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Research Paper

Inactivation of *Listeria monocytogenes* on and within Apples Destined for Caramel Apple Production by Using Sequential Forced Air Ozone Gas Followed by a Continuous Advanced Oxidative Process Treatment

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ABSTRACT

This study evaluated the efficacy of using sequential forced air ozone followed by an advanced oxidative process (AOP) treatment to inactivate *Listeria monocytogenes* on and within Empire apples. The forced air ozone treatment consisted of a reactor that introduced ozone (6 g/h) into an airstream that flowed through an apple bed (ca. 30 cm in depth). Before treatment, the apples were conditioned at 4°C to ensure that condensate had formed before the apples were transferred to the reactor. The condensate ensured sufficient relative humidity to enhance the antimicrobial action of ozone. Air was passed through the apple bed at 9.3 m/s, and the ozone was introduced after 10 min. The ozone concentration measured after exiting the apple bed reached a steady state of 23 ppm. A 20-min ozone treatment supported a 2.12- to 3.07-log CFU reduction of *L. monocytogenes,* with no significant effect of apple position within the bed. The AOP-based method was a continuous process whereby hydrogen peroxide was introduced as a vapor into a reactor illuminated by UV-C and ozone-emitting lamps that collectively generated hydroxyl radicals. Operating the AOP reactor with UV-C light (54-mJ cm^2 dose), 6% (v/v) hydrogen peroxide, 2 g/h ozone, and a chamber temperature of 48°C resulted in a 3-log CFU reduction of *L. monocytogenes* on the surface of the apples and internally within the scar tissue. Applying a caramel coating, from a molten solution (at 80°C), resulted in a 0.5-log CFU reduction of *L. monocytogenes* on the apple surface. In apples treated with the sequential process, *L. monocytogenes* could only be recovered sporadically by enrichment and did not undergo outgrowth when the caramel apples were stored at 22°C for 19 days. However, growth of *L. monocytogenes* within the core, but not the surface, was observed from caramel apples prepared from nontreated control fruit.

Key words: Advanced oxidative process; Apples; Decontamination; Hydrogen peroxide; *Listeria*; Ozone

In 2014, caramel apples contaminated with *Listeria monocytogenes* were linked to a foodborne illness outbreak in which 35 people contracted listeriosis, 7 of which died *(5)*. The specific source of *L. monocytogenes* was not identified, but it was hypothesized that the contamination was introduced on the apples and then spread throughout the processing facility *(5)*. Although cases of listeriosis were reported for the consumption of whole apples, the majority were linked to caramel apples prepared from the contaminated fruit *(5)*. In the course of caramel apple production, the inserted stick was thought to introduce *L. monocytogenes* into the inner core whereupon the pathogen population grew to infectious levels. This hypothesis was supported by the findings of Glass et al. *(10),* who reported growth of *L. monocytogenes* within caramel apples during postproduction storage. The experimental approach was to spot inoculate the surface, stem, and calyx areas of the apple with a five-strain cocktail of *L. monocytogenes* to achieve a final cell density of 4.2 log CFU per apple. The inoculated apples were allowed to air dry for 5 to 10 min before inserting a stick and then being dipped into molten caramel held at 95°C *(10)*. When stored at 7°C, *L. monocytogenes* within the core of the apples experienced a 7-day lag period before growing to 3 log CFU during a 28-day period. When caramel apples were stored at 25°C, the internalized *L. monocytogenes* grew without a lag period, reaching 7 log CFU within a 2-day period. The results of the study were confirmed by Salazar et al. *(22),* who also reported growth of *L. monocytogenes* within the core of caramel apples. The main difference in the Salazar et al. *(22)* study compared with the Glass et al. *(10)* study was that the *L. monocytogenes* was stress adapted before being introduced onto and within the apples. On this occasion, caramel apples stored at 25°C exhibited a shorter (24-h) lag phase compared with 6 days at a 5°C storage temperature. In a similar manner, Gustafson and Ryser *(14)* also reported that *L. monocytogenes* introduced into the core of apples grew more rapidly when stored at 22°C compared

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with 4°C, although there was only a 1- to 2-day extension of the lag phase at the lower storage temperature. Based on the findings of the aforementioned studies, researchers recommended to store caramel apples under refrigerated conditions, despite only marginal differences in the lag period of the pathogen at the different temperatures. Clearly, a more effective strategy would be to decontaminate apples before going through the caramel apple–making process.

The traditional approach for decontaminating produce, such as apples, is to undertake a postharvest wash in a sanitizer solution *(12)*. However, postharvest washing can lead to cross-contamination between batches and potentially introduce pathogens into the core of the apple via infiltration of the stem scar tissue *(1, 13, 19)*. In addition, residual moisture on apples prevents adherence of the caramel layer, thereby leading to production problems *(14)*. Therefore, aqueous-free decontamination methods would be more compatible with caramel apple production.

The objectives of the current study were to develop and validate the efficacy of a sequential treatment based on gaseous ozone treatment, followed by an advanced oxidative process (AOP) for inactivating *L. monocytogenes* on both the surface of apples and in the internal tissue. Ozone was selected based on its high oxidation capacity, low by-product formation, and historical use as an antimicrobial gas *(8, 16)*. There have been previous studies that have demonstrated that ozone introduced into the atmosphere of storage rooms can reduce microbial loading on fruit *(27)*. However, ozone in storage rooms is applied at a low level (0.5 to 2 ppm) to prevent excessive corrosion of fittings and to reduce the hazard to workers *(11)*. Consequently, an extended exposure time is required to achieve microbial reductions, although the ability of the ozone to contact each individual apple within large bins represents a challenge *(6)*. Therefore, in the current study an alternative approach of introducing ozone into an airstream, which passes through the apple bed, was undertaken. The approach is referred to as forced air ozone and has the potential of delivering the gas at high concentrations homogenously through the apple bed, while minimizing its release into the working environment.

AOP is a generic term for reactions generating antimicrobial, albeit short-lived, hydroxyl radicals *(28)*. The radicals are formed by the degradation of ozone, hydrogen peroxide (H_2O_2), or both by UV-C light to form singlet oxygen or hydroxyl radical. The formed hydroxyl can interact with the target (i.e., microbial cell) or react to form antimicrobial by-products *(28)*.

The combination of ozone and hydrogen peroxide can also generate radicals in the absence of UV light. To date, AOP has primarily been applied to degrade organic compounds (e.g., pesticides) in wastewater and to decontaminate carton packaging *(3, 4, 20, 25, 26)*. An AOP-based process has also been described for decontaminating fresh produce *(15)*. Here, the combination of UV light and hydrogen peroxide was demonstrated to inactivate *Escherichia coli* and *Salmonella* introduced on the surface or subsurface of a range of produce types such as lettuce, cauliflower, and onion *(15)*. From optimization studies, the efficacy of the AOP-based treatment was dependent on the hydrogen peroxide concentration (1.5% [v/v]), the UV-C dose (37.8 mJ cm^2), and the operating temperature (48°C). Although effective, the system described by Hadjok et al. *(15)* was a batch process that was incompatible with continuous production. Therefore, in the current study a continuous AOP was developed for apple decontamination.

MATERIALS AND METHODS

Bacterial cultures and growth conditions. *L. monocytogenes* serotypes 4a, 4b, 1/2b, 1/2a, and 3a were obtained from the Public Health Agency of Canada (Guelph, Ontario). The isolates were preserved at –80°C and recovered on tryptic soy agar (Thermo Fisher, Whitby, Ontario, Canada). Each *L. monocytogenes* strain was cultivated individually in 50 mL of tryptic soy broth at 37°C for 24 h. Cells were harvested by centrifugation (Sorvall ST 8 benchtop centrifuge, Thermo Fisher) at 5,000 × *g* for 10 min, and the pellet was resuspended to a final optical density at 600 nm of 0.2 (ca. 8 log CFU/mL). Equal volumes of each suspension were then combined to form the inoculating cocktail that was held at 4°C for 48 h to allow for stress adaptation. A dilution series of the cocktail was prepared in saline and plated onto modified Oxford agar (MOx, Thermo Fisher) that was incubated at 30°C for 48 h.

Produce samples and inoculation. Nonwaxed Empire apples (with an approximate weight range of 105 to 114 g and a circumference range of 18 to 21 cm) were provided by Moyers Apple Products Ltd. (Lincoln, Ontario, Canada) and stored at 4°C until required. Only intact apples with no bruising or visible signs of spoilage were included in the study. Apples were spot inoculated with the *L. monocytogenes* cocktail around the equatorial surface of each fruit to give a final loading of 7 log CFU per apple. When *L. monocytogenes* was to be internalized into the core, 0.1 mL of the inoculum was dispensed onto the stem scar tissue and the apple was placed in a vacuum chamber. All inoculated apples were then subjected to two vacuum cycles (operating at 10^3 Pa) to facilitate internalization into the core *(15)*. The apples were then dried in a biosafety cabinet for 2 h before being tested with the different intervention methods.

Forced air ozone reactor. The reactor consisted of an ozone generator (ozone output of 6 g/h; Netech, Guangshou, People's Republic of China) that was positioned at the bottom or top of a 1.34-m^3 plywood (1.5-cm-thick) box lined with corrugated plastic (0.2 cm in thickness). Inoculated apples were placed in a perforated container (40 cm in depth, 29.5 cm in diameter) (Fig. 1). The ozone was pulled up or down through the apple bed, via internal and external fans blowing at a velocity of 9.3 m/s (Exitch AN100, FLIR Commercial Systems, Nashua, NH). The reactor was an enclosed system with the humidity being maintained above 85% via a setting on the humidifier unit (4.5-L capacity; Honeywell, Palatine, IL). The temperature, humidity, and ozone concentration were measured using an Aeroqual series 940 monitoring unit (Aeroqual, Auckland, New Zealand). The air was exhausted from the chamber via a fan and passed over four UV-C lamps (12 W, 254 nm) to decompose residual ozone after it left the reactor. For treatment runs, the apples were loaded into the perforated container and the door of the reactor was closed. Air was blown through the unit for 10 min before introducing the ozone, at which point the treatment time was started. The ozone concentration was monitored as it left the apple pile but before entering the UV chamber. At the end of the treatment, the ozone unit was switched off and the air was allowed to flow through the bed to vent the system.

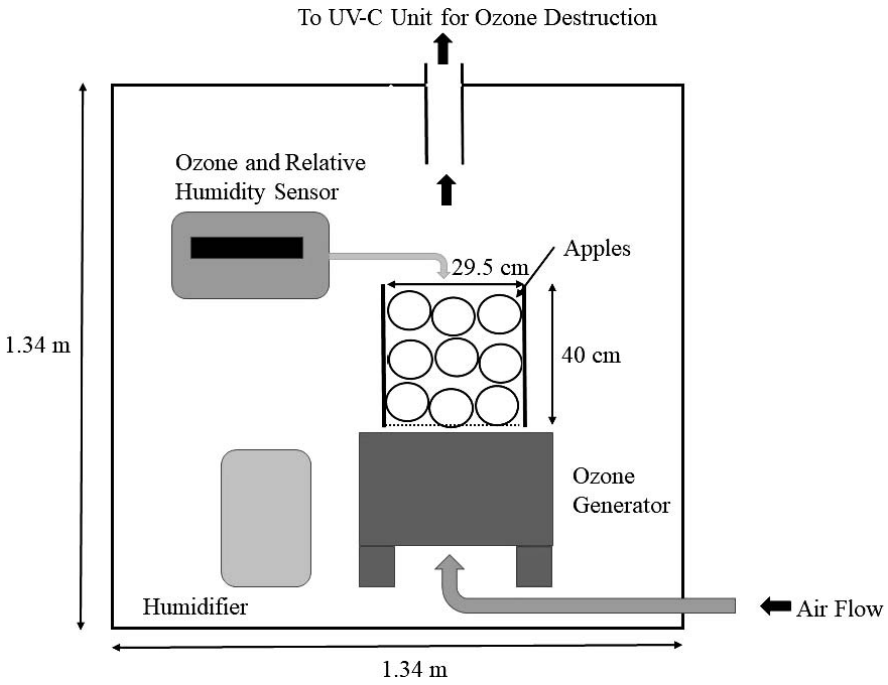

FIGURE 1. *Forced air ozone reactor used to treat Empire apples inoculated with* Listeria monocytogenes. *The reactor consisted of an ozone unit that was placed above or below the perforated container holding the apples. The excess ozone was drawn into the UV unit to degrade residual concentrations of the antimicrobial gas. The unit was contained within a plastic-lined sealable box, and the internal humidity was maintained via a humidifier. The relative humidity and ozone concentration were monitored and recorded.*

AOP. The AOP reactor consisted of a UV fixture (SaniRay lightbox, Atlantic Ultraviolet Corporation, Hauppauge, NY) containing three 25-W (254-nm), 54-cm-long UV-C lamps (Atlantic Ultraviolet Corporation) that were placed 16 cm above a rolling conveyer onto which the sample passed (Fig. 2). The UV-C fluence at the point the sample passed was measured using a UVX radiometer (UVP, Upland, CA). On occasions when ozone was to be included, a 185-nm-emitting, 26-W lamp with an estimated ozone output of 2 g/h (Atlantic Ultraviolet Corporation) was placed in between two of the UV-C lamps.

The hydrogen peroxide (30% [v/v]; Sigma-Aldrich, Oakville, Ontario, Canada) was diluted with distilled water to the required working concentration (1 to 6% [v/v]). The hydrogen peroxide was prewarmed to 48°C before being added to the vaporizing unit (Honeywell). The temperature within the reactor space was maintained at 48°C by a fan heater that also functioned to distribute the peroxide vapor within the chamber (Fig. 2).

Inoculated apple samples were placed on a plastic tray with the stem scar tissue facing the UV lamps. The tray was transferred to the center of the reactor and removed when the treatment time had elapsed.

Preparation of caramel apples. Wooden sticks (15 cm in length, 0.5 cm in diameter) were inserted into the midway point of the apples (both the treated and the control groups), through the stem scar tissue. The apples were then dipped into caramel (Gold Medal Minneapolis, Burnsville, MN) that was maintained at 80°C per commercial practice. The caramel coating was allowed to harden before the apples were dipped into molten chocolate. The caramel apples were then transferred to an incubator maintained at 22°C. On storage days 0, 2, 5, 7, 12, and 19, caramel apples ($n = 3$) were removed to determine *L. monocytogenes* levels.

Recovery and enumeration of *L. monocytogenes* from apples. *L. monocytogenes* was recovered from apples, including those that had been caramel coated, by submerging them individually into 100 mL of One-Step *Listeria* Enrichment media (Oxoid, Basingstoke, UK) and then manually massaging them for 60 s to release the surface bacteria. An apple was then taken out and the core (containing the internalized *L. monocytogenes*) was removed using a sterile corer that was sterilized between uses by flaming in alcohol. The core was transferred to a sterile stomacher bag placed in One-Step Enrichment media to a ratio of 1:10. Both the core sample and surface rinse were homogenized with stomachers (Seaward, London, UK) for 60 s. A dilution series was prepared in saline and plated onto MOx agar that was then incubated at 30°C for 48 h. In parallel, the homogenates were enriched at 37°C for 24 h and then streaked onto MOx agar that was incubated at 30°C for 48 h. A presumptive-positive colony from each plate was subjected to confirmation using API test strips (bioMérieux, Inc., Laval, Quebec, Canada).

Statistical analysis. Triplicate samples were tested for each treatment variable and, unless otherwise stated, at least two independent validation trials were performed. In storage trials, each data point represents the average of three samples with caramel apples being withdrawn over a 19-day period. The bacterial counts were transformed into log values, with statistical differences between means performed using analysis of variance in combination with the Tukey test (SPSS, IBM, Armonk, NY). Enrichment was undertaken on samples that did not return countable colonies on plates, with the lower detection limit being designated for a positive sample and zero being assigned for a negative sample.

RESULTS

Inoculation and recovery of *L. monocytogenes* from apples. When *L. monocytogenes* was inoculated onto the surface of apples and left to dry over a 24-h period, there was a significant reduction of 3 to 4 log CFU of the original inoculum (7 log CFU). Therefore, the wait period between inoculating the apples to treatment with ozone or AOP was limited to 2 h. By using this inoculation regime, it was possible to recover more than 10% of the *L. monocytogenes* that had been spot inoculated onto the apple surface. The vacuum infiltration method used to introduce *L. monocytogenes* into the core yielded a recovery of 4.11 to 5.03 log

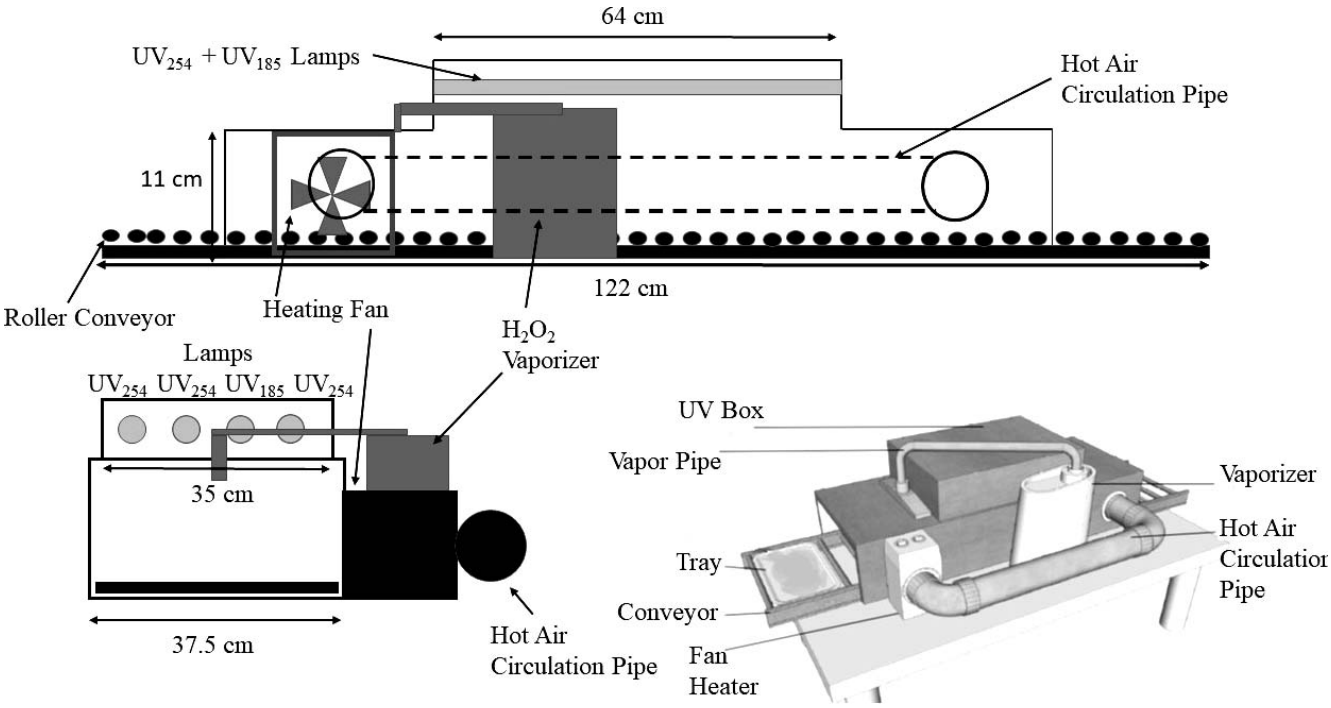

FIGURE 2. *Advanced oxidative process reactor used to treat surface- and vacuum-infiltrated* Listeria monocytogenes*–inoculated apples. The unit consisted of a UV lightbox that housed three UV-C lamps (254 nm) and an ozone-generating lamp (185 nm). The hydrogen peroxide vapor was introduced into the headspace of the reactor. The sample was placed on a tray and then treated for the designated time before removing.*

CFU, verifying that the method was sufficient to use for validating the AOP.

Forced air ozone reactor. Inoculated apples, held at 4°C, were transferred to a perforated container (with a depth of ca. 30 cm) within the reactor, and ozone gas was introduced at the top or bottom of the container. The relative humidity was provided by the condensation on the apple surfaces (due to the change in temperature after leaving the 4°C environment) and a humidifier that maintained the humidity within the reactor at >85% (Fig. 1). The ozone concentration at the outlet (after apple bed but before passing though the UV lights) increased to 23 ppm with a 60-s lag period and a 10-min rise period (Fig. 3). The ozone concentration stabilized with no significant change in concentration over 40 min (Fig. 3).

It was found that the log CFU count reductions supported by a 20-min ozone treatment varied between 2.12 and 3.07 log CFU per apple, leaving residual populations of 4.26 to 5.21 log CFU per apple (Table 1). The log reductions achieved were independent of the ozone being introduced at the top or the bottom of the container, in addition to the placement of fruit within the pile (Table 1). Increasing the treatment time to 40 min did not significantly ($P > 0.05$) increase the log count reduction of *L. monocytogenes* on the surface of apples compared with the 20-min exposure (data not shown).

AOP for inactivation of *L. monocytogenes* on the surface and within apples. UV light applied alone (54 mJ cm^2) to inoculated whole apples resulted in a 0.86 ± 0.62-log CFU reduction of *L. monocytogenes* on the surface but no decrease within the core. However, when UV light was combined with hydrogen peroxide, a concentration-dependent increase in the inactivation of *L. monocytogenes* was recorded (Fig. 4). The log CFU reduction of *L. monocytogenes* on the surface when water vapor (i.e., 0% H_2O_2) was substituted for hydrogen peroxide was significantly ($P < 0.05$) lower compared with when H_2O_2 was applied during UV treatment (Fig. 4). However, there was no significant ($P > 0.05$) increase in the efficacy of UV–hydrogen peroxide when 2% (v/v) H_2O_2 was used compared with 6% (v/v) H_2O_2 (Fig. 4).

L. monocytogenes that had been vacuum infiltrated into the stem scar tissue of apples decreased by 1.15 log CFU when UV was combined with 6% (v/v) hydrogen peroxide (Fig. 4). In comparison, with concentrations of hydrogen peroxide <4% (v/v), significantly ($P < 0.05$) lower decreases of *L. monocytogenes* within the core were observed (Fig. 4).

To enhance the AOP, additional trials were undertaken by inclusion of an ozone-generating lamp within the reactor. In the absence of hydrogen peroxide, UV-C light and ozone supported an approximately 1-log CFU reduction of *L. monocytogenes* on the surface and subsurface of inoculated apples for a 30-s treatment (Table 2). The extent of *L. monocytogenes* inactivation within the core significantly increased with exposure time, with treatments in the order of 90 to 120 s being significantly ($P < 0.05$) more effective compared with 30 s. A treatment time of 120 s of the UV–hydrogen peroxide treatment supported a log CFU reduction of *L. monocytogenes* within the core, although it was not

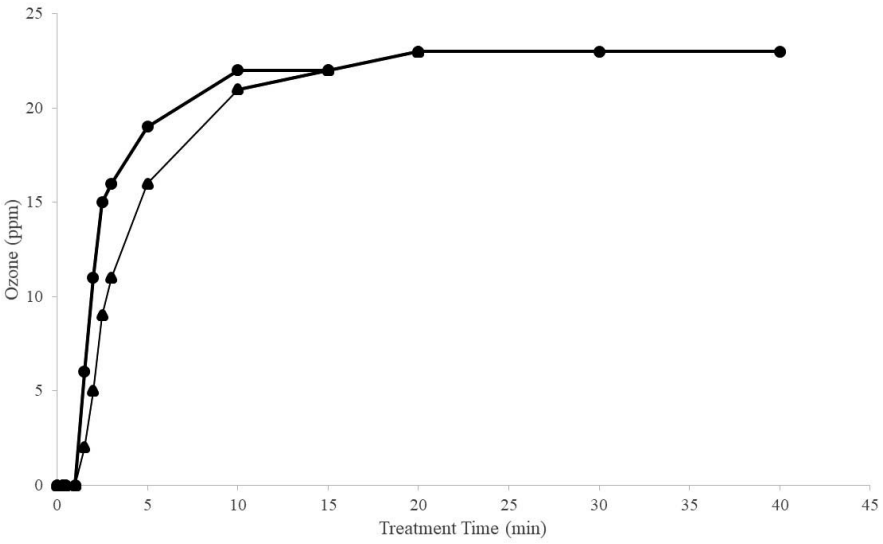

FIGURE 3. *Typical ozone concentration profile at the point exiting the apple bed but before passing through UV-C lights. Apples were loaded into the reactor's holding container to a depth of ca. 30 cm. Air was blown through the apple bed for 10 min before introducing ozone at which point the unit was switched on and run for 20 min (▲) or 40 min (●).*

significantly different compared with when UV-ozone was applied alone (Table 2).

The AOP combining UV-C light, ozone, and hydrogen peroxide supported significantly ($P < 0.05$) higher log reductions of *L. monocytogenes* on the surface of apples compared with when H_2O_2 was omitted (Table 2). The efficacy of the combined treatment was independent of treatment time, with no significant ($P > 0.05$) difference between the *L. monocytogenes* log CFU count reductions obtained at 30 s compared with at 120 s (Table 2).

By using the three agents in combination, a more rapid inactivation within the core was observed. Yet, increasing the treatment time to 120 s did not significantly ($P > 0.05$) increase the log CFU reductions obtained when UV–ozone–hydrogen peroxide was used in combination with residual *L. monocytogenes* being recovered (Table 2).

Inactivation of *L. monocytogenes* on and within apples by using a sequential ozone gas treatment followed by AOP before coating in caramel-chocolate. Empire apples were inoculated to give an *L. monocytogenes* loading of 5.10 ± 0.20 log CFU per apple on the surface and 4.20 ± 0.36 log CFU/g within the subsurface. The apples were subjected to an ozone treatment in the forced air reactor (20 min) followed by UV–hydrogen peroxide–ozone for 60 s before inserting sticks and then coating the apples with caramel. The combined processes of forced air ozone, AOP, and caramel coating decreased *L. monocytogenes* levels on the surface, with enrichments returning with negative results representing a 5-log CFU reduction (Table 3). The *L. monocytogenes* within the core samples was also reduced, with one sample testing positive by enrichment (Table 3).

In comparison, *L. monocytogenes* levels on the surface of control apples (i.e., apples not subjected to forced air ozone or AOP) decreased by 0.5 log CFU by the caramel-coating process. In the control group, *L. monocytogenes* levels increased within the core after a 5-day lag phase (Table 3). The levels attained after 19 days of storage at 22°C were significantly ($P < 0.05$) higher compared with the numbers on day 1. In contrast, *L. monocytogenes* on the apple surface remained unchanged throughout the 19-day storage period (Table 3). *L. monocytogenes* derived from decontaminated apples did not undergo repair and outgrowth, with sporadic samples testing positive by enrichment throughout the 19-day storage period (Table 3). In all cases, the caramel apples did not exhibit spoilage symptoms (browning or softening) during storage.

TABLE 1. *Log count reductions of* Listeria monocytogenes *inoculated onto apples and then treated for 20 min in the ozone reactor with the gas being introduced as an upward or downward airstream*[a]

Location of inoculated apple within bed[b]	*L. monocytogenes* (log CFU/apple)	
	Upward ozone airstream	Downward ozone airstream
Bottom	5.21 ± 0.94 A (2.12)	4.79 ± 0.37 A (2.54)
Middle	4.71 ± 0.80 A (2.62)	4.70 ± 0.96 A (2.63)
Top	4.78 ± 0.25 A (2.55)	4.26 ± 0.45 A (3.07)

[a] Values are the means ± standard deviations (log count reductions) of duplicate trials with three apples sampled per location within the apple bed. Means followed by the same letter are not significantly different. The initial cell density of inoculum introduced onto the apple was 7.33 ± 0.16 log CFU per apple.

[b] Location of the apples within the container placed in the forced air ozone reactor.

DISCUSSION

The study illustrated that by using a combination of a forced air ozone reactor with a sequential AOP treatment, it was possible to inactivate *L. monocytogenes* on and within apples. Additional inactivation of *L. monocytogenes* on the surface was supported by the molten caramel coating step.

Critically, only by using a combination of interventions could the inactivation of *L. monocytogenes* be achieved, as opposed to relying on a single process.

Although ozone gas has been evaluated for decontamination of produce, it has been restricted to being introduced at low concentrations into the headspace of storage rooms or at higher concentrations for short exposure times within

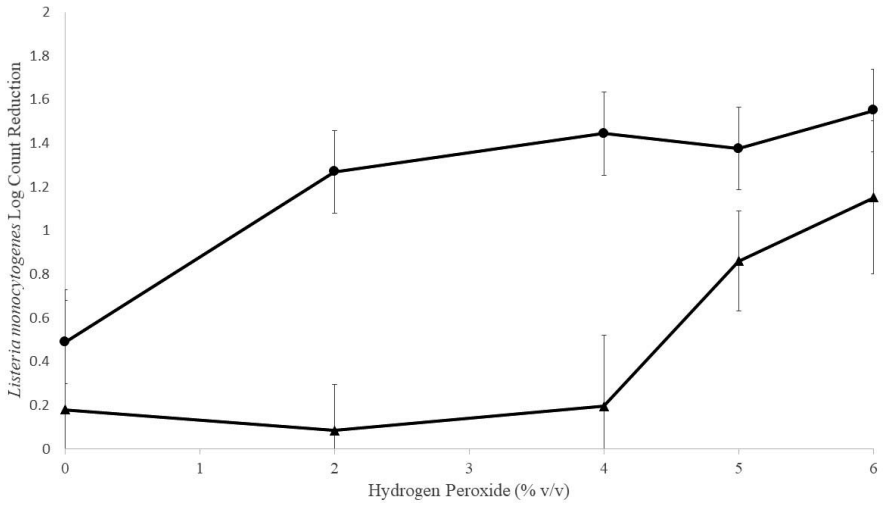

FIGURE 4. *Inactivation of* Listeria monocytogenes *on (●) and within (▲) apples by UV–water vapor (no hydrogen peroxide) or UV–hydrogen peroxide from 1 to 6% (v/v) applied in the reactor depicted in Figure 2. The initial loading of* L. monocytogenes *on the apple surface was 4.67 ± 0.53 log CFU per apple and the internal core was 5.03 ± 0.13 log CFU per apple. The apples were placed in the reactor chamber that operated at 48°C and were treated for 60 s. Upon completion of the treatment, the apples were removed and* L. monocytogenes *survivors were recovered.*

sealed chambers *(9, 11, 21, 24)*. For example, *L. monocytogenes* on blueberries was reduced by 2 to 3 log CFU when stored for 10 days under 90 to 95% relative humidity, with the ozone gas concentration being maintained at 4 ppm *(7)*. In another study, Akata et al. *(2)* described an ozone treatment whereby the gas (5.3 ppm) was introduced into a chamber and flowed over the sample (button mushrooms), with excess gas being bled through an outlet valve to avoid backpressure within the system. These authors reported a 1-log CFU reduction in *E. coli* O157:H7 and *Salmonella* levels with a 30-min treatment by applying 5.3 ppm of ozone, with *L. monocytogenes* requiring up to 40 min to achieve the equivalent level of inactivation. In this respect, the reactor applied in this study resulted in a 2- to 3-log CFU reduction within 20 min. This could have been attributed to the following: the more intimate contact between ozone and pathogen by virtue of gas flow within the apple bed, the relatively high ozone concentration (23 ppm), the maintenance of high relative humidity (>85%) that is required to increase the susceptibility of pathogens to the antimicrobial gas, or a combination *(7, 17)*. Comparing the antimicrobial action of ozone between studies should be done with caution, given the differences in concentration and method of ozone gas delivery, along with temperature and relative humidity control. Differences between strains, along with methodology to inoculate and recover pathogens, must also be considered. Nevertheless, the results of the current study illustrated that *L. monocytogenes* levels could be reduced within a shorter time compared with times for fumigation or modified storage atmosphere approaches.

It was noted in the current study that increasing the ozone contact time beyond 20 min (up to 40 min) did not result in a significant increase in log CFU count reductions of *L. monocytogenes* on apples. It is possible that surviving *L. monocytogenes* populations were located within clumps of cells or on subsurface structures of the apples. However, additional *L. monocytogenes* inactivation could be achieved by applying a sequential AOP-based method. The AOP, based on using a combination of UV–hydrogen peroxide, only resulted in a 1.6-log CFU reduction of surface populations of *L. monocytogenes* and a 1-log CFU reduction of internalized populations within the stem scar tissue. The level of reduction of internalized populations is comparable to that obtained in a previous study using the same UV–hydrogen peroxide combination *(15)*. However, in the current study, 6% (v/v) hydrogen peroxide was required, whereas in the previous study, 1.5% (v/v) was sufficient to support pathogen reduction. It was also noted that the log

TABLE 2. *Inactivation of* Listeria monocytogenes *on the surface or core of apples within an advanced oxidation process reactor generating hydroxyl radicals from UV-ozone alone or with hydrogen peroxide vapor at 48°C*[a]

Treatment time (s)[b]	Surface[c]		Core[d]	
	UV-ozone[e]	UV–ozone–hydrogen peroxide[f]	UV-ozone	UV–ozone–hydrogen peroxide
30	1.05 ± 0.05 A a	2.32 ± 0.31 AB b	1.00 ± 0.42 A a	2.95 ± 0.35 A b
60	1.90 ± 0.20 B a	2.28 ± 0.52 AB a	1.05 ± 0.25 A a	2.25 ± 0.21 B b
90	1.10 ± 0.15 A a	1.95 ± 0.41 A b	2.15 ± 0.16 B a	3.10 ± 0.35 A b
120	1.35 ± 0.25 A a	2.53 ± 0.28 B b	2.95 ± 0.18 C a	2.94 ± 0.20 A a

[a] Values are means ± standard deviations. Means followed by the same uppercase letters in the same column are not significantly different. Means with the surface or core category followed by the same lowercase letters within rows are not significantly different. The surface initial loading was 5.03 ± 0.13 CFU per apple, and the core initial loading was 4.67 ± 0.53 CFU per apple.
[b] Transition time to pass through the AOP reactor.
[c] *L. monocytogenes* inoculated onto and recovered from the surface of apples.
[d] *L. monocytogenes* vacuum infiltrated and recovered from the apple core.
[e] Treatment with UV (254 nm) and UV (185 nm) (ozone) lamps.
[f] Treatment with UV (254 nm), UV (185 nm) (ozone), and hydrogen peroxide (6% [v/v]) vapor.

TABLE 3. Listeria monocytogenes *counts on the surface and core of caramel apples prepared from treated or nontreated fruit and then stored at 22°C for up to 19 days*[a]

	L. monocytogenes, log CFU/apple (positive by enrichment/total tested)			
	Inoculated control caramel apples[c]		Treated caramel apples[d]	
Storage day[b]	Surface[e]	Core[f]	Surface	Core
1	4.60 ± 0.01 A	4.17 ± 0.60 A	0 (0/3)	0.67 ± 1.15 (1/3)
3	3.40 ± 0.10 B	3.73 ± 0.92 A	0 (0/3)	0.67 ± 1.15 (1/3)
5	4.68 ± 0.12 A	3.64 ± 0.38 A	0 (0/3)	0 (0/3)
8	4.33 ± 0.06 A	4.39 ± 0.16 A	0.50 ± 0.87 (1/3)	0.67 ± 1.15 (1/3)
13	4.51 ± 0.31 A	5.45 ± 0.73 AB	0.50 ± 0.87 (1/3)	1.33 ± 1.15 (2/3)
19	4.88 ± 0.09 A	6.31 ± 0.09 B	0 (0/3)	0 (0/3)

[a] Values are means ± standard deviations. Means followed by the same letter within columns are not significantly different.
[b] Caramel apples stored at 22°C with three units being withdrawn at each timed sampling point.
[c] Apples were inoculated, but not treated, using ozone or AOP before applying caramel-chocolate layer.
[d] Inoculated apples treated within a forced air ozone reactor for 20 min followed by AOP treatment (54 mJ/cm^2, UV, ozone, 6% [v/v] hydrogen peroxide, 48°C).
[e] *L. monocytogenes* recovered from the surface of caramel apples.
[f] *L. monocytogenes* recovered from the core of caramel apples.

reduction of surface counts of *L. monocytogenes* was significantly lower compared with those reported by Hadjok et al. *(15)* that were >3 log CFU for *E. coli* or *Salmonella*. Yet, it should be noted that there were differences between the studies. Specifically, neither *L. monocytogenes* nor apples were included in the study performed by Hadjok et al. *(15)*. More critically, the batch-based system had a greater proportion of hydrogen peroxide vapor within the headspace compared with the continuous process where H_2O_2 was introduced at the entry point into the unit. As a consequence, the hydroxyl radicals may not have contacted the entire surface of apple and may have been primarily restricted to top of the fruit and stem scar tissue. Yet, the inclusion of ozone-generating lamps with UV-C light could substitute for hydrogen peroxide, although synergistic antilisterial activity was observed when all three agents—UV-C light, ozone, and hydrogen peroxide—were used in combination. The increase in the reduction of *L. monocytogenes* on or within the apples can be attributed to a greater volume and oxidative power of hydroxyl radicals generated. It is thought that by using the combination of UV-C light, ozone, and hydrogen peroxide, a stronger oxidation power is obtained that increases the reaction rates with organics, compared with UV–hydrogen peroxide *(20)*. Both attributes could explain the enhanced lethality of the inclusion of ozone with UV light and hydrogen peroxide over the UV–hydrogen peroxide system.

The final intervention was the caramel coating that, in principle, would be expected to inactivate *L. monocytogenes* on the apple surface. However, in agreement with the findings of other studies, the caramel applied at 80°C only supported a negligible reduction of *L. monocytogenes (10, 14, 22)*. It is possible to achieve higher log reductions by increasing the temperature of the molten caramel to >82°C *(14)*; however, the functionality and quality would likely be negatively affected at higher coating temperatures.

In agreement with other studies, in nondecontaminated control caramel apples, no *L. monocytogenes* growth occurred on the interface between the caramel and the fruit surface *(10, 22)*. Growth was observed within the core of caramel Empire apples, despite the exudate juice being at a pH of 3.22. In this regard, the exposure to the acidity of the apple, along with stress imparted by the ozone and AOP treatments, could be the reason for the lack of outgrowth of *L. monocytogenes* during storage.

It was evident that applying the treatments individually resulted in residual population reductions, but the treatments were more effective when used sequentially. It is becoming established that the reduction of pathogens, such as *L. monocytogenes,* is greater when sequential antimicrobial treatments are delivered compared with individual treatments *(18, 23)*. Although it has not been studied in depth, the increased lethality of sequential treatments is thought to be due to the cells becoming more susceptible to stress or having different modes of inactivation, thereby having different targets. In the current study, both the forced air ozone treatment and AOP-based interventions were oxidative, although DNA damage via UV-C exposure could not be discounted. Also, decreasing the *L. monocytogenes* numbers by using the forced air ozone treatment led to less of a bioburden for the AOP and caramel-coating steps to inactivate.

In commercial practice, the forced air ozone treatment would be applied to treat apples from cold storage, thereby taking advantage of the high relative humidity caused by the formation of condensate on the fruit surface. The AOP method was primarily designed to decontaminate the scar tissue of the apples before inserting the sticks, before going onto the caramel-coating process. It is conceivable that the order in which the interventions were performed or the use of additional hurdles (e.g., sanitizer dip) could have further increased the log reduction of *L. monocytogenes* achieved. This aspect of the hurdle concept should be explored in future studies in relation to decontaminating apples and also for fresh produce in general.

In conclusion, this study has demonstrated that a caramel apple–making process based on forced air ozone followed by AOP interventions can be used to reduce *L. monocytogenes* on the surface and scar tissue of apples. By introducing ozone into an airstream that flowed through the apple bed, it was possible to achieve a homogenous gas distribution, although residual survivors remained. The continuous AOP intervention could reduce internal populations, especially when UV-C light, ozone, and hydrogen peroxide are used in combination. The caramel-coating process further contributed to decreasing surface *L. monocytogenes* levels. When the treatments were applied in sequence, low residual levels were encountered on the surface and internal tissue of caramel apples, but they did not undergo outgrowth during a 19-day storage period at 22°C. The current study focused on caramel Empire apples, and it is possible that the efficacy of treatments may be influenced by varietal differences. Therefore, future studies should verify the decontamination efficacy of the dual treatments against *L. monocytogenes* introduced onto other apples commonly used for candy and caramel apple production. It is anticipated that the same treatments could be extended to other fresh produce, thereby providing supplemental or alternative interventions to postharvest washing.

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