Inactivation of *Listeria innocua*, *Salmonella Typhimurium*, and *Escherichia coli* O157:H7 on Surface and Stem Scar Areas of Tomatoes Using In-Package Ozonation†

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ABSTRACT

A novel in-package ozonation device was evaluated for its efficacy in inactivating three microorganisms (viz., *Listeria innocua*, attenuated *Salmonella Typhimurium*, and *Escherichia coli* O157:H7) on tomatoes and for its effect on fruit quality. The device produced ozone inside sealed film bags, reaching a concentration of 1,000 ppm within 1 min of activation. The three bacterial cultures were inoculated onto either the smooth surface or the stem scar areas of the tomatoes, which were then sealed in plastic film bags and subjected to in-package ozonation. *L. innocua* on tomatoes was reduced to nondetectable levels within 40 s of treatment on the tomato surface, with inactivation of ca. 4 log CFU per fruit on the stem scar area. An increase in treatment time did not result in a proportional increase in bacterial reduction. For *E. coli* O157:H7 and *Salmonella*, there was little difference (≤1 log) in the effectiveness of the system when comparing surface and scar-inoculated bacteria. Both bacteria were typically reduced by 2 to 3 log CFU per fruit after 2- to 3-min treatments. No negative effects on fruit color or texture were observed during a 22-day posttreatment storage study of ozone-treated tomatoes. These results suggest that the three bacteria responded differently to ozonation and that in-package ozonation may provide an alternative to chemical sanitizers commonly used by the industry.

The microbial safety of fresh fruits and vegetables continues to be a major concern; recalls of fresh produce and outbreaks of foodborne illness associated with their consumption occur every year. Fruits and vegetables frequently implicated in outbreaks include leafy greens, tomatoes, and melons (9). Currently, the produce industry relies on washes with chemical sanitizers such as chlorine to reduce the risk of pathogen contamination; however, aqueous chemical sanitizers have very limited effectiveness (1, 20). Pathogens on the surfaces of fresh fruits and vegetables may reside in protected sites such as crevices, stomata, or cracks that aqueous sanitizers cannot reach; therefore, nonaqueous antimicrobials may be an option to enhance the microbial safety of fresh produce such as tomatoes. Gaseous antimicrobials are attractive for use on fresh produce because they tend to dissolve in wound sites on fruits and vegetables, and microbes hiding in those areas are likely to be inactivated.

An expert panel declared ozone a generally recognized as safe substance for use in food processing (8), which has encouraged broader use of this gas by the food industry. Ozone was approved by the U.S. Food and Drug Administration for the treatment of raw agricultural commodities such as fresh fruits and vegetables (6). The biocidal effect of ozone is accomplished by a combination of high oxidation potential reacting with organic material up to 3,000 times faster than chlorine (5), as well as the ability of ozone to diffuse through biological cell membranes. Ozone gas has been applied to several fresh fruits and vegetables and was effective in inactivating *Salmonella* spp. and *Escherichia coli* O157:H7 on blueberries (2), tomatoes (4), cantaloupe (23), and spinach (13). Yousef et al. (29) reported that inactivation of microflora on food by ozone depends greatly on the nature and composition of food surfaces, the types of microbial contaminants and initial bacterial load, and the degree of attachment or association of microorganisms with a food. Bialka and Demirci (2) found that a 5% ozone treatment for 64 min reduced cocktails of *Salmonella* spp. and *E. coli* O157:H7 on blueberries by 3.0 and 2.2 log CFU/g, respectively. Han et al. (11) reported that more than a 5-log reduction of *E. coli* O157:H7 occurred on green peppers after treatment with 7 mg/liter ozone for 20 and 40 min at 22°C. Similarly, Zhao and Cranston (31) reported that a 3- to 6-log reduction of *E. coli* O157:H7, *Salmonella*
spp., and *Staphylococcus aureus* on black pepper could be achieved by passing ozonized air (6.7 mg/liter at 6 liters/min) through black pepper for 60 min. Klockow and Keener (13) were able to generate ozone in plastic bags and reduce the *E. coli* O157:H7 population by 3 to 5 log CFU per leaf of cut spinach after a 5-min treatment and 24 h of subsequent storage. Almost all ozone technologies, except that described by Klockow and Keener (13), are currently designed to treat unpackaged foods. Schwabedissen et al. (22) developed an in-package ozonation technology in which ozone was generated inside packages by applying plasma-generating labels on both surfaces (inside and outside) of the package. The labels placed inside the packages support a dielectric barrier discharge that generates ozone from oxygen in the air in flexible or rigid packages when high voltage is applied to the outside labels. Compared with the technology used by Klockow and Keener (13), the in-package ozonation system by Schwabedissen et al. (22) used electricity-conductive labels and is much more flexible in terms of commercial applications and parameter changes. In addition to ozone being produced by the system, other oxygen species may be generated (22). The effects of this technology on inactivating foodborne pathogens have not been investigated.

The objectives of this present study were to (i) evaluate the formation of ozone in packages of cherry tomatoes, (ii) investigate efficacy of an in-package ozonation system in inactivating *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. innocua* inoculated on the smooth surface and stem scar area of tomatoes, and (iii) assess quality changes of tomatoes during post-ozone-treatment storage.

**MATERIALS AND METHODS**

**Preparation of tomatoes.** Cherry tomatoes were obtained from local supermarkets. Fruits were washed with 200 ppm of chlorine (pH 6.5) for 2 min, rinsed with water, and dried on paper towels for 2 to 3 h in a laminar hood at ambient temperature before being inoculated.

**In-package ozonation system and measurement of ozone.** The in-package ozonation system (PlasEt PS 500) and initial electron-conductive labels were provided by JE PlasmaConsult GmbH (Wuppertal, Germany). The system consisted of a power supply and treatment chamber (Fig. 1). Additional electron-conductive labels were purchased from Dausend and Steuernagel GmbH and Co. KG (Wuppertal, Germany). Ozone was generated from the labels placed inside film bags via dielectric barrier discharge. In addition to the ozone produced by the device, other reactive oxidative chemical species such as H₂O₂ (from water vapor), NO₂, and singlet oxygen may be produced (22). Tomatoes in a 24-oz (710-ml) rigid polymer tray (C24DER, Dart Container Corp., Mason, MI) were placed into polymer film bags (19.5 by 25 cm; PD961 EZ, Sealed Air, Duncan, ND) and sealed using an impulse heat sealer (MP-16, J. J. Elemer Corp., St. Louis, MO). The purpose of the trays was to support upright positioning of film bags to ensure that labels did not touch the fruits during treatment. The bags had three electron-conductive labels: one attached on the inside and two on the outside. Packages were placed in a treatment chamber, and electrodes from the power supply were applied to the outside labels, creating an ozone-generating dielectric barrier discharge from air within the package.

A single layer of tomatoes (approximately 200 g) was placed on a 24-oz tray and sealed in film bags (19.5 by 23 cm; PD961 EZ, Sealed Air). Packages were placed in the treatment chamber, and high voltage (5.85 kV) was applied for up to 10 min. Control film bags without fruit were also treated at the same settings. Ozone concentrations were periodically measured using an ozone detector tube (Gastec Corp., Ayase City, Japan) by withdrawing 50 ml of headspace atmosphere through the tube, using a syringe pump (Gastec Corp.). All experiments were conducted at ambient temperature (22 ± 2°C). To study the effect of voltage on ozone production, various voltages (5.85, 6.00, 6.30, and 6.60 kV) were applied to control packages without tomatoes, and ozone concentrations were measured after 1 min of treatment.

**Bacterial strains.** Three strains of *Listeria innocua* (ATCC 33090, 33091, and 51742) were obtained from the American Type Culture Collection (Manassas, VA). *L. innocua* was used as a nonpathogenic surrogate for *L. monocytogenes*. *L. innocua* and *L. monocytogenes* have demonstrated comparable inactivation kinetics in published experiments involving thermal, nonthermal, chemical, and ozone treatment (14, 16, 24, 25). *L. innocua* strains were incubated on tryptic soy agar (TSA; Difco, BD, Sparks, MD) at 37°C and stored on agar slants at 4°C prior to experiments.

Four strains of Shiga toxin–negative *E. coli* O157:H7 used in this study include 6980-2, 6982-2, CV267, and ATCC 700728. Strain 700728 (strain BDMS T4169) was purchased from ATCC. Ampicillin-resistant *E. coli* O157:H7 6980-2 (isolated from beef), 6982-2 (isolated from beef), and CV267/gfp (isolated from cattle) were graciously provided by Drs. Cathy Webb and Michael Doyle (University of Georgia, Center for Food Safety, Griffin). Ampicillin resistance of ATCC 700728 was induced by a CaCl₂ heat shock method (19).

Four strains of *Salmonella* Typhimurium were used in the study. Three attenuated strains of *Salmonella* Typhimurium (χ3985, χ4096, and χ8089) came from Dr. Roy Curtiss III (Center for Diseases and Vaccinology, Arizona State University, Tempe (3)). One strain of nonpathogenic *Salmonella* Typhimurium (ATCC 700720) was attained from ATCC. Bacterial identification was
confirmed using biochemical profiles based on *Salmonella* O antiserum poly A-I and Vi (Difco, BD), API 20E (bioMérieux Vitek, Hazelwood, MO), and Microgen GN-ID identification kit (Microgen Bioproducts Ltd., Camberley, UK). *Salmonella* strains were selected for nalidixic acid–resistant mutants by successive transfers into tryptic soy broth (TSB; Difco, BD) with increasing concentrations of nalidixic acid to a final concentration of 100 μg/ml over 10 days. Strains were incubated in TSB containing 100 μg/ml nalidixic acid at 37°C and maintained at 0 to 2°C until they were used.

**Bacterial inoculation.** Each bacterial isolate was grown for 16 to 18 h at 37°C with agitation (150 rpm) in 10 ml of TSB for *L. innocua*, in TSB supplemented with 100 μg/ml ampicillin for *E. coli*, and in TSB with 100 μg/ml nalidixic acid for *Salmonella* strains. Cultures were sedimented by centrifugation (5,000 × g for 10 min at 4°C), washed with 0.1% peptone water, and resuspended in 0.1% peptone water, resulting in cell populations of ca. 10⁹ CFU/ml. Equal volumes of bacterial cultures were combined to produce three- or four-strain composites. Multistrain composites (25 μl) of each genus were deposited on either a demarcated smooth-surface area ca. 0.5 cm in diameter or on the stem scars of tomatoes at ambient air temperature. Fifteen fruits were used for the smooth area inoculation, and 10 fruits were used for scar inoculation for each replicate experiment. After inoculation, tomatoes were dried in an aseptic, continuously circulating laminar flow hood for up to 3 h. Fruits were placed in a single layer on 24-oz plastic trays, sealed in film bags, and subjected to in-package ozonation treatments within 10 min of sealing (see below).

**Treatment with the in-package ozonation system.** Tomatoes (25 fruits placed in a single layer on a 24-oz plastic tray) were sealed within film bags (PD961 EZ, Sealed Air). The total weight for tomatoes in sealed plastic bags was between 180 and 230 g, depending on the fruit size, over an experimental period of >6 months. Film bags were affixed with labels on the inside and outside of the top portion of the bags. The film bags (19.5 by 23 cm) were placed into the treatment chamber, where electrodes were placed in contact with the labels. Samples were treated at ambient temperature for different times (up to 3 min) at 5.85 kV. Dried bacterial inoculation spots on tomatoes were generally positioned upward during the treatment. Two hours after treatment, fruits were removed from bags and the surviving bacteria were enumerated.

**Enumeration of bacteria.** The inoculated tomato skin and stem scars were excised from tomatoes using sterile scissors and placed in Whirl-Pak filter bags (Nasco, Ft. Atkinson, WI). Skin and stem scar weights were 4 to 6 g and 7 to 11 g, respectively. Peptone water (0.1%) was added to sample bags to bring the volume up to a total of 15 and 40 ml for the skin and stem scar samples, respectively. Stem scars were carefully macerated in the bag on the laboratory bench with a rubber mallet. Sample bags containing macerated stem scar and skin samples were then pummeled using a model 400 Stomacher (Seward Stomacher Lab System, London, UK) for 1 min at 260 rpm. Sample filtrates were serially diluted and spread plated onto plates of polyoxymyin, acriflavine, lithium chloride, cetazidime, aesculin, and mannitol agar for *L. innocua*, xylose lysine Tergitol 4 (XLT-4) agar with 100 μg/ml nalidixic acid for *Salmonella* strains, and sorbitol MacConkey agar with 100 μg/ml ampicillin for *E. coli* O157:H7; these were incubated at 37°C for 24 h (48 h for *L. innocua*). A thin agar layer method, in addition, was used to assess the injury of inoculated *Salmonella* strains and *E. coli* (27, 28). Briefly, bacteria recovered from tomatoes were inoculated onto a medium consisting of approxi-

![Ozone concentration in packages without (open circle) and with (closed circle) tomatoes as a function of treatment time. Data represent means of two independent replicate experimental trials. Curves were fitted using the exponential decay–exponential linear combination function of Sigmaplot version 11 (Systat Software, Inc., San Jose, CA).](image)

**RESULTS AND DISCUSSION**

**Ozone concentration.** The ozone concentration in the packages increased rapidly with increasing treatment time,
regardless of the presence of tomatoes (Fig. 2). One minute of treatment resulted in \(1,000\) ppm of ozone detected in the packages with or without tomatoes, while a 3-min treatment time yielded ozone concentrations of ca. \(1,500\) ppm. Maximum ozone concentrations were obtained after ca. 4 min of treatment. Following treatments, ozone concentrations decreased gradually over time. This concentration reduction may be due to increases in the temperature of ozone-generating labels during dielectric discharge; increasing temperatures are known to destabilize ozone. These levels decreased rapidly, reaching levels of only ca. 10 ppm within 60 min (Fig. 3). Two hours after treatment, ozone levels fell to 1 ppm. Ozone, in addition to thermal lability, may be destabilized by combining with itself or with \(\text{NO}_2\) produced during the treatment. Additionally, ozone is a strong oxidizer that is able to react with bacteria, food components, and even polymer films; it can also diffuse through the film and out of the food and the food packaging. The film used in this study has an oxygen transmission rate of 6,000 to 8,000 \(\text{ml/m}^2\text{/24 h, 1 atm (101 kPa)}\) at \(22^\circ\text{C}\). While the ozone permeability of the film is an unknown, it is possible that some ozone diffused out of the packages; nevertheless, ozone probably has a lower diffusion rate through the polymer films compared with oxygen, due to differences in molecular structure and size. Voltage has a significant effect on ozone concentrations (data not shown). Ozone concentrations in packages without tomatoes increased proportionally with increasing voltage. Ozone concentration in the packages treated at 6.0, 6.3, and 6.6 kV was 9, 39, and 175% higher, respectively, than in those treated at 5.85 kV.

**Bacterial reduction.** *L. innocua* inoculated on the smooth surface of the fruit was very sensitive to ozone treatments (Fig. 4). Increasing treatment time from 0 to 20 s decreased *L. innocua* populations by more than 4 log CFU per fruit. After 40 s of treatment, no *L. innocua* (detection limit 0.3 log CFU per fruit) was recovered. *L. innocua* inoculated onto the scar area of tomatoes also decreased rapidly during the first 20 s of treatment, achieving ca. a 3-log CFU per fruit reduction; however, a further 40 s of treatment yielded less than 1 additional log reduction. Populations of *L. innocua* did not decrease further when treatments were extended from 1 to 3 min.

The population of *Salmonella* strains was assessed using three media: TSA, XLT-4, and TSA/XLT-4 overlay. When *Salmonella* strains were inoculated on the smooth surface, populations were reduced by 2.3 to 3.0 log CFU per fruit after 1 to 3 min of treatment, as assessed on all three media (Table 1). The population of *Salmonella* strains on XLT-4 fell below 1 log CFU per fruit after ozone treatment at all three treatment times (which was up to 1.5 log lower than populations recovered on TSA), indicating that some bacteria were injured by ozone treatments. The efficacy of the in-package ozonation technology in reducing *Salmonella* strains or in increasing the proportion of injured cells did not significantly increase \((P > 0.05)\) with increasing treatment time from 1 to 3 min.

*Salmonella* strains inoculated on tomato stem scars were reduced by 0.8 to 3.3 log CFU per fruit following 1 to...
TABLE 1. Effect of in-package ozonation on populations of Salmonella Typhimurium inoculated on the surface and stem scar area of tomatoes

<table>
<thead>
<tr>
<th>Treatment time (min)</th>
<th>TSA</th>
<th>XLT-4</th>
<th>TSA/XLT-4 overlay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface inoculated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4.33 ± 0.40 A</td>
<td>3.25 ± 0.46 A</td>
<td>4.00 ± 0.25 A</td>
</tr>
<tr>
<td>1</td>
<td>1.92 ± 0.64 B</td>
<td>0.45 ± 0.33 Bc</td>
<td>0.96 ± 0.55 B</td>
</tr>
<tr>
<td>2</td>
<td>2.02 ± 0.70 B</td>
<td>0.96 ± 0.21 B</td>
<td>1.26 ± 0.15 B</td>
</tr>
<tr>
<td>3</td>
<td>1.77 ± 0.27 B</td>
<td>0.24 ± 0.19 C</td>
<td>1.11 ± 0.21 B</td>
</tr>
<tr>
<td>Scar area inoculated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5.83 ± 0.19 A</td>
<td>5.04 ± 0.29 A</td>
<td>5.42 ± 0.20 A</td>
</tr>
<tr>
<td>1</td>
<td>4.75 ± 0.76 B</td>
<td>4.26 ± 0.37 B</td>
<td>4.61 ± 0.41 B</td>
</tr>
<tr>
<td>2</td>
<td>4.59 ± 0.80 B</td>
<td>1.76 ± 0.66 C</td>
<td>2.08 ± 0.74 C</td>
</tr>
<tr>
<td>3</td>
<td>3.18 ± 0.45 C</td>
<td>1.96 ± 0.38 C</td>
<td>2.58 ± 0.45 C</td>
</tr>
</tbody>
</table>

Inoculated fruits were treated with in-package ozonation for 0, 1, 2, and 3 min. Data represent means (log CFU per fruit) ± standard deviations (n = 4). Means with the same letter in the same column and within the same surface area inoculation location are not significantly different (P > 0.05).

3 min of ozonation. Treatment times of 2 and 3 min resulted in a greater inactivation (P < 0.05) of the bacteria than at 1 min when enumerated on XLT and overlay media, while only a 3-min treatment resulted in a greater reduction of Salmonella strains as assessed by TSA. Initial populations of Salmonella strains on the tomato scar area were higher than on the smooth surface, suggesting that Salmonella strains were able to survive better or penetrate into the scar tissue. Reduction of Salmonella strains by ozone on the scar area was not significantly different from that on the smooth surface (2.1 versus 2.7 log).

Populations of E. coli O157:H7 inoculated on the smooth surface of tomatoes were reduced by 1.8 to 2.6 log CFU per fruit after a 1- to 3-min treatment (Table 2). Pathogen reduction did not increase as treatment time increased from 1 to 3 min, indicating that there was a leveling-off effect, or diminishing returns for lethality, at times over 1 min. Populations of E. coli on the stem scars of tomatoes were reduced by 2.1 to 3.0 log CFU per fruit after 1 to 3 min of treatment; furthermore, similar to results on the smooth surface, increasing treatment times from 1 to 3 min did not increase the inactivation of E. coli O157:H7. Mean reductions of E. coli O157:H7 on the smooth surface and scar area of Salmonella were 2.2 and 2.5 log CFU per fruit, respectively, and higher reductions (P < 0.05) of E. coli were achieved on the scar area as compared with the smooth surface.

Inoculated pathogen populations on the stem scar areas were higher than on the smooth surface, in spite of the lower number of tomato samples (10 versus 15 fruits for the smooth surface), suggesting that bacteria did not survive as well on the smooth surface of tomatoes as on the scar area.

Changes in fruit quality. The firmness of tomato fruits was not affected by ozone treatment during 22 days of posttreatment storage at 22°C (Table 3). The only significant difference occurred on day 8, when fruit treated for 3 min required significantly higher maximum force than fruit treated for 1 min. Storage times also did not significantly affect firmness of the fruit.

Tomato color was also not consistently affected by ozone treatment (Table 3). Fruits treated for 3 min had significantly lower L* and a* values than the nontreated control (0 min) after 22 days of storage. No significant differences were found among any other treated and nontreated tomatoes (0 min) at any day of storage. Higher L* and a* values indicate that fruits treated with ozone for 3 min were lighter, with less redness than nontreated controls after 22 days of storage. Fruits treated with ozone always had higher (not always significantly) b* values than nontreated controls (0 min). Significant differences in b* values were found only between nontreated controls and those treated for 1 min on day 1, as well as between the control (0 min) and 3-min–treated fruits on day 8. Higher b* values indicated that the ozone-treated fruits were slightly more yellow than nontreated controls. During the first 8 days of storage, significant decreases in L*, a*, and b* values occurred. No consistent changes were observed for color parameters after subsequent storage. Due to tomato redness, no visual difference in color and appearance was noticed among the fruits during the storage.

Our results demonstrate that L. innocua is more sensitive to ozone treatment than are the other two bacteria tested. L. innocua is a gram-positive bacterium, while Salmonella strains and E. coli O157:H7 are gram-negative bacteria. It is unclear why the same ozone treatments reduced populations of L. innocua more than populations of E. coli and Salmonella strains. The nature of the cell wall, which is more prominent in gram-positive bacteria, perhaps caused L. innocua to be more susceptible to ozonation. Restaino and others (17) concluded that gram-negative bacteria were substantially more sensitive to ozonated water than were the gram-positive bacteria; nevertheless, our
results reveal that, among the three bacteria tested, *L. innocua* is the most sensitive to gaseous ozonation, in agreement with the conclusion of Kim and Yousef (12), who reported that *L. monocytogenes* was more sensitive than *E. coli* to ozone. Disparity in our findings versus those of Restaino et al. (17) might be due to differences in methodology (e.g., aqueous versus atmospheric application of ozone). Further, Robbins et al. (18) found that planktonic cells of *L. monocytogenes* were completely destroyed by exposure to aqueous 0.25 ppm of ozone (8.29 log CFU/ml reduction) in bacterial suspension.

Our results indicate that bacteria were rapidly inactivated during the first minute of treatment; however, as treatment time further increased, reduction of bacterial population decreased. A shielding effect has been used to explain a similar phenomenon observed in aqueous systems (7, 12). It has been hypothesized that, as ozone concentration increases and the population of surviving bacteria decreases, cell lysis of bacteria may occur. Products of cell lysis may compete with living bacteria for ozone, thereby reducing the molecular oxidative products and subsequently shielding the remaining viable organisms. The stem scar area of tomatoes has been identified as an important source of enteric pathogen contamination due to its highly porous nature as well as the inability of sanitizers to effectively penetrate into this area (10). Furthermore, bacteria inoculated into stem scar or wounds of tomatoes appear to have a greater capacity for survival and/or growth than those on the smooth surface of fruits (15, 26, 30). Studies conducted by Wei et al. (26) and Zhuang et al. (32) suggested that 2-min chlorine (100 and 320 ppm) rinses did not completely inactivate *Salmonella* spp. on the surface of tomatoes. Our results suggest that in-package ozonation technology may provide an alternative to chlorine, the most common chemical sanitizer used by the fresh produce industry.

Commercially, fresh and fresh-cut fruits and vegetables are often stored in film bags or rigid containers to create a modified atmosphere. Electrically conductive labels used for the in-package ozonation system can be applied to film bags and rigid containers, making the system applicable to packaging materials currently used by the industry. Commercial packaging of produce leads to stacking and physical contact of fruits, which may prevent uniform ozone distribution to all fruit surfaces. Vibrations or movement of fruits during or immediately after treatment may be used to facilitate the diffusion of ozone and consequent inactivation of microorganisms.

Earlier results have shown the effectiveness of ozone in reducing human pathogens on a number of fruits and vegetables (2, 11, 13, 23). Our present study demonstrates that this technology can be used to enhance the safety of tomatoes in a sealed container without adversely affecting quality parameters. In the present study, tomato fruits were treated with the in-package ozonation system at ca. 22°C. Commercial tomatoes are normally picked green and ripened with gaseous ethylene treatments at 20 to 22°C in a specially constructed room, often followed by repacking (21). The in-package ozonation lends itself to treatment immediately following the ripening step. Many other fresh fruits and vegetables are stored at lower temperatures.

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<table>
<thead>
<tr>
<th>Treatment time (min)</th>
<th>Day 1</th>
<th>Day 8</th>
<th>Day 15</th>
<th>Day 22</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum force (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.02 ± 0.15 AZ</td>
<td>0.99 ± 0.13 ABZ</td>
<td>1.03 ± 0.13 AZ</td>
<td>1.02 ± 0.16 AZ</td>
</tr>
<tr>
<td>1</td>
<td>1.03 ± 0.13 AZ</td>
<td>0.94 ± 0.14 BZ</td>
<td>0.99 ± 0.11 AZ</td>
<td>0.99 ± 0.14 AZ</td>
</tr>
<tr>
<td>2</td>
<td>1.00 ± 0.15 AZ</td>
<td>1.02 ± 0.12 ABZ</td>
<td>0.98 ± 0.13 AZ</td>
<td>0.99 ± 0.16 AZ</td>
</tr>
<tr>
<td>3</td>
<td>1.05 ± 0.09 AZ</td>
<td>1.04 ± 0.13 AZ</td>
<td>1.01 ± 0.15 AZ</td>
<td>1.01 ± 0.10 AZ</td>
</tr>
</tbody>
</table>

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*L*, *a*, *b* and texture were 38.9 ± 1.18, 26.6 ± 2.15, 21.2 ± 1.99, and 1.06 ± 0.12 kg, respectively, on the day (day 0) of treatment. The results represent means (log CFU per fruit) ± standard deviations (n = 4). Means with the same letter in the same column (a and b) and the same row (Z to X) are not significantly different (P > 0.05).
Further research is needed to study the effectiveness of in-package ozonation at increased voltage and lower temperatures. Ozone is more stable at lower temperatures, but its reactivity may be decreased under refrigeration. Studies are also needed to evaluate the effect of ozonation on atmospheric composition during posttreatment storage. During this time oxygen is converted to ozone; consequently, oxygen levels may decrease following treatment. Whether the change will be significant in terms of its impact on the physiology and organoleptic properties of fruits and vegetables needs to be investigated. Furthermore, bacterial populations in the present study were measured immediately (within 3 h) after in-package ozonation; thus, whether further reductions in populations occur during posttreatment storage should be addressed. Future studies will investigate the fate of bacteria during storage after ozonation.

Gaseous ozone is registered as a food contact surface sanitizer, approved for direct application to food products, and has been approved by organic certification and regulatory bodies to treat organic produce. Our results demonstrate that the in-package ozonation system is capable of producing high concentrations of ozone inside sealed packages within a very short time, and it reduces populations of *L. innocua*, *Salmonella Typhimurium*, and *E. coli O157:H7* on the smooth surface and stem scar area of tomatoes. This system is simple and flexible and may be used on film bags and rigid containers, serving as an alternative to aqueous chemical sanitizers for reducing microbial populations on tomatoes and, perhaps, other fruits and vegetables.

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