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Demonstrating the Potential of Industrial Scale In-Package Atmospheric Cold Plasma for Decontamination of Cherry Tomatoes

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ABSTRACT: Increasing process complexity and consumer demands for less processed goods has led to strong demand for new effective decontamination methods. Therefore, atmospheric cold plasma (ACP) technology is finding increasing attention in the food sector and has potential for diverse decontamination applications. The aim of this work was to investigate the antimicrobial efficacy of an open-air dielectric barrier discharge plasma reactor for the treatment of food products after packaging. The approach was tested under both static and continuous modes of treatment against pathogenic microorganisms *Escherichia coli* and *Listeria innocua* inoculated on whole fresh cherry tomatoes and against indigenous microflora of tomatoes. The impact of treatment on produce quality during extended shelf life was also evaluated. Greater than 5 log reductions of *E. coli* population density surface attached on tomatoes was achieved in 150 s of treatment generated during either static or continuous operational mode. *L. innocua* exhibited higher resistance, with a maximum of 3.5 log units reduction achieved. No significant difference in color, firmness, pH, or total soluble solids was observed between control and ACP-treated samples. Therefore, this work demonstrates that useful antimicrobial efficacy can be achieved in tandem with quality retention using a pilot scale system in which the mode of operation was optimized for cherry tomato as a representative fresh product.

KEY WORDS: DBD, cold plasma, antimicrobial efficacy, cherry tomatoes, quality retention

I. INTRODUCTION

Fresh fruits and vegetables are an important part of a healthy diet. Numerous *in vivo* and *in vitro* preclinical and clinical investigations on the beneficial influences of fresh foods have reported on reduced risks of cardiovascular disease, cancer, diabetes, metabolic disease, and Alzheimer's disease.[1–7] In particular, tomatoes and tomato-based products have potential in the prevention of diseases such as cancer, cataracts, and heart diseases due to components such as vitamins A and C, beta-carotene, lycopene, and other antioxi-

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dants.[8] To obtain maximum nutritional benefits from their consumption, fresh fruits and vegetables are eaten raw or minimally processed. However, various factors influence the quality of fresh produce, including bacterial contamination, which can occur during growing, harvesting, post-harvest processing, storage, or distribution. Once attached to the surface of fresh produce, it is difficult to remove pathogens simply by washing.[9,10]

Bacterial pathogens are considered the most important food safety issue for fresh produce, followed by foodborne viruses, bacterial toxins, pesticide residues, and mycotoxins.[11] In 2013, in the European Union, 6043 confirmed cases of verocytotoxigenic *Escherichia coli* infections resulting in 13 deaths were reported.[12]

Minimal processing of fruits and vegetables (washing, cutting, disinfecting, packaging, storage conditions) promotes a faster physiological deterioration, biochemical changes, and microbial degradation of the product, leading to a reduction in produce nutritional quality and shelf-life.[13–15] Therefore, to retain health benefits of bioactive compounds in fruit and vegetables, optimized minimal processing technologies that maintain nutritional quality while promoting microbiological safety are required.

Atmospheric cold plasma (ACP) technology can achieve enhanced gas-phase chemistry without increasing the gas temperature[16] and has demonstrated high efficiency in reducing bacterial pathogens associated with fresh fruit and vegetables while retaining quality attributes. We reported previously that short plasma treatments of 10, 60, and 120 s using a high-voltage dielectric-barrier discharge (DBD) system followed by 24 hours of post-treatment storage reduced populations of *Salmonella*, *E. coli*, and *Listeria monocytogenes* inoculated on cherry tomatoes to undetectable levels inside a sealed package and background populations were not detected after 120–300 s of treatment.[17] Misra et al.[18] demonstrated that 5 min of treatment with ACP was effective for reducing natural microflora of strawberries with 2 log reductions within 24 hours of post-ACP treatment recorded. This was achieved without inducing physiological (respiratory) stress or adversely affecting the color and firmness. Cold plasma contains ions, free electrons, UV light, and reactive species that cause damage to cell walls/membranes and (intra)cellular components of prokaryotic and eukaryotic organisms. The in-package plasma technology is a low-energy, water light, non-thermal, post-package treatment. Generating cold plasma discharges inside food packages can achieve useful antimicrobial effects on fresh produce, with the additional safety advantages of mitigating against recontamination and efficacy with the retention of bactericidal molecules in contact with the food surface.

A common approach employed across challenge studies with plasma decontamination systems reported in the literature is utilization of single units of produce. There is little information available on the antimicrobial efficacy of plasma for larger-scale or industrial operation. Therefore, the aim of this work was to evaluate the pilot-scale SAFEBAG plasma system under conditions more representative of industrial practices. The SAFEBAG prototype uses ACP for continuous in-package decontamination of fruits and vegetables. The system was tested under both static and continuous modes of treatment to represent fresh produce packages after sealing in the production line. The target microorganisms selected for this study were *E. coli* and *Listeria innocua* and

the produce considered included cherry tomatoes. Instrumental and sensory techniques were used to characterize the effects of the ACP treatment on the quality and nutritional profiles, including color, texture, pH, total soluble solids (TSS), and firmness, of plasma-treated tomatoes.

II. MATERIALS AND METHODS

A. Plasma System

The prototype SAFEBAG system (Fig. 1) is based in a DBD reactor operating in open air. It consists of 1-m-long electrodes and an adjustable discharge gap (up to 4.5 cm) allowing for several flexible packages (from 4 to 10, depending on the bag size) to be treated simultaneously in static mode or continuously; that is, when a conveyor belt carries the bags through the plasma discharge. The control panel allows for control of applied voltage (0–100 kV) and is provided with an interactive meter displaying readings of root mean square input voltage and discharge current. A Bergoz current probe placed in the circuit allows for readings of discharge current waveforms via connection of an oscilloscope to the Bayonet Neill–Concelman socket.

Two side-grip belts displace sealed bags filled with fresh-cut produce into the treatment zone, where they pass through the discharge gap. Use of high voltages facilitates

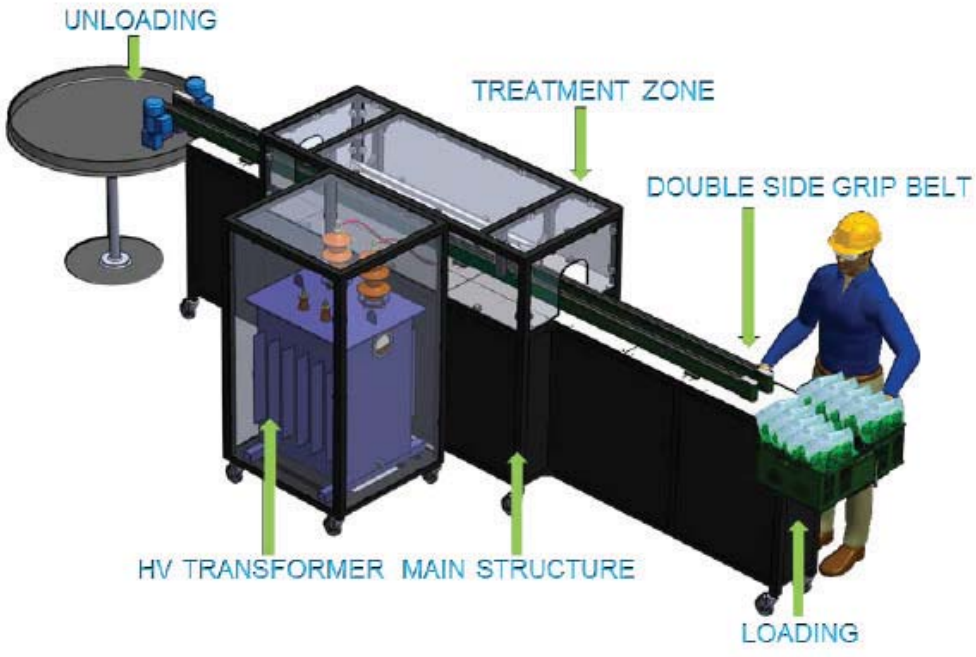

FIG. 1: Schematic diagram of SAFEBAG prototype

ionization of the gas contained within the package, resulting in the generation of significant amounts of reactive species that convey a bactericidal effect on the fresh product. The speed of the belts can control the duration of the treatment, after which the bags are released onto the unloading platform.

B. Produce Selection

Cherry tomatoes were used to evaluate the effect of ACP on microbiological quality and physical characteristics of produce treated using SAFEBAG. Whole fresh cherry tomatoes (class 1; origin: Spain) were purchased from the local supermarket and stored at 4°C until use. The tomatoes were 2 ± 0.5 cm in diameter and 10–15 g in weight. The same produce cultivar was used for each microbiological and quality experiment.

C. Experimental Design

To assess the effect of treatment on pathogen reduction in large samples, a series of eight inoculated samples of tomatoes (80–100 g) were sealed within polyethylene terephthalate bags (~25 × 25 cm). All samples were packaged using compressed air. Samples were treated immediately with 100 kV for 150 s in stationary mode or while in continuous movement on the conveyor belt. During continuous treatment, the gap was always filled with the bags containing tomatoes. For static treatment, up to two bags were used. All samples were subjected to a post-treatment storage time of 24 hours at 4°C. To assess the effect of treatment on the produce background microflora, uninoculated samples were subjected to the same treatment and post-treatment storage conditions. In order to evaluate any possible effect of the 24 hours storage on the bacterial growth, inoculated but untreated samples were stored for 24 hours under identical temperature conditions. In addition, an extended 7-day storage at 4°C was used for quality assessment after continuous processing of tomatoes. All experiments were performed in duplicate and replicated twice.

D. Microbiological Quality Analysis

1. Bacterial Strains and Inocula Preparation

Two challenge microorganisms were used: *E. coli* NCTC 12900 was obtained from the National Collection of Type Cultures of the Health Protection Agency (UK), and *L. innocua* NCTC 11299 was obtained from the microbiology stock culture of the School of Food Science and Environmental Health of the Dublin Institute of Technology (Ireland). The cells of overnight cultures were harvested by centrifugation, washed twice, and resuspended in sterile phosphate buffered solution (Oxoid Ltd, UK), resulting in the suspension with cell concentration of 8–9 $\log_{10}$ colony-forming units (CFUs)/mL, which was further used as the working inoculum. The concentration of inoculum was confirmed by plating appropriate dilutions on TSA, followed by incubation at 37°C for 24 hours for *E. coli* and 48 hours for *L. innocua*.

2. Preparation of Produce and Pathogen Inoculation

For inoculation purposes, tomatoes were placed with the blossom end down on sterile Petri dishes. The samples were spot-inoculated with bacteria using 50 µl of a culture. The droplets were deposited in several different locations, ensuring that the inoculum did not flow to the side of the samples. Inoculated samples were dried for 1 hour in laminar flow safety cabinet to allow the attachment of bacteria on the surface of produce before the ACP treatment.

3. Post-Treatment Microbiological Analysis

For microbiological analysis, inoculated untreated control samples (to estimate initial attached bacterial population), inoculated untreated samples stored for 24 hours (to assess the effect of storage on microbial growth), uninoculated untreated control samples (to determine initial background microflora), and either inoculated or uninoculated ACP treated samples were analyzed. The samples were transferred aseptically into sterile stomacher bags (BA6041, Seward Ltd, UK) containing 10 mL of sterile maximum recovery diluent (MRD) and hand rubbed for 2–3 min. The resulting suspension was diluted serially in MRD. Surviving *E. coli* and *L. innocua* populations were determined using the agar overlay method. Briefly, aliquots of an appropriate dilution were surface plated on TSA, incubated for 2–4 hours, and overlayed with the appropriate selective media: sorbitol MacConkey agar (Scharlau Chemie, Spain) supplemented with cefixime tellurite (Oxoid Ltd, UK) for *E. coli* and polymyxin-acriflavine-LiCl-ceftazidime-aesculin-mannitol (PALCAM, Scharlau Chemie, Spain) supplemented with PALCAM Listeria-Selective Supplement (Oxoid Ltd, UK) for *L. innocua*. Plates were then incubated for 24–48 hours at 37°C. Surviving background microflora of the uninoculated samples was evaluated using nonselective medium TSA for estimation of total aerobic mesophilic bacteria and potato dextrose agar (Scharlau Chemie, Spain) for estimation of yeasts and molds, with further incubation of agar plates at 37°C and 25°C for 48 hours and 5 days, respectively. The limit of detection for bacterial recovery on food samples was 1.0 $\log_{10}$ CFUs/sample.

E. Physico-Chemical Quality Analysis

1. Color Measurement

Color was quantified using an L^*-a^*-b^* colorimeter (Colour Quest XE Hunter Lab, Northants, UK) for control and treated cherry tomato samples. The color measurement was performed on each tomato (along four symmetrical sections) and average values reported. The instrument was calibrated using white ($L^* = 93.97$, $a^* = 0.88$ and $b^* = 1.21$) and green ($L^* = 56.23$, $a^* = 21.85$, $b^* = 8.31$) standard tiles. The hue angle was calculated as (1) $h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right)$ and chroma as (2) $C^* = \sqrt{(b^{*2} + a^{*2})}$.

2. pH Measurement

The pH of the cherry tomatoes was determined by using a hand-held pH meter with a spear electrode (Eutech Instruments, Thermo Fisher Scientific Inc., the Netherlands). The pH was measured for fresh tomatoes before packaging and for the control and treated groups on daily basis in triplicate for two tomatoes.

3. Firmness

The firmness of control and treated samples was analyzed using an Instron texture analyzer (Instron 4302 Universal Testing Machine, Canton, MA). The texturometer was mounted with a 500 N load cell and equipped with a 2 mm flat head stainless steel cylindrical probe that punctures the cherry tomato sample at a downloads speed of 200 mm/min and a distance of 20 mm. A single whole tomato was placed on the stage for each measurement. The maximum force (in Newtons) required to puncture the sample was used as an indication of firmness. Data were analyzed with Bluehill software. The firmness of three tomatoes from each package was measured individually and an average firmness value was reported. Experiments were conducted in duplicate.

4. TSS

TSS were measured using a hand-held refractometer (Bellingham and Stanley Ltd, UK). Refractive index was recorded and converted to °Brix. Measurements were performed at room temperature. Distilled water was used to clean the refractometer prism after each analysis.

F. Ozone Measurements

Ozone concentration inside the sealed package was measured using ozone detector tubes (18M, Gastec Corporation, Japan). Measurements were taken immediately after plasma treatment and after 24 hours of post-treatment storage.

G. Statistical Analysis

Statistical analysis was performed using SPSS version 21.0 (IBM). Changes in the selected physical and microbiological quality indices of tomatoes after ACP treatment were subjected to analysis of variance (ANOVA). Parameters of the untreated controls (0 and 24 hours) and ACP-treated samples were compared according to the method of Fisher's least significant difference at the 0.05 level.

III. RESULTS AND DISCUSSION

ACP technology is a relatively new approach aiming to improve microbiological safety in conjunction with maintenance of sensory attributes of the treated foods. In recent

years, ACP treatments of fresh fruits, vegetables, and other products have been the subject of much research demonstrating that this process may offer an effective alternative to conventional methods within food production settings. Key process advantages of ACP application include minimal water usage, broad-spectrum microbial control, lack of residue, reduction of operating costs (if an atmospheric air is used as a working gas), and the development of large-scale systems for continuous treatment of different produce commodities. This study examined industrial operation parameters, including static and continuous modes of ACP treatment generated by using high-voltage SAFEBAG prototype on a processing line of packaged cherry tomatoes in terms of microbiological safety and physico-chemical quality parameters.

A. Effect of ACP Treatment on Microbiological Quality of Tomatoes

The influence of either the static or continuous mode of ACP treatments on *E. coli* and *L. innocua* inoculated on cherry tomatoes are presented in Fig. 2A. The target cell characteristic appeared to be an important factor in achieving efficient produce decontamination using the SAFEBAG prototype system. It can be observed that *L. innocua* exhibited higher resistance to ACP treatment than *E. coli*. After 150 s of either static or continuous mode of ACP treatment, populations of *E. coli* were outside detection limits, with an average of 5 log reduction achieved, whereas *L. innocua* was reduced by a maximum 3.5 log units. In general, numerous comparative studies reported that Gram-positive bacteria were more resistant to ACP treatment than Gram-negative bacteria, which is explained by the thicker membrane of the Gram-positive bacteria that may present a barrier to the diffusion of plasma reactive species through the bacterial cell wall, thus impacting antimicrobial efficacy. Previous work demonstrated that Gram-negative *Salmonella* and *E. coli* were inactivated more rapidly on the surface of cherry tomatoes compared with Gram-positive *L. monocytogenes*. After 10 and 60 s of treatment, respectively, *Salmonella* and *E. coli* cells could not be recovered, whereas 120 s of treatment was required to inactivate populations of *L. monocytogenes*.[17] Similarly, Jayasena et al.,[19] investigating the effect of bacterial cell wall structure on inactivation activity of oxygen/nitrogen plasma generated on flexible thin-layer DBD, reported 2.04, 2.54, and 2.68 log CFUs/g in pork butt samples and 1.90, 2.57, and 2.58 $\log_{10}$ CFUs/g in bee-loin samples for *L. monocytogenes*, *E. coli*, and *Salmonella*, respectively, after 10 min of treatment, demonstrating higher reductions for Gram-negative pathogens. However, some studies have reported no clear patterns of sensitivity to plasma between Gram-positive and Gram-negative species. Min et al.[20] reported that 5 min of ACP treatment at 34.8 kV resulted in reduction of *E. coli*, *Salmonella*, and *L. monocytogenes* inoculated on lettuce by 1.1, 0.4, and 1.0 $\log_{10}$ CFUs/g, respectively. Higher initial attached population of *L. innocua* (~6.5 $\log_{10}$ CFUs/sample) cells in the current study could be another factor potentially contributing to lower inactivation levels for *L. innocua* compared with *E. coli* (average 5.2 $\log_{10}$ CFUs/sample). A number of studies have demonstrated clearly that initial microbial contamination levels play a crucial role in achieving effective decontamination of food surfaces by cold plasma technology.[21,22] In addition, in the current study, there

was an effect of operational mode on the efficacy of the system tested noted in the case of *L. innocua*. Although both static and continuous operation reduced cell populations significantly compared with the untreated controls ($p < 0.05$), continuous treatment appeared was more effective, reducing populations of *L. innocua* by 3.5, whereas, after static treatment, the number of cells was reduced by 2.3 $\log_{10}$ CFUs/sample.

Figure 2B presents the reduction of mesophilic bacteria and yeasts/molds on cherry tomatoes due to static and continuous ACP treatments. An average initial population of mesophiles and yeast/molds on cherry tomatoes was 5.7 $\log_{10}$ CFUs/sample. After 150 s of static ACP treatment, an average reduction of 3.5 $\log_{10}$ CFUs/sample of tomato spoilage microflora was recorded using the industrial prototype. Similarly, Baier et al.,[23] using indirect treatment generated on a microwave-driven air plasma torch, achieved reductions

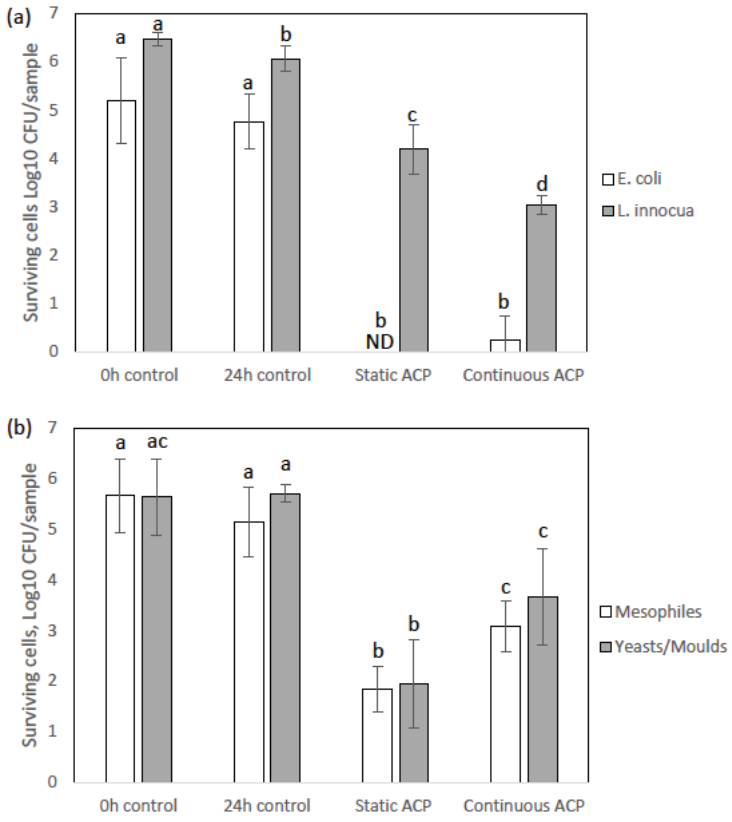

FIG. 2: Effect of either static or continuous mode of ACP operation on *E. coli* and *L. innocua* inoculated on cherry tomatoes (A) and on background microflora of cherry tomatoes (B). "ND" indicates not detected. Limit of detection: 1 log10 CFUs/sample. Vertical bars represent standard deviation. Different letters indicate a significant difference in population levels when mean values were compared within the same bacterial type.

of total mesophilic counts by 3.3 log cycles after 5 min of treatment and no CFUs were detected after 10 min of treatment. In contrast to pathogen inactivation, lower reduction rates were achieved with continuous treatment for the populations of mesophiles and yeast/molds, with reductions of 2.6 and 2.0 $\log_{10}$ CFUs/sample, respectively.

B. The Effect of ACP Treatment on Quality Characteristics of Tomatoes

1. Color

The effect of 150 s of static mode of ACP treatment for 150 s and 24 hours of storage post-treatment at 4°C on the color of cherry tomatoes is presented in Fig. 3 and the change in color of cherry tomatoes over 7 days of storage at 4°C after continuous mode treatment is depicted in Fig. 4. For both treatment types, there was no significant difference ($p \geq 0.05$) in the mean L*, a*, and b* values for fresh, control, and treated tomatoes at a 95% confidence level throughout the extended post-treatment storage. Other studies have also reported insignificant changes in the color of tomatoes subjected to ACP treatment.[21,23]

2. Firmness

Figure 5 represents the effect of static ACP treatment on the firmness of cherry tomatoes stored after treatment for 24 hours at 4°C. A slight loss in firmness of the cherry toma-

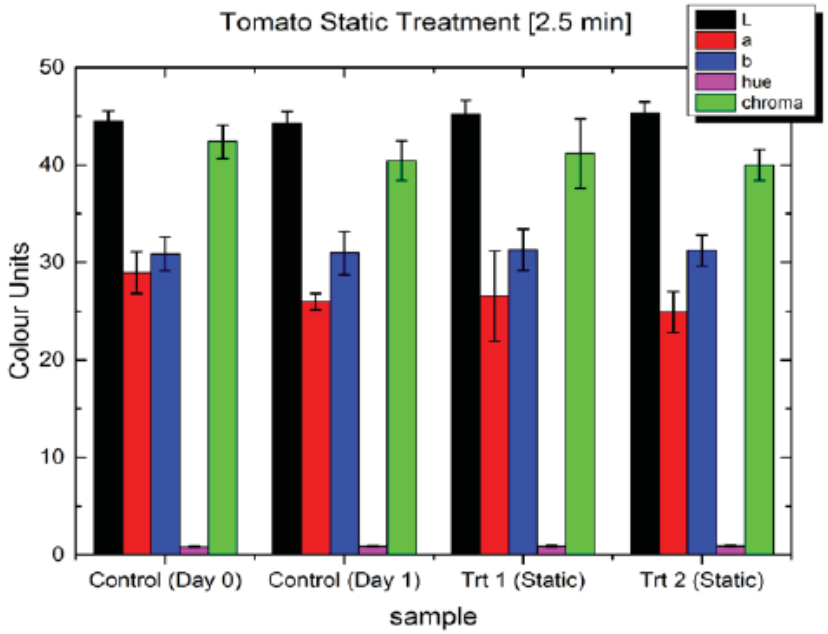

FIG. 3: Change in color of cherry tomatoes after treatments under static mode: Tr 1 and Tr 2 refer to two independent treatments.

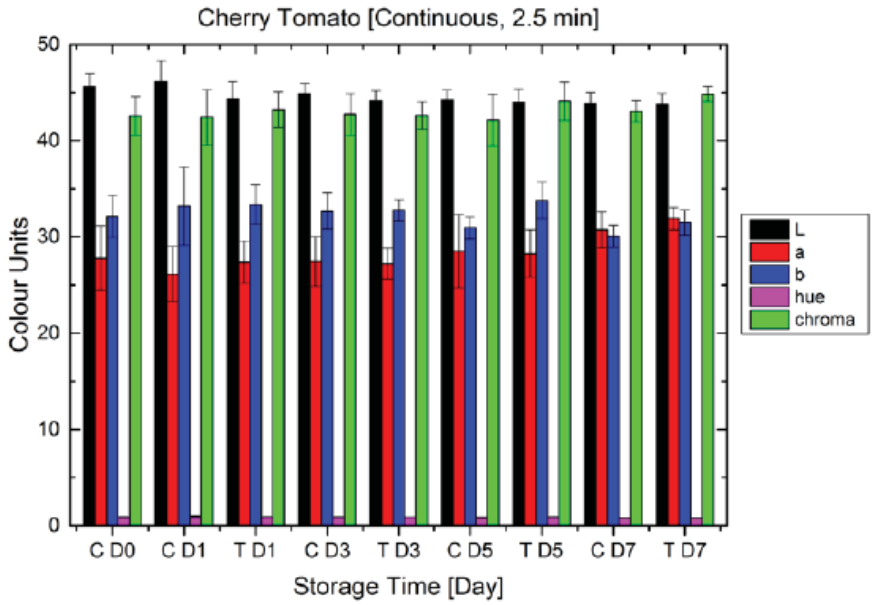

FIG. 4: Change in color of cherry tomatoes during storage after treatments under continuous mode

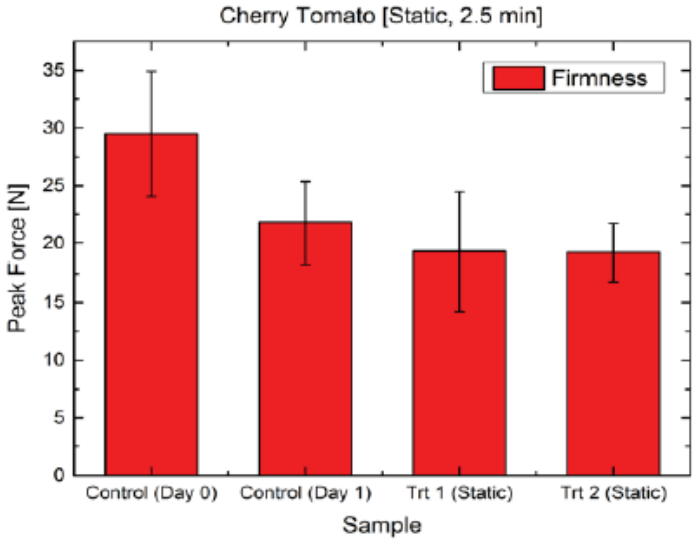

FIG. 5: Peak force required to puncture the control and treated (static mode) cherry tomatoes

toes was observed after 24 hours of storage for control samples. However, the difference between day 1 control and respective treated tomatoes was insignificant. This observation, deriving from pilot scale treatment, is consistent with our previous observations

on a laboratory scale device.[24] Good repeatability is noticeable from the independent repetition of the experiment. The storage study indicated that the firmness of cherry tomatoes decreased over time (Fig. 6). However, the difference between the firmness of the control and treated group of cherry tomatoes was insignificant on any day of storage. Again, this trend is in agreement with previous studies using a laboratory scale DBD plasma source.[18,24]

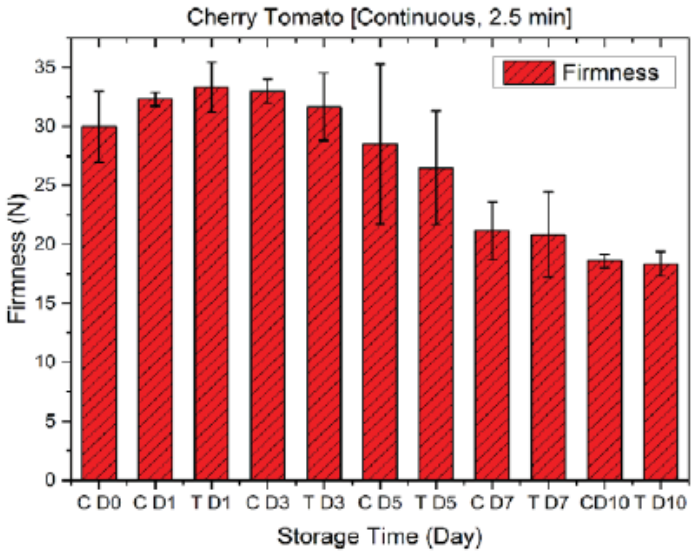

FIG. 6: Firmness of control and plasma-treated cherry tomatoes over a storage period of 10 days

3. pH and TSS

After 24 hours of storage (day 1), a significant change in pH or TSS of control samples was not observed (Table 1). The change in pH and TSS of the day 1 control and samples treated under static mode was also insignificant. In addition, there was no significant change in tomato pH and TSS (Figs. 7A and 7B, respectively) over extended storage as a result of continuous treatment.

TABLE 1: TSS and pH of control and treated (static mode) cherry tomatoes

	Parameters	
Sample	**pH**	**TSS**
Control (day 0)	4.13 ± 0.23	6.58 ± 0.49
Control (day 1)	4.34 ± 0.11	6.50 ± 0.50
Treatment 1 (static)	4.16 ± 0.14	6.33 ± 0.58
Treatment 2 (static)	4.39 ± 0.35	7.00 ± 0.00

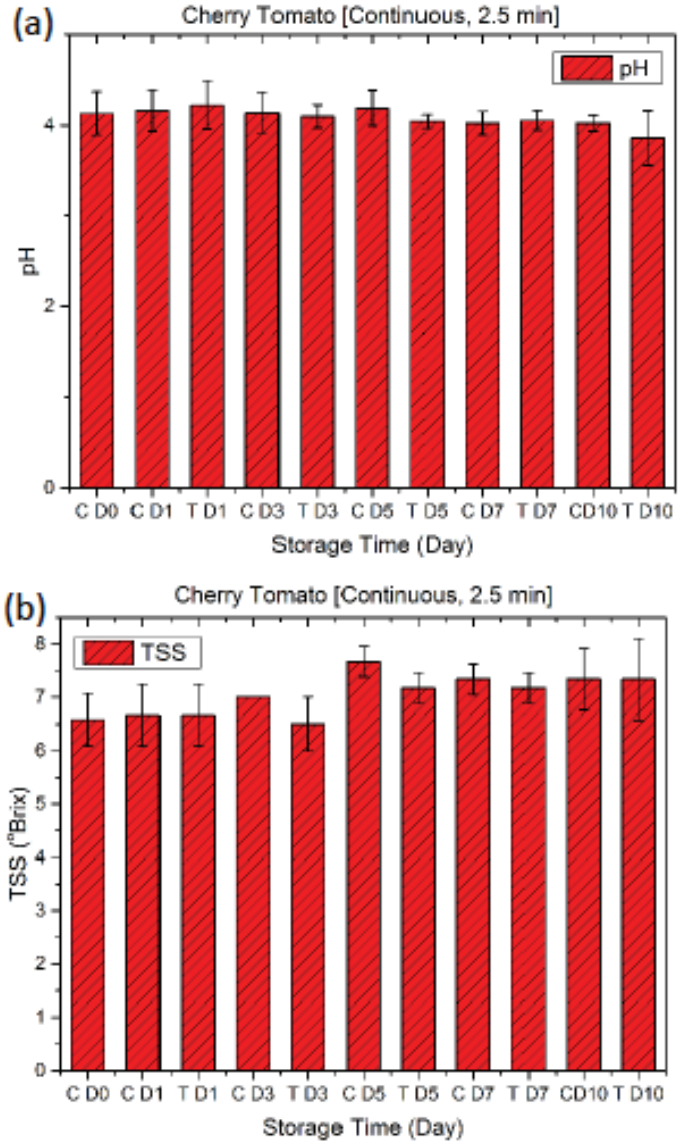

FIG. 7: (A) pH and (B) TSS of control ("C") and plasma-treated ("T") cherry tomatoes over a storage period of 10 days ("D")

C. Ozone Concentration

The overall ozone concentrations were within the range of 450–900 ppm depending on the mode of operation, with lower ozone levels generated during static than continuous mode of treatment (Fig. 8). Ozone in both the gaseous and aqueous states is effective against a wide range of pathogenic microorganisms, including bacteria, yeasts, molds,

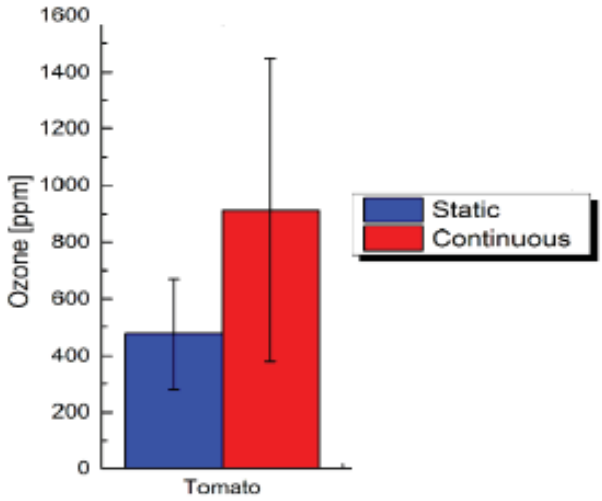

FIG. 8: Ozone concentrations inside the packages for static and continuous mode of operation (150 s) when loaded with cherry tomatoes

and viruses. Its oxidant reduction potential is much higher than that of chlorine, acids, and hydrogen peroxide.[25] In this work, an enhanced inactivation effect of continuous ACP treatment was demonstrated for *L. innocua*, which could be attributed to the higher levels of ozone concentration generated inside the sealed bags during continuous treatment compared with during the static treatment. Recent findings suggested that >2 log reduction in the microbial population can be obtained on dried oregano by gaseous ozone treatments with an acceptable taste, flavor, and appearance.[26] Alexopoulos et al.[27] analyzed fresh-cut lettuce and green bell papers for reduction of their surface microflora by ozonated water. The most effective treatment in this work was when vegetables were dipped in continuously ozonated water (0.5 mg/L), leading to 3.5 log of microbial reductions after 30 min of exposure, which is higher than after chlorination. Fan et al.[28] reported 2 to 3 log reductions of *E. coli* and *Salmonella* inoculated on tomatoes after 2–3 min of ozonation.

In general, ozone is one of the most long-lived reactive species and is important in determining antimicrobial effects of ACP treatment that utilizes extended post-treatment storage. The concentration of ozone as an example showing that longer-lived species can be influenced by a number of extrinsic and intrinsic treatment parameters that need to be considered to achieve successful inactivation of pathogens associated with fresh produce. These parameters include plasma system design, electrode material, and geometry, as well as produce characteristics, humidity brought by the produce, and the use of atmospheric air for induction of the plasma and the associated dynamic plasma chemistry. In terms of bacterial inactivation, ozone destroys microorganisms through widespread oxidation of internal cellular components, causing rapid cell death. The major mechanisms involve oxidation of sulfhydryl groups and amino acids of enzymes, peptides, and proteins to shorter peptides; oxidation of unsaturated fatty acids to acid

peroxide; degradation of cell envelop lipids resulting in leakage of cellular components; increase in cell permeability protein disruption; and damage of nucleic acid resulting in cell lysis.[29] In addition, ozone has elevated diffusion capabilities that enable its rapid diffusion through biological cell membranes.[27] Previous studies demonstrated that high concentrations of ozone generated during 5 min of in-package high-voltage indirect DBD ACP treatment (4420 ppm), and other generated chemically reactive species, completely disintegrated *Salmonella* cells present on the surface of lettuce.[30] Toyokawa et al.[31] demonstrated that plasma treatment generated using a DBD plasma roller conveyer and atmospheric air induced slight morphological changes to *Xanthomonas campestris* cells; however, the treatment caused significant degradation of lipopolysaccharides and oxidation of genomic DNA, leading to cell death.

In conclusion, this work demonstrated that a pilot scale in-package cold plasma system could induce discharges inside food packages during both static and continuous modes of treatment. It was possible to achieve rapid bactericidal effects against background microflora through the retention of bactericidal species during the produce storage stages while retaining important produce quality characteristics. Challenge pathogens *E. coli* and *L. innocua* inoculated on whole cherry tomatoes were reduced by an average of 5.0 and 3.5 $\log_{10}$ CFUs/sample, respectively, after 150 s of continuous operation, whereas maximum reductions for background microflora of 3.7 log units were achieved using static mode. This work also demonstrated that factors such as type of bacteria, cell concentration, and mode of treatment may also influence plasma treatment decontamination effects and should be taken into consideration to achieve maximum advantages during industrial adoption of this technology. Moreover, the design of ACP food processing systems, in which biological decontamination forms part of the remit, should include working gas composition, gas flow rate, electrode configuration, and voltage levels as determinants of the efficacy of treatment. Importantly, it is likely that different produce commodities will require specific application of treatment parameters in order to improve microbiological safety and physico-chemical quality as they present diverse microflora and biochemical characteristics.

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