment times and conditions were chosen based on our earlier study on bacterial reductions *(18)*. To inactivate *Salmonella* spp. on the smooth surface of tomatoes and apples, a treatment time of 8 s was sufficient. However, to achieve maximum reduction of the bacteria on the stem scar area of tomatoes, 3 cycles composed of a 20-s treatment time followed by a 20-min dwell time per cycle were needed. A 30-s treatment was required to achieve maximum reductions on lettuce and cantaloupe.

Storage. The produce items were placed into polymer film bags with two perforated holes (0.5 cm) and then stored at 10°C and ambient temperature (22°C). Fresh produce items were removed from storage and measured periodically, with higher frequencies in the earlier storage periods.

Comparison with 1% H_2O_2 wash. Because most earlier studies applied H_2O_2 as a wash treatment of fresh produce *(8, 14, 19),* we conducted a study to evaluate the residue of H_2O_2 on fresh produce after washing with 1% H_2O_2 for 1 min to compare the residue levels with those from iHP treatments. The produce items were dipped into 150 to 1,500 mL of 1% H_2O_2 solution for 1 min. Then, romaine lettuce and tomatoes were spun by a salad spinner. Unwaxed Gala apples and cantaloupe were dried on a stainless steel pan for 1 min. The residue H_2O_2 was then measured.

Measurement of H_2O_2. To measure H_2O_2 residue on fresh produce, the produce items were placed in plastic film bags containing deionized water at ambient temperature and agitated for 1 min. The amounts of water to rinse the H_2O_2 residue from produce surfaces depended on the type of produce, level of H_2O_2 residues (based on preliminary experiments), and storage time. Initially, a large amount of water was used for lettuce and tomatoes; later, when the levels of H_2O_2 decreased, less water was used to increase detection sensitivity. Thus, for sampling cantaloupe, apples, tomatoes, and lettuce during storage, 500, 50, 50 to 100, and 50 to 200 mL of water were used, respectively. For each measurement, 1 cantaloupe, 1 apple, 6 tomatoes, and 2 pieces of lettuce were used. H_2O_2 leached into the water from the produce surface was measured using a H_2O_2 test kit (model HYP-1, Hach, Loveland, CO). The leachate from the produce was serially diluted to the sensitivity range (0 to 10 mg/L) of the test kit. The test uses a titration method based on the reaction of thiosulfate with peroxide in diluted sulfuric acid. The accuracy of the test kit was verified using known concentrations of H_2O_2.

Statistical analysis. Experiments were repeated three times independently. Data were subjected to statistical analysis using analysis of variance (version 9.4, SAS Institute Inc., Cary, NC). Duncan's multiple range test was used to separate the means with a significant level of $P = 0.05$. The detection limit varied because of variations in the weights of the fresh produce items for each measurement.

RESULTS AND DISCUSSION

In a previous study, we determined H_2O_2 residue levels after treatments with iHP at conditions that achieved

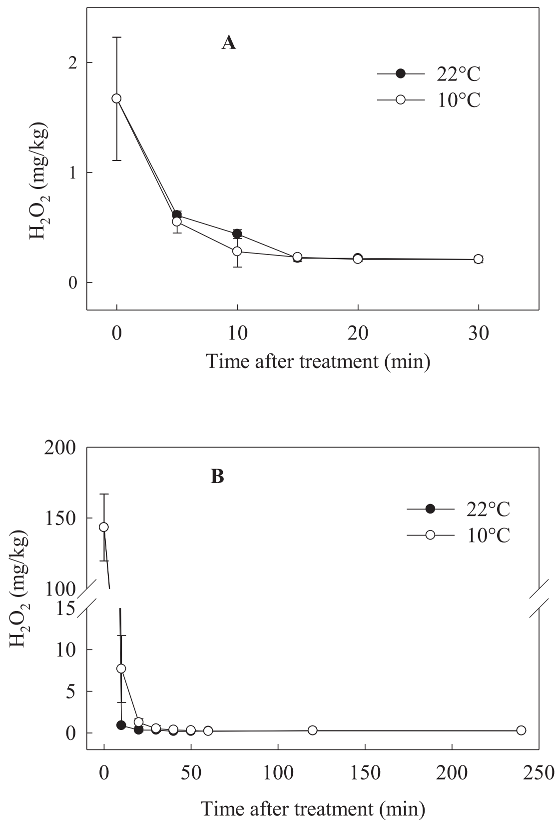

FIGURE 1. *Changes in the level of H_2O_2 residue on tomato fruit stored at 22 and 10°C after iHP treatment of a 8-s spray time followed by a 30-min dwell time (A) and 3 cycles of 20-s spray time and a 20-min dwell time (B). Vertical bars represent standard deviations.*

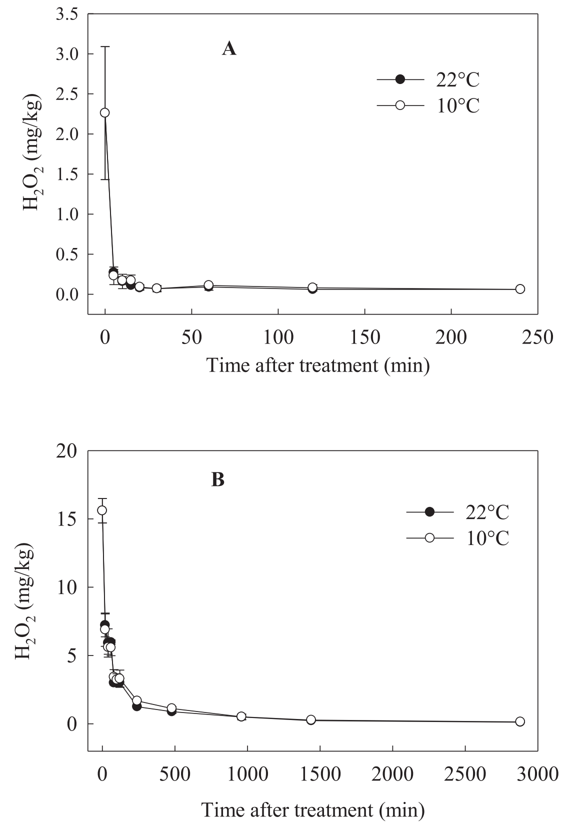

FIGURE 2. *Changes in the level of H_2O_2 residue on apples (A) and cantaloupe (B) stored at 22 and 10°C after 30-s spray time followed by a 30-min dwell time. Vertical bars represent standard deviations.*

maximum reductions of bacteria on the produce items *(18)*. Results showed that an 8-s treatment with iHP was able to reduce the populations of inoculated *Salmonella* Typhimurium on smooth surfaces of apples and tomatoes to levels below the detection limit (0.70 log CFU per piece), achieving a more than 5-log reduction of *Salmonella*. Reductions were recorded around 1 log CFU per piece on the stem scar area of tomatoes. As the treatment time increased, greater reductions were generally achieved; however, even after 60 s of treatment, the reduction was only 2.16 log CFU/g. In testing, the highest reductions of *Salmonella* populations in tomato stem scars were achieved with an iHP treatment of three cycles of a 20-s spray time, plus a 20-min dwell time, which reduced *Salmonella* populations on the stem scar by 2.73 log CFU per piece.

For romaine lettuce and cantaloupe, the efficacy of iHP increased with treatment time from 0 to 30 s, and 30 s of treatment achieved reductions of *Salmonella* of 3.63 and 2.48 log CFU per piece, respectively. Further increases in treatment time failed to achieve significant additional reductions. Therefore, 30 s of treatment followed by a 30-min dwell time was regarded as the optimized condition for romaine lettuce and cantaloupe.

The H_2O_2 residue level measured immediately following an 8-s iHP treatment on the tomato surface was 1.67 mg/kg (Fig. 1A). The level decreased rapidly at both 22 and 10°C, with less than half of the initial level detectable after 5 min. After 30 min, the level fell below the detectable level (detection limit: 0.21 mg/kg). The residue on tomatoes after three cycles of 20-s treatments was 143.3 mg/kg when measured immediately after treatment (Fig. 1B). The level decreased to less than 0.9 mg/kg and less than 7.7 mg/kg after 20 min at 22 and 10°C, respectively. The level continued to decrease during storage to below the detection limit (0.26 mg/g) after 30 and 60 min at 22 and 10°C, respectively. It appears that declines in residual H_2O_2 levels were more rapid at 22°C than at 10°C. Similar rapid reductions during post-H_2O_2 treatment storage were observed by Back et al. *(1),* in that the residue declined over time from an initial level of >1,000 to 6.67 mg/kg after 24 h at 4°C.

The residual level and its changes on apples were similar to those of tomatoes, with 2.3 mg/kg of the initial H_2O_2 level after 8 s of treatment followed by decreasing to a level below 0.2 mg/kg within 20 min at both 22 and 10°C (Fig. 2A). The initial residual H_2O_2 level on cantaloupe was 15.6 mg/kg (Fig. 2B). The level decreased during storage, but the decrease was slower compared with those on apples or tomatoes. It took more than 24 h for the level to fall below 0.2 mg/kg. Storage temperature did not affect the changes in H_2O_2 residue level during storage. Because of the large size of cantaloupe compared with apples and cherry tomatoes, it took longer for the cantaloupe to reach the targeted temperature of 10°C after the fruit was placed in a 10°C refrigerator. Therefore, the temperature (10°C) did

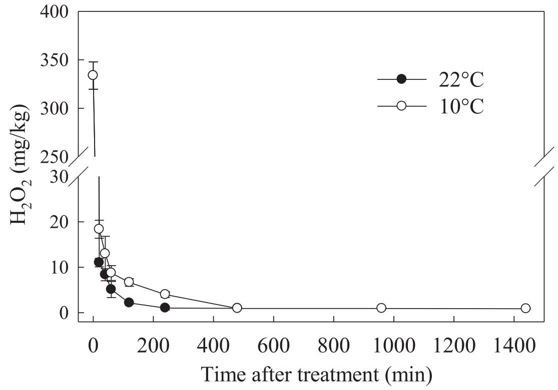

FIGURE 3. *Changes in the level of H_2O_2 residue on romaine lettuce stored at 22 and 10°C after iHP treatment of 30-s spray time followed by a 30-min dwell time. Vertical bars represent standard deviations.*

not represent the true temperature of the fruit. Cantaloupe has a rough surface with netting that retains more liquid compared with the surfaces of apples and tomatoes, which are mostly smooth and hydrophobic.

The residual level was the highest on romaine lettuce, with an initial level of 333.7 mg/kg (Fig. 3). The level decreased rapidly during storage, especially during the first 20 min, and the decrease was more rapid at 22°C than at 10°C. The level decreased to below the detection limit (<1 mg/kg) after 8 h at both temperatures. Our results demonstrated that H_2O_2 residue levels were the highest on lettuce on a weight basis. This could be because lettuce has a higher surface area–to–mass ratio than the other three types of produce items, which allows greater attachment of H_2O_2. H_2O_2 decomposes to water and oxygen in two ways: nonenzymatically and enzymatically. Both enzymatic and nonenzymatic decompositions are affected by temperature. Therefore, it is not surprising that H_2O_2 is more stable at 10°C than at 22°C.

H_2O_2 as a wash has been studied and applied to many fresh produce items and has shown effectiveness in reducing populations of pathogens, especially when applied at an elevated temperature or combined with other antimicrobials. The application of 1% H_2O_2 wash was shown to be an effective decontamination technique for *E. coli*–infected apples *(14)*. To compare H_2O_2 residue with the result of washing with H_2O_2 solution, the samples were submerged into 1% H_2O_2 solution for 1 min. Results showed that the level of residual H_2O_2 on tomatoes, apples, cantaloupe, and romaine lettuce was 5.41, 11.68, 21.66, and 944.71 mg/kg, respectively (Table 1). The levels were higher than those after the treatment with iHP generated from 7.8% H_2O_2 solution. The results also showed that the amount of residual H_2O_2 was the highest on lettuce followed by cantaloupe, apples, and tomatoes, which was a trend similar to that seen with iHP.

According to U.S. Food and Drug Administration regulations, H_2O_2 is a generally recognized as safe antimicrobial agent *(23)*. The U.S. Environmental Protection Agency established an exemption from the requirement of a tolerance for residue of H_2O_2 on food when used in solutions at concentrations $\leq$ 1,100 ppm *(22)*. In the present

TABLE 1. *Residue H_2O_2 on tomatoes, apples, cantaloupe, and romaine lettuce after dip treatment with 1% H_2O_2*

Produce	Residue H_2O_2 (mg/kg)
Tomato	5.41 ± 0.62 D[a]
Unwaxed Gala apple	11.68 ± 2.36 C
Cantaloupe	21.66 ± 1.63 B
Romaine lettuce	944.71 ± 84.29 A

[a] Values are means ± standard deviations ($n = 3$). Means with different letters are significantly different (Duncan's multiple range test, $P = 0.05$).

study, H_2O_2 solution was applied at a higher concentration, although in an aerosolized and plasma-activated form, than the exemption levels. Information generated from the present study may be used to conduct risk analysis of H_2O_2 related to the application of the technology. Caution should also be taken to avoid worker exposure to aerosolized H_2O_2 during treatment and handling of fresh produce items immediately after treatment.

In another study, dried prunes were treated with vaporized H_2O_2 solution at 35% (w/w) for up to 60 min *(17)*. H_2O_2 residues were detected 24 h after exposure in all treated samples. The H_2O_2 residues only declined to levels of less than 5 mg/L 90 days after exposure. The iHP system used in the present study produced nano- and micrometer droplets via a nozzle by pressurized air. Furthermore, the droplets were activated by cold plasma and the treatment time was short ($\leq$60 s). Therefore, H_2O_2 tended not to condense on the surface of the fresh produce and lower amounts of H_2O_2 residue were deposited on produce surfaces compared with washing or with sprayed or vaporized H_2O_2.

When H_2O_2 aerosols pass through the cold plasma arc, other reactive species, such as nitrates and nitrites, may be produced. However, their amounts would be lower than that of H_2O_2 (7.8%). The aerosols of the H_2O_2 solutions were only in the plasma arc for a fraction of a second, which may not produce detectable amounts of nitrate or nitrite. For example, it has been shown that the amounts of nitrate and nitrite were only 250 μm (0.00155%) and 12.63 μm (0.000058%) in water treated for 1 min with a submerged cold plasma system with applied voltage of up to 15 kV *(13)*. Nevertheless, studies have been planned to identify and quantify other reactive species that may be generated in the cold plasma arc. Furthermore, the toxicity of the reactive species may be evaluated.

In summary, our results showed that the levels of H_2O_2 residues after treatments with iHP or wash depended on the nature of fresh produce surface and the surface area–to–mass ratio. Fresh produce items with a rough surface (cantaloupe) and with more surface area (lettuce) retained higher amounts of H_2O_2 residue. H_2O_2 residues decreased rapidly after iHP antimicrobial treatment, and within 24 h the levels fell below 1 mg/kg for all samples. Higher storage temperature accelerated the decomposition of H_2O_2. The H_2O_2 residue amounts deposited on fresh produce surfaces after iHP treatments were lower than those found after washing with 1% H_2O_2. Overall, our results demonstrated that H_2O_2 residue is not a major concern after a short period of posttreatment storage.

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