Cold Plasma Inactivates *Salmonella* Stanley and *Escherichia coli* O157:H7 Inoculated on Golden Delicious Apples†

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ABSTRACT

Cold plasma generated in a gliding arc was applied to outbreak strains of *Escherichia coli* O157:H7 and *Salmonella* Stanley on agar plates and inoculated onto the surfaces of Golden Delicious apples. This novel sanitizing technology inactivated both pathogens on agar plates, with higher flow rate (40 liters/min) observed to be more efficacious than were lower flow rates (20 liters/min), irrespective of treatment time (1 or 2 min). Golden Delicious apples were treated with various flow rates (10, 20, 30, or 40 liters/min) of cold plasma for various times (1, 2, or 3 min), applied to dried spot inoculations. All treatments resulted in significant (*P < 0.05*) reductions from the untreated control, with 40 liters/min more effective than were lower flow rates. Inactivation of *Salmonella* Stanley followed a time-dependent reduction for all flow rates. Reductions after 3 min ranged from 2.9 to 3.7 log CFU/ml, close to the limit of detection. For *E. coli* O157:H7, 40 liters/min gave similar reductions for all treatment times, 3.4 to 3.6 log CFU/ml. At lower flow rates, inactivation was related to exposure time, with 3 min resulting in reductions of 2.6 to 3 log CFU/ml. Temperature increase of the treated apples was related to exposure time for all flow rates. The maximum temperature of any plasma-treated apple was 50.8°C (28°C above ambient), after 20 liters/min for 3 min, indicating that antimicrobial effects were not the result of heat. These results indicate that cold plasma is a nonthermal process that can effectively reduce human pathogens inoculated onto fresh produce.

Fresh and fresh-cut fruits and vegetables are increasingly implicated in outbreaks of foodborne illness (6, 7, 17). Produce-associated outbreaks in the United States have risen from less than 20 throughout the 1970s to more than 100 in the 1990s (14). Human pathogens such as *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* are more commonly associated with produce than has historically been recognized. Proper adherence to good agricultural practices and good manufacturing practices in the course of production and processing can reduce the likelihood of contaminated produce reaching the consumer. Conventional surface treatments based on antimicrobial chemicals are inadequate for assuring the safety of these commodities. Therefore, research efforts are ongoing to develop an alternative, robust antimicrobial process (a "kill step") suitable for application to fresh and fresh-cut produce. As an example of a kill step being investigated, irradiation has been consistently shown to be effective in inactivating bacterial human pathogens on produce (12). However, issues such as uncertainties about costs, throughput capacity, governing regulations, and consumer acceptance have prevented widespread adoption of this technology.

Cold plasma is a relatively new sanitizing technology in the field of food processing. A wide array of plasma-generating technologies has been in use for a number of years in such applications as lighting, electronics, and materials processing. Within the context of food processing, however, this is a first-generation technology, with many basic technological hurdles yet to be addressed. Although plasma has many gas-like qualities, and may be considered for practical purposes to be an energetic form of gas, it is technically a distinct state of matter. As energy is added to materials, they change state, going from solid to liquid to gas, with large-scale intermolecular structure breaking down. As additional energy is added, the intra-atomic structures of the components of the gas break down, yielding plasmas—concentrated collections of ions, radical species, and free electrons (1, 4, 5).

Conventional plasmas, both natural and manmade, are often at very high temperature (as in open flames, lightning, and welding arcs) or very low pressure (as in the aurora borealis, plasma-based video screens, and vapor deposition applications). Newer technologies are able to produce these plasmas at temperatures and pressures closer to ambient. Thus the term cold plasma is a relative term, and in the context of food processing, refers to the ability to generate and apply the plasma nonthermally, at temperatures that foods will tolerate, rather than arising from a need for refrigeration in conjunction with the treatment.

Cold plasma has been evaluated to a limited extent for use in sanitizing foods, although the literature remains sparse. The technologies used to produce cold plasma vary widely, but fall into three general categories: electrode con-
tact (in which the target is in contact with or between electrodes), direct treatment (in which active plasma is deposited directly on the target), and remote treatment (in which active plasma is generated at some distance, and plasma is moved to the target). Using a system of the first type, electrode contact, Deng et al. (3) reduced *E. coli* 12955 on almonds by 5 log after a 30-s treatment (30 kV, 2 kHz). In that study, the almonds were placed in the 10-mm gap between the two electrodes of a dielectric barrier discharge system. Kelly-Wintenberg et al. (9) treated a variety of microorganisms between the electrodes (18 by 15 cm, variable gap spacing) of a radiofrequency-driven plasma system (5 kV, 7 kHz). The cultures (including *E. coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, and bacteriophage ϕX174) were treated on several different substrates, including polypropylene, glass, and agar. The plasma system was able to sterilize the inoculated surfaces for all microorganisms and materials tested, with treatment times ranging from 30 s to 15 min. In that study, plasma was more effective against wet-mounted cultures on the surface, but required longer exposures to achieve the same kill levels for dried cultures or cultures embedded within agar plugs.

Research with systems of the second type, direct treatment, has shown that a radiofrequency-driven plasma (0.2 to 0.5 kV, 13.56 MHz) applied across a narrow gap (1 mm) reduced *E. coli* by 4.6 and 5.1 log CFU/ml after treatment of 10 and 60 s, respectively (15). As the space between the plasma emitter and the treated culture was increased, antimicrobial efficacy was reduced, until at 10-mm spacing, no reductions were observed at any power level tested. A 10-kV gliding arc plasma applied to inoculated Golden Delicious apples from a distance of 10 mm reduced *Listeria innocua* by 0.39 log CFU/ml after a 2-min treatment at 115 mA. A treatment of 150 mA for 4 min reduced *L. innocua* by 1.1 log CFU/ml (13).

Using systems of the third type, remote treatment, Montie et al. (11) used the flow of feed gas to move a relatively high volume of plasma away from the parallel electrode plates where it was generated, across a distance of 20 cm to the target. A 10-s treatment reduced *E. coli* and *S. aureus* inoculated on polypropylene by 4 and 2 log CFU/ml, respectively. Using a related technology, one atmosphere uniform glow discharge plasma (OAUGDP), Kayes et al. (8) treated a variety of microorganisms inoculated onto agar, and calculated D-values (in seconds). For pathogens freshly inoculated onto pH 7.0 agar, D-values ranged from 22 s (Shigella flexneri and Vibrio parahaemolyticus) to 51 s (*E. coli* O157:H7). On slightly acid agar, pH 5.0, D-values were lower, and ranged from 19 s (V. parahaemolyticus) to 31 s (*Salmonella Enteridis*). The OAUGDP reactor has also been shown to effectively reduce pathogens inoculated onto produce (2). A 2-min treatment reduced *E. coli* O157:H7 on Red Delicious apples by approximately 3 log CFU, reduced *Salmonella Enteridis* on cantaloupe by approximately 3 log CFU, and reduced *L. monocytogenes* on iceberg lettuce by approximately 2 log CFU. Additional treatment of lettuce for up to 5 min led to an ultimate reduction of *L. monocytogenes* of 5 log CFU, although comparable extension of treatment of cantaloupe did not yield comparable additional reductions of *Salmonella enteridis*. Treatment using the OAUGDP involves placing the sample into the reactor chamber and flooding the chamber with plasma.

For treatment of bulky or irregularly shaped foods such as fresh and fresh-cut produce, an appropriate plasma technology will allow for remote generation of the plasma, and application across a distance of several centimeters. The objectives of this study were (i) to characterize the behavior of a gliding arc plasma system, (ii) to establish basic parameters of inactivation of bacterial human pathogens, using agar plates, and (iii) to further optimize cold plasma treatment of these pathogens inoculated onto the surface of Golden Delicious apples.

**MATERIALS AND METHODS**

**Plasma equipment.** The gliding arc cold plasma system consists of two components, the power supply and the plasma emitter. The power supply is a customized ground referenced, high-voltage transformer, center tapped. The system runs on standard 60-Hz AC power, with rated maximum operating outputs of 60 mA at 15 kV. External wiring connecting the power supply to the plasma emitter was high-voltage-insulated cabling, rated for 30 kV. The plasma emitter is a custom-made modification of a gas-injected gliding arc system constructed at the Drexel Plasma Institute, Philadelphia, Pa. (Fig. 1). Unlike other cold plasma—generation technologies, this system is designed to be operated on an open-air bench top, and does not require a closed-batch process of placing the samples into an enclosed treatment chamber. The electrodes used in this application was unpolished, 2-mm-thick rods of oxygen-free copper (alloy 101). The electrodes were attached to the emitter body at top and bottom with stainless steel lugs, either fixed (top) or mounted on adjustable ceramic set screws (bottom). The rods were fixed at 3 mm apart at the plasma generation point, and bent away at a 45° angle. The plasma generation point was 8 mm above the gas inlet. The feed gas for the plasma emitter was dried, filtered air. Studies were conducted at key gas flow rates, described below.

**Microorganisms.** The isolates utilized in this study were from the U.S. Department of Agriculture, Agricultural Research Station, Eastern Regional Research Center culture collection: *E. coli* O157:H7 ATCC 43894 (American Type Culture Collection, Manassas, Va.), and *Salmonella Stanley* H0558 (Centers for Disease Control and Prevention, Atlanta, Ga.). The isolates were maintained in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.). Fresh cultures (10 ml) of each isolate were grown overnight in TSB at 37°C for use in experiments. The cell concentration of these individual cultures was approximately 10⁶ CFU/ml, as determined by serial dilution and plate count on tryptic soy agar (TSA; Difco, Becton Dickinson) incubated overnight at 37°C.

**Media treatments.** Cultures were diluted to approximately 10⁶ CFU/ml, using sterile Butterfield's phosphate buffer. Aliquots of 100 μ1 (~10⁴ CFU) were spread on the surface of TSA plates and allowed to completely absorb onto the agar—approximately 30 min. Each plate to be treated was positioned under the gliding arc plasma emitter, which was mounted and oriented in a downward-pointing configuration, with the edge of the emitter shield wall 1 mm from the lip of the plate and 8 mm from the surface of the agar. A mark was made on each plate to denote the ori-
FIGURE 1. The gliding arc cold plasma system as applied to Golden Delicious apples. Flow rates are 10 (A), 20 (B), 30 (C), or 40 (D) liters/min. Note decreasing gap between plasma plume and treated surface, and rise of plasma discharge along supporting electrodes.

entation of the plate with respect to the roughly rectangular field of plasma deposition. In addition to the untreated control, plates were exposed to the plasma emitter for either 1 or 2 min at a flow rate of 20 liters/min (20 or 40 liters total volume of plasma deposited, respectively). Additional plates were treated for either 1 or 2 min at a flow rate of 40 liters/min (40 or 80 liters total volume of plasma deposited, respectively). Three plates were treated for each combination. After treatment, the plates were covered, inverted, and incubated at 37°C overnight. The plates were evaluated qualitatively to determine visible indications of suppression of colony formation for each treatment, and to determine if there was a qualitative difference in response for the center of the plate (directly under the plasma gas jet) versus the edge of the plate (oblique to the plasma gas jet). The study was performed twice.

Golden Delicious apples. Based on the results of the qualitative studies using agar plates, a quantitative determination of the effect of cold plasma on E. coli O157:H7 and Salmonella Stanley was conducted, using Golden Delicious apples as the test substrate. As with previous studies of plasma treatments in our laboratory (13), this variety was chosen for its pale color and sensitivity to heat, electrical damage, and abusive conditions. Ripe, waxed apples were purchased at local markets and held at 8°C until the day of the experiment, typically 1 to 3 days. Prior to testing, each apple was washed with 70% ethanol and allowed to air dry. The apples were cut in half with a sterile knife, and placed cut side down onto a sterile Petri plate to facilitate inoculation. An inoculation area was indicated on each apple half with a Sharpie marker. A 100-μl aliquot (~10³ CFU) of fresh overnight culture of either E. coli O157:H7 or Salmonella Stanley was pipetted onto the surface of each apple half and allowed to adhere for 20 min. The inoculation spot was approximately 1 cm in diameter. After the adherence time, any remaining free liquid was carefully wicked off with a Kimwipe. The spot-inoculated apples (controls and those intended for plasma treatment) were allowed to dry at 8°C for 2 h. This period resulted in completely dry inoculation spots for all apples treated. One half of each apple was treated with plasma; one half was an untreated control.

For treatments, the plasma emitter was oriented in an upward-pointing configuration. The apple half was taken from cold storage and placed, inoculated spot down, onto the top of the emitter, with the surface of the apple resting on the edge of the emitter shield wall (Fig. 1). The inoculation spot was oriented within the roughly rectangular field of plasma deposition above the electrodes. The apples were treated with 1, 2, or 3 min of
plasma at a flow rate of 10, 20, 30, or 40 liters/min. After treatment, the inoculation spot of the plasma-treated apple and the untreated control apple were sampled using a sterile cotton swab dipped in sterile Butterfield's phosphate buffer. The swab tip was placed into the dilution tube with 1 ml of Butterfield's phosphate buffer and thoroughly vortexed. Serial dilutions were made with sterile Butterfield's phosphate buffer, and 100-μl aliquots of various dilutions were spread plated onto TSA. The plates were allowed to adhere fully, inverted, and incubated overnight at 37°C. Plates were counted by hand and data scaled for dilution. Clear plates were assigned a value of 10 CFU/ml, the lower limit of detection. Nine separate apples were used for each treatment combination, and the entire study was performed three times.

**Physical parameters.** Plasma-treated apples for all microbiological treatment combinations were examined by lab personnel, and compared with the untreated controls to identify gross changes in color or texture. In separate trials, the temperature of the apples surfaces was measured before and after each treatment combination (time × flow rate). Apple halves were prepared as described and allowed to equilibrate for 2 h at 8°C.

Apples to be treated were taken from cold storage, the temperature of the surface was measured, and the apple was treated with plasma as described. Immediately after treatment, the temperature of the plasma treated surface was measured. Temperatures were measured with an infra-red thermometer (TempTestIR, OAKTON Instruments, Vernon Hills, Ill.) held approximately 6 cm from the surface of the apple. Separate apples were used for each treatment combination, and the measurements were performed five times.

Current draw at each flow rate was determined with 10 separate measurements, using a Pearson Model 1A current transformer loop attached to a Tektronix-320 oscilloscope. The overall shape and luminance of the plasma was evaluated at each flow rate. The distance separating the uppermost part of the region of active plasma from the treated surfaces was measured from scaled photographs taken of the gliding arc plasma discharge at each flow rate of the air injected into the emitter.

**Statistical analysis.** The surviving population of either *E. coli* O157:H7 or *Salmonella* Stanley for each treatment was compared with that recovered from its respective untreated spot inoculation control (typically 10^5 CFU/ml). This was done to account for expected loss of CFU resulting from desiccation of the spotted inoculum and to account for possible day-to-day variation in inoculum strength over the course of the experiments. Reductions from the control were compared among the various treatment combinations using two-way analysis of variance (ANOVA), Tukey's test (P < 0.05) (SigmaStat v. 4.0, SPSS, Inc., Chicago, Ill.), using data pooled from the three plates for each of the three replications. Temperatures of plasma-treated apple surfaces were similarly compared with two-way ANOVA, using pooled data. Current draw at each power level was compared using one-way ANOVA, using pooled data.

**RESULTS**

**Media treatments.** By visual inspection of the plates, it is evident that cold plasma reduced the viable populations of both pathogens on the surfaces of treated agar plates. In general, *Salmonella* Stanley was observed to be more sensitive than was *E. coli* O157:H7 in the plate assay. For *Salmonella* Stanley (Fig. 2), distinct zones of clearing, corresponding to the field of plasma deposition, were evident on plates treated with 40 liters/min for 1 or 2 min, treated with 20 liters/min for 2 min, and to a lesser extent, on plates treated with 20 liters/min for 1 min. For *E. coli* O157:H7 (Fig. 3), zones of clearing were evident at for the 2-min treatments of both 20 and 40 liters/min, to a lesser extent on plates treated with 40 liters/min for 1 min. Zones of clearing were generally less distinct for *E. coli* O157:H7 than for *Salmonella* Stanley.

For each pathogen, inactivation was more influenced by the flow rate and time than by the total volume of plasma deposited onto the surface. Treatments of 20 liters/min for 2 min and 40 liters/min for 1 min deposited equal amounts of plasma (40 liters), yet 40 liters/min was more effective.

**Golden Delicious apples.** The reduction of pathogens on apples followed the same general pattern as that observed qualitatively on agar plates—that *Salmonella* Stanley is more effectively inactivated by cold plasma than *E. coli* O157:H7 is, and that higher flow rates and longer times increase efficacy. However, the plate counts obtained from the treated apples indicated that *E. coli* O157:H7 was more readily inactivated on apple surfaces than on the agar plate, particularly at the 40 liters/min flow rate. Plate counts from the control spot inoculations were typically 10^5 CFU/ml.

Cold plasma was effective in inactivating both *E. coli* O157:H7 and *Salmonella* Stanley on apples. All treatment combinations in this study significantly (P < 0.05) reduced the surviving population for both pathogens, relative to the untreated control. In general, higher flow rates resulted in greater inactivation at shorter times.

Inactivation of *Salmonella* Stanley followed a time-dependent reduction for all flow rates (Fig. 4). For the lowest flow rate (10 liters/min), each successively longer treatment yielded significant additional increases in pathogen inactivation. For 30 liters/min, the 2-min treatment was significantly more effective than the 1-min treatment was, but lengthening the treatment to 3 min did not result in significantly more inactivation. For 20 and 40 liters/min, in contrast, the reductions obtained at 1 and 2 min did not differ, but reductions obtained at 3 min were significantly greater than at either of the shorter times.

Plasma produced at 40 liters/min was more effective than all of the lower flow rates for 1 min of treatment, and was generally the most effective throughout. After 1 min at 40 liters/min, *Salmonella* Stanley populations were reduced by 2.4 log CFU/ml, significantly greater reductions than reductions at 10, 20, or 30 liters/min (1 to 1.4 log CFU/ml). After 3 min, reductions for 10 or 20 liters/min (3.1 to 3.3 log CFU/ml) were intermediate between those obtained for 30 liters/min (2.9 log CFU/ml) and 40 liters/min (3.7 log CFU/ml). The most extreme reductions were close to the experimental limit of detection for these studies (a 4-log CFU/ml reduction).

Inactivation of *E. coli* O157:H7 was uniformly effective for 40 liters/min, but was time dependent for 10, 20, and 30 liters/min (Fig. 5). At 40 liters/min, treatments of 1, 2, or 3 min gave statistically similar reductions of 3.4, 3.6, and 3.4 log CFU/ml, respectively. For 30 liters/min, each successively longer treatment yielded significant additional increases in pathogen inactivation. At lower flow...
rates, the pattern of response was mixed. For 20 liters/min, the 2-min treatment was significantly more effective than the 1-min treatment was, but lengthening the treatment to 3 min did not result in significantly more inactivation. For 10 liters/min, in contrast, the reductions obtained at 1 and 2 min did not differ, but reductions obtained at 3 min were significantly greater than at either of the shorter times.

The flow rate of 40 liters/min was significantly more effective than all lower flow rates at 1 or 2 min. At 10, 20, or 30 liters/min, inactivation of *E. coli* O157:H7 was related to exposure time, with 3 min resulting in the highest reductions of 2.6 to 3 log CFU/ml. As with *Salmonella* Stanley, after 3 min, reductions for 10 or 20 liters/min (3.0 log CFU/ml) were intermediate between those obtained for 30 liters/min (2.6 log CFU/ml) and 40 liters/min (3.4 log CFU/ml). The most extreme reductions were close to the experimental limit of detection for these studies (a 4-log CFU/ml reduction).

For each pathogen, inactivation was more influenced by the flow rate and time than by the total volume of plasma deposited onto the surface. For example, although 10 liters/min for 2 min and 20 liters/min for 1 min deposited the same volume of plasma (20 liters) onto *Salmonella* Stanley–inoculated apples, longer time at the lower flow rate was more effective. In contrast, 20 liters/min for 2 min was less effective than 40 liters/min for 1 min in reducing *E. coli* O157:H7 on the apple surface.

**Physical parameters.** Immediately after removal from refrigerated storage, all apples had the same temperature (8°C). The temperature of the apple surfaces after each plasma treatment was significantly increased over the untreated control at each sampling time (Fig. 6). For 20, 30, and 40 liters/min, but not for 10 liters/min, 1 min of treatment resulted in apple surface temperatures at least 10°C over ambient (22°C). For 10 and 20 liters/min, significant increases in temperature were observed with each successively longer treatment time. However, overall temperatures obtained with 20 liters/min were consistently higher than those obtained at 10 liters/min. Temperatures for the 40-liters/min treatment increased significantly from 1 to 2 min, but were not increased by additional time, to the 3-min treatment.
For the 30-liters/min treatment, the temperature of the plasma-treated apple reached its maximum after only 1 min (40°C) and remained invariant thereafter, up to 3 min. By 3 min of treatment, 10, 30, and 40 liters/min reached statistical parity at 40°C (18°C over ambient), significantly lower than the 20-liters/min treatment. The highest temperature reached was for the 3-min treatment at 20 liters/min flow rate, which was 51°C (29°C above ambient, 43°C over the starting temperature).

Laboratory personnel examined the apples visually and manually after each of the plasma treatments, within 1 to 2 h of treatment application. No gross changes in color or texture were evident for any of the treatment combinations.

The distance separating the plasma and the surfaces treated varied with the feed-gas flow rate (Fig. 1). At 10 liters/min, the plasma was relatively small, bright, and compact, with the highest part of the plasma discharge approximately 5.5 cm from the treated surface. At 20 liters/min, the plasma was somewhat less bright, more diffuse, and more variable in height as transient discharges briefly reached higher than the average height of 4 cm from the treated surface. At 30 liters/min, the discharges were further
diffused, with the characteristic glow relatively uniform throughout the active plasma. At this flow rate, transient discharges were diminished but remained in evidence, and the discharge was observed to climb higher on the supporting electrodes, increasing the overall volume of active plasma, and decreasing the distance separating the plume head from the treated surface to approximately 3 cm. At the highest flow rate, 40 liters/mm, the plasma was maximally diffused, and displayed a glow of uniform intensity. Transient discharges were rarely observed, and the discharge contact extended nearly the entire length of the supporting electrodes, resulting in a maximal volume of active plasma, and making the separation distance approximately 2.5 cm.

It was determined that actual power outputs were above rated maximum of the power supply. Power consumption of the plasma emitter was related to feed gas flow rate. At 10, 20, 30, and 40 liters/min, current draw in the gliding arc system was 59.7, 63.6, 69.6, and 71.2 mA, respectively. Statistical analysis (ANOVA, P < 0.05) indicated that the current draw increase was significant from 10 to 20 liters/mm, and from 20 to 30 liters/mm, but that 40 liters/min did not result in significantly increased current draw over 30 liters/min. The maximum level reached (~70 mA) was approximately 17% above the rated output of the power supply (60 mA).

DISCUSSION

Cold plasma significantly reduced the viable populations of Salmonella Stanley and E. coli O157:H7 on inoculated apple surfaces, for all evaluated combinations of flow rate and time. Reductions for Salmonella Stanley ranged from 1.06 log CFU/ml (10 liters/min for 1 min) to 3.72 log CFU/ml (40 liters/min for 3 min); reductions for E. coli O157:H7 ranged from 0.81 log CFU/ml (30 liters/min for 1 min) to 3.42 log CFU/ml (40 liters/min for 3 min). In general, higher kill rates were obtained with longer times and higher flow rates of the feed gas into the plasma emitter. Salmonella Stanley showed a more time-dependent response for each of the feed-gas flow rates. The 40 liters/min flow rate was significantly more effective at shorter times, but this advantage was reduced with increasing length of treatment. A similar pattern was seen with E. coli O157:H7 with respect to the efficacy of the 40-liters/min treatment as compared with 10, 20, or 30 liters/min, but the response overall was less time dependent for E. coli O157:H7 than for Salmonella Stanley.

The highest flow rate, 40 liters/min, was the most effective. However, the lowest flow rate, 10 liters/min, was not necessarily significantly less effective than 20 or 30 liters/min for either pathogen. For Salmonella Stanley, flow rates of 10, 20, and 30 liters/min each yielded the minimum reduction for treatments of 1 min (1.06 log CFU/ml), 2 min (1.92 log CFU/ml), or 3 min (2.96 log CFU/ml), respectively. For E. coli O157:H7, 30 liters/min consistently resulted in the lowest absolute level of reduction, a difference significant with respect to all others only at the 1-min treatment. The inoculated apples were held for 2 h to allow for drying and adhesion of the inoculum. An important area of future research is to determine the effect of varied inoculation methodologies. Application of cultures more dilute, mixed cultures, abusive holding temperatures, longer holding times prior to plasma treatment, or other variables should be addressed. Interspecies competition, additional opportunity for biofilm formation, or internalization or other mechanisms may interact with the cold plasma treatment.

Although the physical properties of the plasma used herein have not yet been fully characterized, higher flow rates of the feed gas result in a shorter time of flight from the region of active plasma to the target surface. The components of the plasma are reactive and self-quenching, with a relatively short half-life; decreased time of flight would be expected to be one of the major factors in antimicrobial efficacy. As the data from the plasma-treated apples show, however, the precise nature of the inactivation is more com-
plex than a straightforward determination of bulk flow parameters is.

The total volume of plasma deposited on the surface appeared to be less significant a factor than how the plasma was generated and the time of treatment. The readily observed physical differences in the generated plasma field at 10, 20, 30, or 40 liters/min (brightness, dispersion, degree of homogeneity, incidence of transient point discharges, etc.) support the contention that the chemical and physical composition of the plasma is determined by the manner of production, even when the chemical composition of the feed gas remains invariant.

Increased feed gas flow rate tends to increase the heat buildup on the surface when going from 10 to 20 liters/min. Above a critical flow rate, the velocity of the feed gas is sufficiently high that it disperses the inherent heat of the plasma being generated. This effect is influenced by turbulence and by the design, shape, and orientation of the feed gas nozzle. Further development and optimization of the technology is required. Nevertheless, significant reductions in population by both pathogens were observed under conditions where the apple surface was below the temperatures at which thermal kill of the bacteria would be a factor. Even the maximum temperature reached (50.8°C) is below the threshold for thermal kill of either Salmonella or E. coli O157:H7 (7). It is apparent, therefore, that plasma is a nonthermal process, in that kill mechanisms, although as yet poorly understood, are not associated with heat. Further research to determine and quantify precise mechanisms of kill could incorporate controls for heat, ozone, UV, free electrons, or other potential contributors to cold plasma's antimicrobial efficacy.

Maximal reductions obtained for 3-min treatments on apples ranged from 2.96 to 3.72 log CFU/ml for Salmonella Stanley, and were generally greater than those obtained for E. coli O157:H7 (2.6 to 3.4 log CFU/ml). The literature examining the use of nonthermal plasma for inactivation of foodborne pathogens is still relatively sparse. In a study of E. coli 12955 (a nonpathogenic surrogate for Salmonella) inoculated onto almonds, Deng et al. (3) was able to reduce the population by more than 4 log CFU/ml. That result was achieved by placing the almonds in the 10-mm gap between two plasma discharge electrodes and treating for 30 s (25 kV, 2 kHz). The gas medium in that study was not specified and is presumed to have been air. Using defined gas mixes, including the incorporation of noble gases, other technologies have been developed that show antimicrobial efficacy. Helium is commonly used in plasma applications, as it has a lower ionization potential than air has (4, 10, 15). Using a radiofrequency-driven system (0.2 to 0.5 kV, 13.56 MHz), Stoffels et al. (16) developed a plasma emitter that projects 2 to 3 mm from the tip of the discharge point. In that application, increased power levels caused an increase in the resultant plasma temperature, up to 47°C, as did switching from pure helium to mixtures of helium with argon, nitrogen, or air. Sladek and Stoffels (15) used this technology to project plasma a distance of 1 mm from the emitter, and reduced E. coli by approximately 4.6 log CFU/ml after 10 s, and 5.1 log CFU/ml after 60 s. Treatments longer than 60 s did not result in additional inactivation. As the space between the plasma emitter and the treated culture was increased, antimicrobial efficacy was reduced, until at 10-mm spacing, no reductions were observed at any power level tested. Discussions of this system were related to dental and medical applications (13).

The OAUGDP plasma technology has shown promise in several recent studies. Treatment of samples in the OAUGDP system involves sealing the samples within the reactor chamber and saturating the chamber with plasma. For microorganisms freshly inoculated onto agar, calculated D-values were in the range of 20 to 50 s for common foodborne pathogens, depending on the conditions of treatment (8). For microorganisms allowed to incubate on the agar surface for 24 h, the treatment was less effective. D-values for these incubated agar plates varied, depending on pH and temperature of incubation: 38 to 111 s (Salmonella Enteritidis), 37 to 124 s (E. coli O157:H7), 45 to 126 s (L. monocytogenes), or 37 to 181 s (S. aureus). Shielding of the cultures by increased biomass of the treated cultures was cited as one possible mechanism for the reduction in efficacy. This raises the possibility of biofilm-associated protection of pathogens from cold plasma, as with other antimicrobial interventions. Applied to microorganisms on fresh produce samples sealed within the reactor chamber, the OAUGDP reactor effectively reduced E. coli O157:H7, Salmonella Enteritidis, and L. monocytogenes (2). Reductions of 2 to 5 log CFU were obtained in treatments of 2 to 5 min. In that study, the OAUGDP system was more effective when applied to inoculated agar than inoculated produce. The OAUGDP system requires samples to be placed within the reactor chamber. As this field of technology matures, additional applications in food processing will draw on developments in diverse fields.

When examined shortly after treatment, no discoloration, blistering, pitting, bruising, foul aromas, or other gross sensory damage were evident in the plasma-treated apples. These results are consistent with a previous study of Golden Delicious apples treated with gliding arc plasma, which showed no color or textural changes to plasma-treated apples immediately after treatment or during storage at 8°C for 35 days (13). Although using a plasma technology similar to the present work, that study was conducted using an earlier prototype of the gliding arc plasma emitter, operating at 10 kV, using air as the feed gas, but with a more diffuse feed-gas inlet system that provided a flow rate of 300 liters/min. In that study, with feed-gas flow rate fixed, treatment with a power setting of 115 mA for 2 min reduced L. innocua by 0.39 log CFU/ml. A treatment of 150 mA for 4 min reduced L. innocua by 1.1 log CFU/ml. Incubation, treatment, and sampling in that study was similar to that performed in the present work. It should be noted that outbreak strains of Salmonella and E. coli O157:H7 were used in the present work, while a nonpathogenic surrogate was used in the previous study of application of gliding arc plasma to inoculated Golden Delicious apples (13). The potential of cold plasma as an antimicrobial tool is evident in comparing the antimicrobial efficacy achieved in the studies. As with the earlier form of the gliding arc
cold plasma system, the version more advanced used herein is designed to be operated in the open air, rather than within a closed and/or evacuated reactor chamber. The gliding arc process has been developed, on the lab scale, to the extent that orders of magnitude greater reductions are obtained using an order of magnitude less feed gas in shorter times. The present study, using the current version of this cold plasma system, used treatments consuming 0.017 to 0.051 kWh, compared with 0.038 to 0.10 kWh for the earlier study (13). As these improvements were made, the quality of the treated produce remained largely unaffected by the treatments applied. Power consumption in the present study was related to the flow rate of the feed gas.

These results indicate that, with further optimization and technology development, cold plasma has the potential to be an important tool in eliminating pathogens from fresh produce. In addition to determining antimicrobial efficacy and characterization of the antimicrobial compounds and mechanisms of cold plasma, future research must include detailed examinations of the sensory properties of cold plasma treated produce and more information on the economics of the process using larger scale equipment.

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