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Comparing inactivation of *Escherichia coli* O157:H7 on fresh produce using plasma-activated mist

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ABSTRACT

Plasma-activated mist (PAM) is a novel method that has shown potential for the decontamination of fresh produce. In this study, we evaluated the decontamination of *Escherichia coli* O157:H7 on nutrient agar plates and various types of fresh produce, both individually and stacked layers. Single pieces of kale achieved a notable 3-$\log_{10}$ reduction, while spinach and lettuce showed reductions of over 1.5-$\log_{10}$, and strawberry exhibited the lowest reduction of 0.8-$\log_{10}$. The efficacy on stacked multiple layers of spinach, kale, and strawberry was shown to achieve just over 0.9-$\log_{10}$ reduction of *E. coli* but always worse than the inactivation observed on a single piece. A mathematical diffusion model was employed to explore scaling up PAM for industrial applications, particularly on stacked spinach layers.

Industrial relevance

This study investigated a scalable and efficient method for reducing pathogens on produce surfaces. The potential of plasma-activated mist for large-scale application, emphasizing its relevance to industrial food processing.

1. Introduction

The demand for fresh and minimally treated produce has skyrocketed in recent years, which can be attributed to the innumerable health benefits of consuming fresh fruits and vegetables. However, with this increased demand for fresh produce, comes a higher risk of foodborne illnesses (Kilonzo-Nthenge et al., 2018). According to the Congressional Research Service, in the past ten years, produce has been linked to over 30 foodborne illness outbreaks, sickening over 1500 people annually in the USA. The leading causes of illness are linked to Norovirus, *Salmonella*, and *Escherichia coli.* Specifically, *E. coli* O157:H7 has been linked to outbreaks involving leafy greens (Johnson, 2019). Leafy greens present greater microbial hazards, as they account for up to 40% of all produce-related illnesses and outbreaks (Johnson, 2019). A 2008 United Nations World Health Organization study ranked leafy greens as the Level 1 (highest) Priority in microbial hazards in the fruit and vegetable category; berries, green onions, melons, sprouted seeds, and tomatoes were ranked Level 2, and all other produce was ranked Level 3 (WHO, 2008).

Apart from the public health risk, fresh produce has a limited shelf life and begins to decrease in initial quality after harvesting due to a plethora of problems, including—but not limited to—microbial contamination, spoilage, and decay. Norovirus, *Salmonella*, and *Escherichia coli* are some of the common pathogenic microorganisms found on fresh produce due to contamination (Johnson, 2019); while *Erwinia carotovora, Pseudomonas spp., Corynebacterium, Xanthomonas campestris*, and lactic acid bacteria are some of the bacteria responsible for spoilage (Tournas, 2005). Spoilage problems manifest themselves in produce attributes, such as appearance, texture, flavor, and nutritional quality, which can limit their shelf life (Mahajan et al., 2017). This limited food shelf life due to spoilage bacteria exacerbates food waste, with a 2009 Food and Agriculture Organization study estimated that 32% (weight based) of all produced food is lost or wasted (Lipinski et al., 2013). Because of the limited time produce handlers can offer fresh produce, there is a substantial financial benefit for such companies to find ways to extend produce shelf life and reduce waste (Pan, Cheng, & Sun, 2019).

One of the methods employed during postharvest fresh produce

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handling to increase shelf life is chlorination. Chlorination refers to three U.S. Environmental Protection Agency (EPA) approved postharvest techniques using: Chlorine gas, Calcium hypochlorite, and Sodium hypochlorite (Suslow, 2000). Chlorine gas is the cheapest, but has the most rigorous safety demands, because of its toxic nature. Chlorine gas is, however, rarely used as it is reserved for large-scale decontamination, not easily adaptable by postharvest fruit and vegetable operations (Boyette et al., 1993). Calcium hypochlorite is the most common method employed for postharvest purposes. Calcium hypochlorite tablets or granulated powder is dissolved in tepid water and then added to wash tanks or hydrocoolers. Sodium Hypochlorite, the active ingredient in most household liquid bleaches, is only used on a limited postharvest scale, because it is hard to store and more expensive than Calcium hypochlorite. While chlorination has been the method of choice for many decades, it has limited effectiveness and can create undesirable byproducts such as chloroform or other trihalomethanes that are suspected carcinogens at high concentrations (Suslow, 2000). Being more aware of the potential risks of chlorination and customer advocacy for minimal produce treatment, there is an urgent need to find other methods of produce treatment.

In recent years, there have been advancements in shelf-life extension methods, including high-pressure processing, pulsed electric field, pulsed light application, and ozone washing (Pan et al., 2019; Pan, Sun, & Han, 2017). Despite their progress in non-thermal physical processing, these novel approaches have some limitations. For instance, high-pressure processing applies pressures between 50 and 1000 MPa to liquid or solid foods, with or without packaging. While it is less intense than heat treatments like drying, it can still cause issues such as a loss of structure stability and changes in fruit tissue properties, particularly concerning high-porosity foods like strawberries (Tao et al., 2014). Pulsed electric field involves applying short bursts of high voltage electric fields to achieve microbial inactivation or modify food structure. However, it is not well-suited for solid foods, as they cannot form the necessary air bubbles for this treatment (Syed et al., 2017). Another method, pulsed light application, uses intense, short-duration pulses of white light to eliminate bacteria on food, food contact surfaces, and packaging. However, it suffers from inefficiencies due to the irregularities on food surfaces, leading to some microbes escaping the treatment (shadowing) (Mandal et al., 2020). Considering these drawbacks, further research is necessary to explore additional decontamination methods that can overcome these challenges.

One new non-thermal method of food decontamination and preservation is the use of non-equilibrium plasma. Non-equilibrium plasma is also called non-thermal plasma or "cold" plasma. Air plasma at atmospheric pressure delivers localized heat, charged particles, electric fields, ultraviolet (UV) photons, and reactive oxygen and nitrogen species (RONS) (Laroussi, 2009; Laroussi & Leipold, 2004). Besides applying plasma directly to materials for disinfection, plasma-activated water (PAW) can also act as an effective bactericidal solution (Kamgang-Youbi et al., 2009; Thirumdas et al., 2018). PAW is generated by allowing non-thermal plasma to interact with water, leading to the transfer of reactive species from the gas phase to the liquid. PAW derives its antimicrobial properties from the interactions between the microorganism and the RONS within the solution, including hydrogen peroxide (H_2O_2), nitric acid (HNO_3), and peroxynitrite (ONOO-), among others (Girard et al., 2016; Lu et al., 2016; Zhou et al., 2018). PAW treatments only involving reactive species can avoid damaging the food structure and can maintain the quality of the harvested fresh fruit (Sakudo, Yagyu, & Onodera, 2019). Previous research has considered that Plasma-activated mist (PAM) act as a more concentrated form of PAW which can more efficiently transport plasma-generated reactive species to the produce surface because of their high interfacial surface area (Ranieri et al., 2019). PAM contains the same antimicrobial properties as PAW but is more compatible with the emerging industry trends because of its low water consumption. It is also proposed that PAM could maintain an even higher level of food structural integrity as it would avoid unnecessary contact with excess water, which can adversely affect some types of fresh produce such as strawberries.

In this study, PAM was generated using a microsecond pulsed dielectric barrier discharge system (mspDBD), in order to evaluate its efficacy for inactivation of *E. coli* O157:H7 on leafy greens such as spinach, kale, and lettuce and high-porosity fruits such as strawberries. Both single pieces and multiple layers of fresh produce were tested in the PAM system. In addition, a diffusion model was developed to describe the transportation of PAM and provide the possibility of calculating the distance PAM can travel—creating a path toward to scale-up of PAM.

2. Materials and methods

2.1. Plasma-activated mist (PAM) system

All experiments were performed using the mspDBD plasma treatment system that was described in detail by Ranieri et al. (2019). In brief, the system consisted of several parts, including three concentric electrodes to generate plasma, nebulizers to generate average 0.3 μm water droplets from tap water, and a 100 L container (mini refrigerator) where fresh produce was treated in. The mini refrigerator contains an internal circulation fan, aiding in achieving homogeneity within the chamber. The high voltage pulses were delivered at 26 kV, 3500 Hz, with a 100% duty cycle. The nebulizers (Model 241 V, Sonaer Ultrasonics, USA) were used to generate droplets inside the bucket. Unfiltered compressed air carried the droplets from the bucket to the plasma discharge. The air flowrate was varied at 10–100 SLPM range. A vertical tube connecting the bucket and electrodes was used to remove larger droplets by gravity. Three identical DBD electrodes were employed. Droplets were treated in a 12.5 cm discharge zone and entered the refrigerator through the top inlet (Fig. 1).

2.2. Bacterial culture

Rifampicin resistant strain of *Escherichia coli* O157:H7 (ATCC 700728) was kindly provided to us by Dr. N. Nitin, University of California at Davis, Davis, CA, USA. Strain O157:H7 was cultured by inoculating 2 mL of Tryptone Soy Broth (Becton Dickinson, USA) containing 25 μL of 10 mg/mL rifampicin (Sigma Aldrich, USA) with 0.4 mL of *E. coli* O157:H7 suspension and incubated at 37 °C overnight. The final bacteria concentration in colony-forming units per mL (CFU/mL) was adjusted for each experiment by using McFarland standards (Sutton, 2011), which was confirmed by plating on Tryptone Soy Agar (TSA).

2.3. Preparation of fresh produce

Strawberries, chopped kale, iceberg lettuce, and baby spinach were purchased from a local supermarket and stored at 4 °C a day before the experiment. Strawberries, spinach, and kale leaves of similar size were selected and placed on a petri dish for experiments. Iceberg lettuce was torn into pieces with similar sizes of spinach and kale. *E. coli* O157:H7 was spot inoculated on the leaf and the non-cut side of the strawberry using 0.1 mL aliquots with concentration of 10^7–10^8 CFU/mL. After inoculation, samples were left in a safety cabinet to completely dry (approximately 2 h).

2.4. Decontamination tests

A single piece of fresh produce was placed on a petri dish and then treated in the systems for up to 60 min. In each experiment, replicates were placed on each shelf, and a total of 8 samples were in the refrigerator. The refrigerator was turned on during each experiment that resulted periodic fluctuations of humidity and temperature inside the chamber (Fig. S3). After running for 10 min, the temperature ranged between 7 and 11 °C, while humidity fluctuated between 40% and 70%.

For multiple layers of fresh produce tests, *E. coli*-inoculated spinach

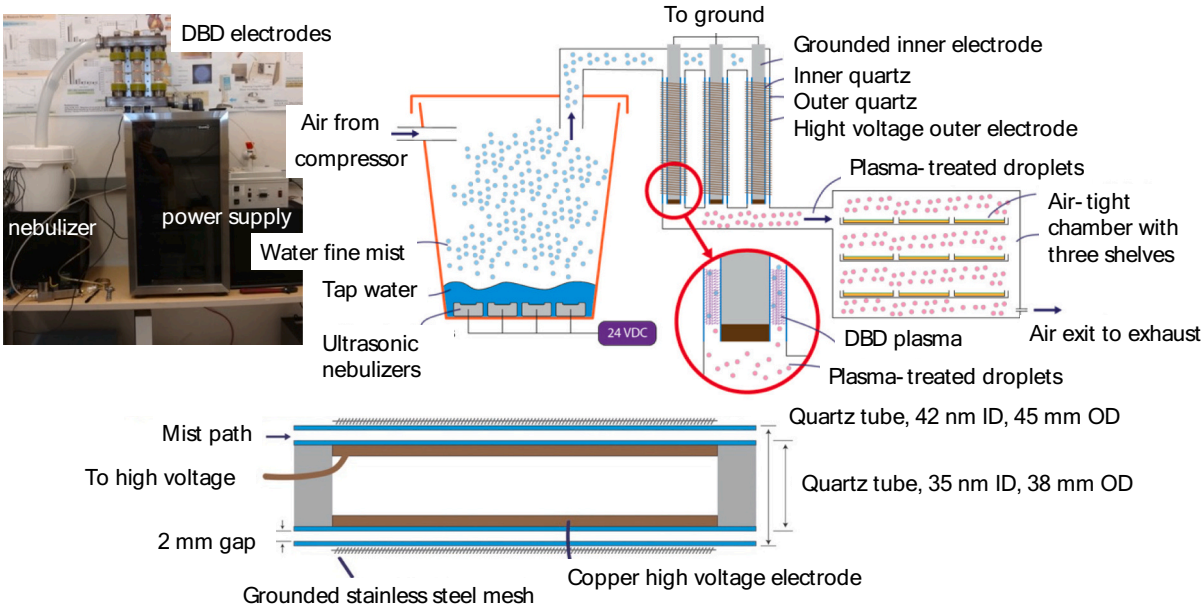


Fig. 1. (top) Photograph and schematic of the 4 ft^3 plasma misting chamber; and (bottom) schematic of a single cylindrical DBD electrode (Ranieri et al., 2019).

was manually stacked and placed in two types of containers and then treated with PAM. Thirty six grams of spinach were placed in a 500 mL glass beaker (diameter 9 cm) and set in the mini refrigerator directly (Fig. 2 D). After treatment, the spinach was sampled from three locations (top, middle, and bottom) (Fig. 2 C). Secondly, 100 g of spinach was placed in a plastic tube open on both sides (diameter 12 cm) (Fig. 2 A). The plastic tube was connected to the entrance of PAM. PAM went through the tube from the top and left from the bottom of the tube. There were plastic shelves with small holes in the tube to split spinach equally into 3 groups. Similarly, 58 g of kale or 400 g of strawberry were placed in the tube for experiments.

2.5. Microbial analyses

After treatment, produce samples were placed in bags and stomached with 10 mL of sterile phosphate buffered saline (PBS) for 120 s in a stomacher (Seward, UK) at normal speed. A volume of 0.5 mL of appropriate dilutions in PBS or undiluted suspension from each stomacher bag was plated on replicate rifampicin-coated TSA plates and cultured overnight at 37 °C for colony counting.

2.6. Data analysis

Data sets were analyzed using GraphPad Prism 9. The *P*-value of <0.05 is considered significant. The data are means ± standard deviations (SDs).

3. Results and discussion

3.1. Inactivation of E. coli on tryptic soy agar (TSA)

To adjust the plasma system, the inactivation efficacy of PAM was measured *via* treating the *E. coli* inoculated TSA in the system at different plasma power and flowrate settings. The concentration of *E. coli* inoculated TSA was approximate 10^5 CFU/plate. The *E. coli* inoculated TSA was placed in different shelves in the fridge system and treated for 15 or 30 min at three flowrates from 10 to 100 SLPM. We observed that at 10 SLPM, the PAM inactivated *E. coli* more effective (Supplementary Material Fig. S1). Longer treatment time and lower flowrate had a significant higher antimicrobial efficacy which was agreed by previous study (Ranieri et al., 2019). There was no notable difference in the count of *E. coli* on TSA observed after treatment across each shelf which indicated PAM was homogenized in the chamber.

We tested the inactivation efficacy by changing the plasma power

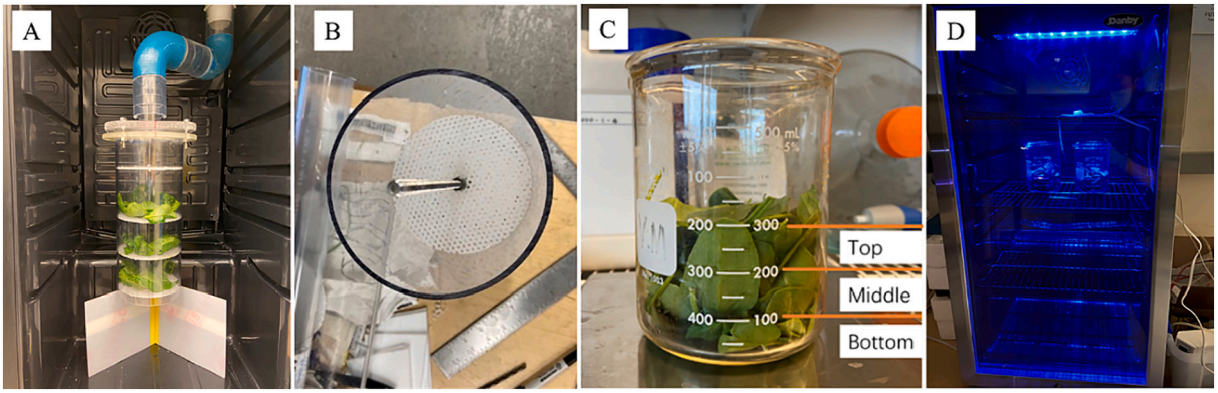


Fig. 2. Experiment setup of multiple layers of spinach treated in PAM. A and B are experiments with the tube, and C and D are experiments with the beaker.

while keeping the same gas flowrate (10 SLPM). The cell counts of *E. coli* on treated and untreated plates were compared (Fig. 3). We observed that at 10 SLPM of gas flowrate and 10 min plasma residence time, 30–40 W had an average of 500 colonies (2.7-log) left on the plate, 40–50 W had an average 108 colonies (2.0-log), and 60–65 W had 24 colonies (1.4-log) remaining, while untreated TSA had approximately 10^5 colonies (5-log). Replicates of plates were located on each shelf of the 4 shelves in the mini refrigerator and no significant difference of the remaining cell count was found between each shelf after treatment ($P = 0.6684$, ANOVA). In addition, higher power of PAM resulted in lower cell counts remained after treatment ($P < 0.001$, ANOVA). Our results of a higher power having better antimicrobial efficacy is in agreement with Xu et al. (2017), which found higher frequency of DBD plasma, in other word, in higher energy can produce more H_2O_2 in droplets that can have a greater impact on microbes.

3.2. Inactivation of E. coli on single pieces of fresh produce

The inactivation of *E. coli* deposited on fresh produce is shown in Table 1. The gas flowrate was 10 SLPM and power was 60–65 W. The inactivation of *E. coli* on spinach resulted in 1-log reduction in 10 min treatment and increased the efficiency to 1.8-log in 60 min treatment. The treatment achieved approximately a 3-log reduction in inactivation efficiency of *E. coli* on kale after 60 min. *E. coli* on lettuce and strawberry has relative lower efficiency that was low as 1.5-log and 0.8-log removal, respectively. The lower removal of *E. coli* from lettuce and strawberry compared to kale may be due to their higher roughness and lower hydrophobicity. Bacteria tend to attach or be entrapped in grooves or cavities, providing protection against washing treatments. This is supported by the positive correlation between roughness and adhesion rate of bacteria, and the negative correlation between roughness and inactivation efficiency by various treatments such as electrolyzed water and peroxyacetic acid (Wang et al., 2009). Studies have reported that the surface roughness of kale, spinach, lettuce, and strawberry ranges from 1 to 2, 3, 2–5, to 287 μm (Adhikari et al., 2015; Kinsinger et al., 2017; Palma-Salgado et al., 2020), respectively. Additionally, the hydrophobicity (contact angle) of these produce items also varies, with kale being the most hydrophobic with a measurement of 110°, followed by strawberry (76°), lettuce (60°), and spinach being the least hydrophobic with a measurement of 56° (Palma-Salgado et al., 2020; Syamaladevi et al., 2015). In a study by Tan et al., the inactivation efficiency of *Enterobacter aerogenes* on lettuce, kale, and baby spinach surfaces using PAM generated by DBD plasma was highest for spinach and lowest for lettuce (Tan & Karwe, 2021), which is in an agreement with our results using *E. coli*. In addition to surface characteristics of the produce that may impact inactivation, it has also been shown that *E. coli* cells are able to enter the tissue of lettuce leaves to protect themselves from disinfectant exposure (Seo & Frank, 1999).

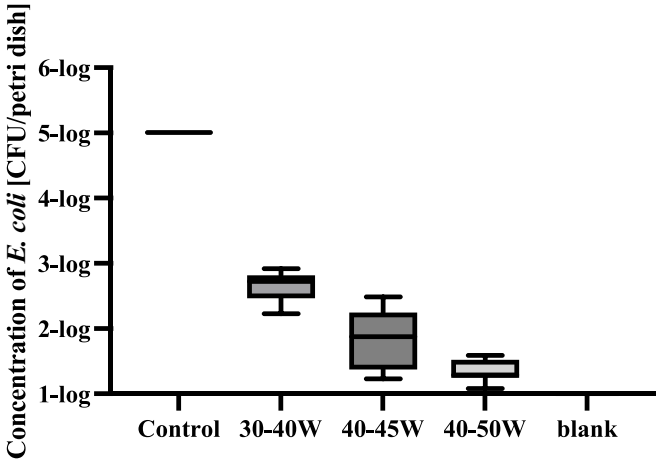


Fig. 3. Plasma inactivation of *E. coli* at different power setting at flowrate 10 SLPM for 10 min ($n = 1$). Eight *E. coli* inoculated plates were placed on 4 shelves in the fridge system. Two plates were on each shelf. Two *E. coli* inoculated untreated plates were set as positive control, and a blank plate (no *E. coli* inoculated) was set as negative control.

Table 1
PAM inactivation of *E. coli* (log reduction) on single piece fresh produce (60–65 W) and on multiple layers of spinach in beaker (140 W) with gas flowrate 10 SLPM. * indicates that the viability of *E. coli* on treated fresh produce were below the limit of quantification (LOQ) (20 CFU/sample, 1.3-log CFU/sample). The values that below LOQ were replaced by LOQ/2 (10 CFU/sample,1-log CFU/sample) for calculation of log reduction. N is the number of the replicates in each experiment.

Treatment Time[min]	Spinach (Single piece)	Kale (Single piece)	Lettuce (Single piece)	Strawberry (Single piece)	Spinach (Multiple layers in beaker)
10	1.0 ± 0.2 n = 4	2.9 ± 0.3 *n* = 4	1.2 ± 0.5 n = 4	0.5 ± 0.4 n = 4	
30		2.7 ± 0.2 *n* = 8	1.1 ± 0.3 n = 8		
60	1.8 ± 0.03* n = 8	3.3 ± 0.4* n = 8	1.5 ± 0.3 n = 8	0.8 ± 0.6 n = 8	0.9 ± 0.1 *n* = 18

Strawberries are a unique type of produce due to their irregular shape, surfaces covered with seeds, and high levels of organic acids (Kallio et al., 2000). Consequently, it has been difficult to effectively decontaminate strawberries using traditional aqueous sanitation methods, such as washing with chlorinated water (Kallio et al., 2000). Research has shown that gaseous ozone and chlorine dioxide can also effectively reduce *E. coli* on strawberries. A 5% (wt/wt) ozone treatment achieved a 1.8-log reduction without affecting the color of the fruit (Bialka & Demirci, 2007), while a 0.6 mg/L chlorine dioxide gas treatment for 30 min achieved a 3-log reduction but caused some bleaching on the green leaves of the strawberry caps (Han et al., 2004). However, the potential formation of harmful by-products or residues from chlorine dioxide treatment requires further investigation. In a study by Trinetta et al., it was found that lettuce treated with 5 mg/L chlorine dioxide for 20 min can achieve 4-log reduction of *E. coli*, but had high levels of residues, including chloride, chlorate, and chlorite. These levels exceeded the Maximum Contaminant Level (MCL) for chlorite in drinking water, which could potentially raise health concerns for consumers (Trinetta et al., 2011).

The efficacy of PAM to inactivate microorganisms is correlated to the formation of RONS. Previous studies examined the pH, oxidation-reduction potential (ORP), electrical conductivity (EC) and the concentrations of hydrogen peroxide (H_2O_2), nitrites (NO_2^-), and nitrates (NO_3^-) in PAM condensate and compared with tap water mist (no plasma treatment) condensate (Tan & Karwe, 2021); He et al., 2022. The presence of H_2O_2 and NO_3^-, coupled with a decrease in pH, suggests that RONS are formed, which is thought to be the key factor in PAM disinfection (Liu et al., 2016).

3.3. Inactivation of E. coli on multiple layers of fresh produce

It is important to note that there is a lack of studies exploring the potential of gaseous treatment methods for the sterilization of fresh produce in bulk. Therefore, in addition to the examination of single spinach leaves, a study was also conducted to evaluate the effectiveness of PAM on piled spinach. Two types of containers, glass beaker and plastic tube, were utilized in the experiment. The beaker containing 36 g of *E. coli*-inoculated spinach were subjected to PAM treatment in the same reaction chamber as single piece fresh produce test at different flow rates ranging from 10 to 80 SLPM and power of 140 W for 1 h. The

results showed that the inactivation efficiency of multiple layers of spinach in the beaker was lower, with a 0.9-log reduction, compared to single piece spinach treated at 60 W (Table 1, independent *t*-test, $p < 0.001$). Furthermore, the inactivation efficiency was measured at different locations within the beaker (top, middle and bottom), and a significant difference was found (ANOVA, $p = 0.0068$) (Fig. 4). No significant difference in inactivation efficiency was observed across the entire group when treated in PAM at different flow rates ($p = 0.3582$). Due to the closed bottom of the beaker and the entry of PAM from the top, it is possible that the top portion of the leaves had more exposure to PAM, resulting in higher inactivation efficiency.

When treated spinach in the tube, the PAM entered the container from the top and left from the bottom. The inactivation efficiency of *E. coli* on spinach were measured by the location in the tube (Supplementary material Table S1). The dosage of PAM was determined by multiplying the power by the duration of exposure (Fig. 5). Results indicated that when treated with a dose of PAM exceeding 2000 W •min, a 1-log reduction in *E. coli* on spinach was achieved, whereas a dose of 1000 W•min resulted in an inactivation of approximately 0.5-log reduction.

Additionally, strawberry and kale were subjected to PAM treatment in the tube generated at a power of 56 W (Fig. 6). The gas flowrate was 20 SLPM and the contact time was 20 min. The results showed that the inactivation efficiency of *E. coli* on spinach after PAM treatment was 0.5-log, while the inactivation efficiency on kale and strawberry was 1-log. The analysis indicated that there was a significant difference between the inactivation efficiency of these three types of fresh produce (ANOVA, $p < 0.001$). Compared to single piece of fresh produce, multiple layers of spinach had lower inactivation efficiency. One possible reason is that the smaller spacing between the leaves results in a reduction in the exposure of the spinach surface to PAM. In contrast, a single piece of spinach placed on the petri dish experiences full exposure to PAM. Additionally, the reduced spacing between the leaves could accelerate the velocity of PAM and more collisions with the produce surface, resulting quicker condensation of PAM on the surface. Except the surface property which may affect the inactivation efficiency (Wang et al., 2009), when fresh produce piled up, different spacing between each produce may affect the circulation and led to the difference of the inactivation efficiency.

3.4. Quality of produce after treatment of multiple layers

The quality of spinach, kale and strawberry after PAM treatment was determined by visual observation (Supplementary material Fig. S2). Results indicated that no significant difference were found between untreated and treated kale and strawberry by 56 W PAM for 40 min, while regional discoloration and signs of surface drying were observed in spinach treated with plasma generated at 140 W and 96 W for 20- and 30-min contact times, respectively. Despite similar doses of plasma treatment (Supplementary material Table S1), spinach treated with 140 W plasma for 20 min (2800 W•min) showed a greater extent of appearance changes compared to spinach treated with 96 W plasma 30 min (2880 W•min) and 56 W plasma for 40 min (2240 W•min), which exhibited minor changes and no noticeable changes, respectively. These visual changes suggest the potential for chemical reactions between the plasma and surface components of the spinach. Furthermore, higher power of plasma treatment, even at the same dose (*i.e.*, lower power but same total energy input), may result in more extensive damage to the spinach.

Our result suggested that using longer treatment time and lower power of plasma can reduce the damage of the surface but achieving the same inactivation effects as shorter treatment time and higher power. The system can be adjusted to fit different needs by various produce. On the other hand, the composition of PAM can be adjusted by adding chemicals into the water reservoir. For example, addition of H_2O_2 can increase the inactivation effects (Golkowski et al., 2012; He et al., 2022) and have a safe level of H_2O_2 residue after one day storage (Song & Fan, 2021).

4. Development of diffusion model for explaining inactivation efficiency during treatment of multiple layers by PAM

In order to assess the effectiveness of PAM for treating large volumes of fresh produce, a transport model was developed for multiple layers of spinach and its inactivation kinetics were evaluated experimentally.

The PAM is the mixture of fine droplets and reactive species. The movement of PAM follows the Fick's Law. It is suggested that the diffusion over a distance is

$$< x^2 >= q_i D t \tag{1}$$

Where x is the mean distance a molecule will have diffused in a certain time, t. $<x^2>$ is the mean-square displacement. D is the diffusion coefficient and assumed to be 0.3 cm^2/s. q_i is numerical constant which depends on dimensionality: $q_i = 2$, 4, or 6, for 1, 2, or 3 dimensional diffusion. In our study, a 2D model for the PAM transport is built. So the diffusion time is

$$t = \frac{x^2}{q_i D} = \frac{x^2}{4D} \tag{2}$$

We introduced a coefficient η to the equation because PAM has a probability to collide, stick or react to the surface of fresh produce. If PAM stick on the surface of produce, we considered the life-time of it was finished. Life-time (τ)of active species in PAM is

$$\tau = \frac{x^2}{4\eta D} \tag{3}$$

Where η is the probability of active species to stick on or react with the surface of produce. $\eta \approx 1$ for mist droplets size >10 μm, or when the active species has short a life-time such as O_2^-. $\eta \approx 0.1$ for mist droplets size 0.5–5 μm. $\eta \approx 10^{-3}$ for long living species like O_3.

Through the measurement of gas velocity v at the point before

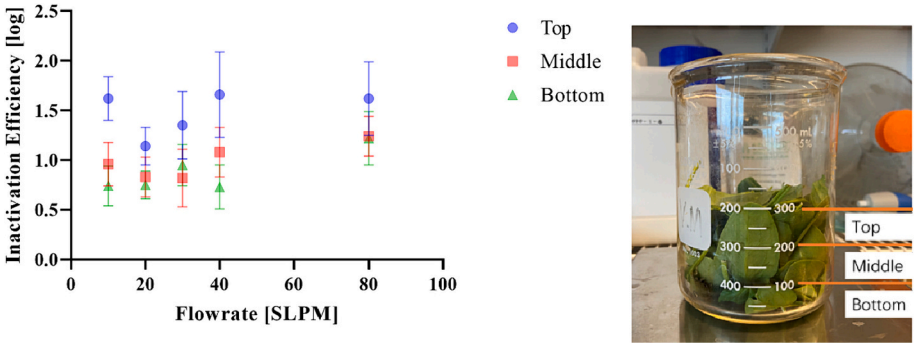


Fig. 4. Inactivation efficiency of *E. coli* on multiple layers of spinach treated in beaker by PAM generated by 140 W power at different flowrate for 60 min.

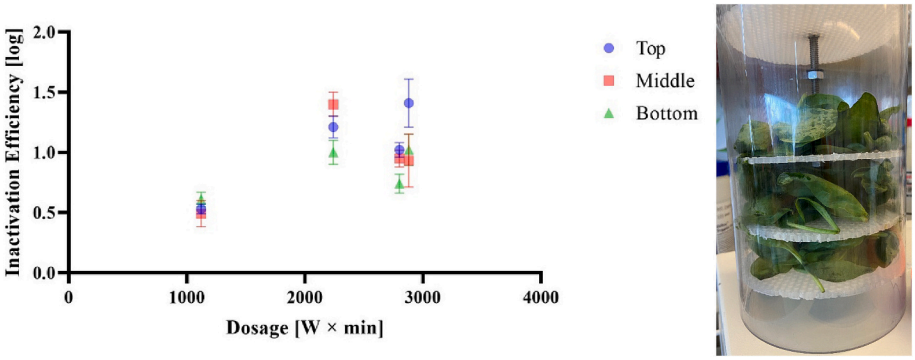


Fig. 5. Inactivation efficiency of *E. coli* on multiple layers spinach in tube at different dosage. Power range in 56 W to 140 W and treatment time range in 20 to 40 min. Flowrate is 20 SLPM for all experiments.

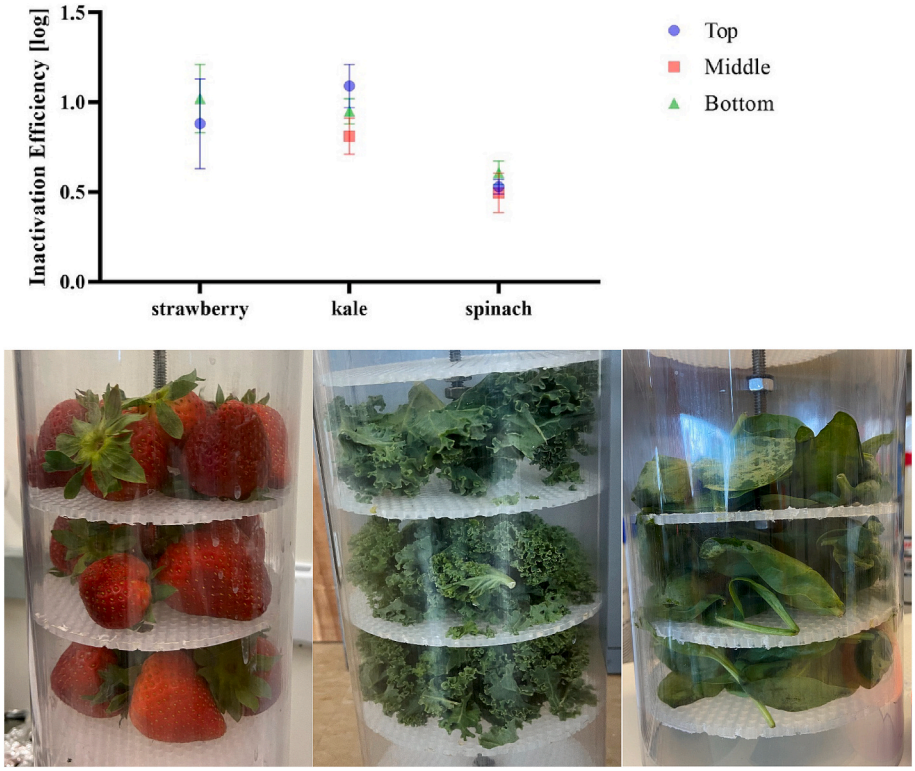


Fig. 6. Inactivation efficiency of *E. coli* on strawberry, kale and spinach treated in tube by PAM generated by 56 W power and 20 SLPM flowrate for 20 min.

spinach, the velocity of PAM in the gap of spinach v' can be estimated (Fig. 7):

$$v' = v/Re_{cr} \tag{4}$$

Re_{cr} is the Reynolds number. The vertical displacement of PAM travel between spinach Z during time t is

$$Z = v't \tag{5}$$

Therefore, the front of PAM displacement Z_f is

$$Z_f = \tau \frac{v}{Re_{cr}} = \frac{R^2 V}{4\eta D Re_{cr}} = R\frac{RV}{D}\frac{1}{4\eta Re_{cr}} \tag{6}$$

Where R is the average distance between spinach leaves. In this study, we assume $R = 0.3$ cm. The probability of disinfection by PAM follows the Poisson Distribution.

$$P_D = P_0 exp\left(-\frac{Z}{Z_f}\right) = D_0 exp\left(-\frac{Z}{R}\frac{D}{RV}4\eta Re_{cr}\right) \tag{7}$$

Where P_0 is the effectiveness of disinfection on the surface at $Z = 0$. The log reduction ratio, denoted as JJ, is a measure used to evaluate the effectiveness of disinfection. In this study, JJ is calculated based on the log reduction at three specific locations within the container where the fresh produce was treated. A JJ value closer to 1 indicates a more even distribution of disinfection efficacy throughout the treated space. The effectiveness of PAM can be evaluated through the calculation of JJ by the following equation:

$$JJ = \frac{logTop - logMiddle}{logTop - logBottom} = \frac{h}{2R}\frac{D}{RV}\alpha \tag{8}$$

$\alpha = 4\eta Re_{cr}$, a parameter for the system and type of fresh produce. Log*Top*, log*Middle* and log*Bottom* represents the log reduction of *E. coli* on top, middle and bottom portion of spinach.

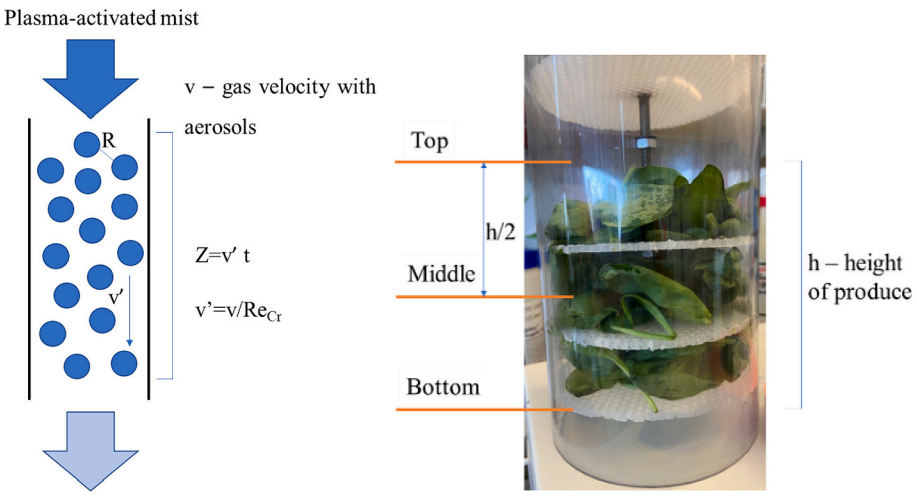


Fig. 7. Schematic of PAM movement in tube and beaker.

5. Modeling results

Mathematical modeling has become popular in recent years as an alternative to conducting complex experimental studies. Researchers have used modeling to explore various aspects of storage rooms such as airflow, heat transfer, temperature distribution, humidity distribution, and chemical distribution like fungicides and chlorine dioxide (Chourasia & Goswami, 2007; Delele et al., 2009; Delele et al., 2012; Hoang et al., 2000; Nahor et al., 2005; Park et al., 2019). However, to our knowledge, there is currently no research on mathematical modeling for PAM disinfection in food applications. Therefore, the goal of this study was to develop a lab-scale PAM treatment chamber to pave the way for constructing a factory-scale treatment module.

In this study, we evaluated the effectiveness of PAM treatment on multiple layers of spinach in two types of containers, a glass beaker with an open top and closed bottom, and a tube with open top and bottom. The log reduction ratio, represented by *JJ*, was calculated from experimental data and the velocity (v) was calculated from the gas flowrate (SLPM) in cm/s (Supplementary material Table S2). The results showed that the relationship between *JJ* and v followed eq. 8, when the spinach was treated in the tube, but deviated from eq. 8 when treated in the beaker, possibly due to the more complicated movement of PAM in the beaker (Fig. 8). In both containers, the relationship between α and v followed the tendency described in eq. 8. The parameter α is dependent on various factors, such as the type of fresh produce being treated, the configuration of the plasma system, and the airflow rate, and is calculated from the experimental data according to the eq. 8. Once α is determined through small scale tests, it can be used to predict the efficacy of the plasma system for larger volumes of fresh produce, which can help to guide the scaling up of the technology for industrial applications.

Although this study modeled PAM transportation, further experiments are necessary to better understand PAM transport and flow dynamics within the reactor. In the future, the model should include more parameters such as bulk density of the fresh produce, saturation time, decomposition time, and half-life of PAM (Pandiselvam et al., 2017). Additionally, testing with different types of fresh produce and bacteria is needed.

In contrast to other laboratory-scale plasma reactors, the mspDBD plasma misting system shows considerable promise for industrial upscaling. This potential arises from its energy-efficient design, concentrating energy use in PAM generation, which facilitates the effective preservation and delivery of reactive species across extended distances. Recent research proposes that PAM can effectively treat large quantities of seeds on an industrial scale (El Shaer et al., 2020), and enhance nitrogen delivery to plant roots, thus improving energy efficiency at a larger scale (Gao et al., 2023). While the effectiveness of PAM in food decontamination is mainly explored in laboratory settings (Song & Fan, 2020; Tan & Karwe, 2021), we believe its scalability for industrial use in the future. However, this transition requires further investigations into factors such as energy cost and environmental impact. The shift from laboratory-scale experiments to industrial-scale deployment is requiring the development of efficient and robust systems capable of handling large volumes, includes considerations for plasma reactor design, treatment chambers, and overall system engineering.

6. Conclusions

This study demonstrated the inactivation efficacy of plasma mspDBD-activated mist for disinfection of fresh produce at a laboratory scale. A lower gas flowrate and higher plasma power input setting demonstrated the best inactivation of *E. coli* O157:H7 on different produce and on nutrient agar plates. Of the different fresh fruits and

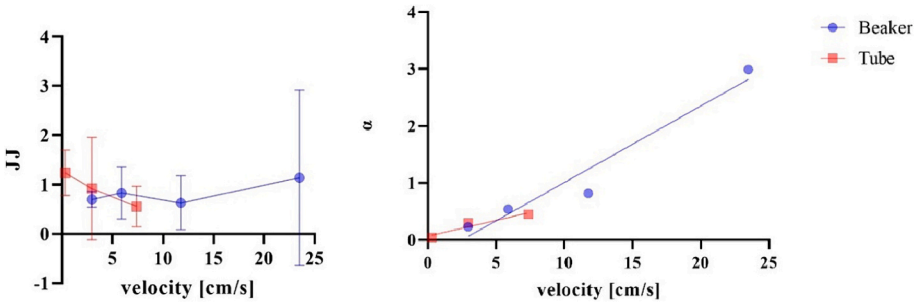


Fig. 8. Relation of velocity with *JJ* and α in beaker and tube.

vegetables examined, strawberry was found to have relatively low inactivation efficiency and slow decay rates when treated by single piece comparing to spinach, kale and lettuce. The efficacy of PAM in inactivating *E. coli* was also evaluated in multiple layers of fresh produce, including spinach, kale, and strawberry. Results indicated a minimum reduction of 1-log in *E. coli* after treatment. Multiple layers of fresh produce had less inactivation efficiency compared to a single piece which may be because of the lower exposure to PAM. To examine the feasibility of scaling up plasma systems for industrial use, a diffusion model was developed that considered the flow velocity of PAM and the inactivation efficiency at different depths of the multiple layers of spinach being treated. Overall, the results from this paper suggest that the mspDBD-generated plasma mist has the potential to effectively reduce pathogens in fresh produce postharvest.

CRediT authorship contribution statement

Jinjie He: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Sidney Ortiz:** Investigation. **Sudipti Attri:** Investigation. **Charles Bailey:** Project administration, Funding acquisition. **Alexander Fridman:** Writing – review & editing, Methodology, Investigation. **Gregory Fridman:** Writing – review & editing, Validation, Funding acquisition. **Christopher M. Sales:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order improve the language. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ifset.2024.103634.

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