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Effects of cold plasma treatments on spot-inoculated *Escherichia coli* O157:H7 and quality of baby kale (*Brassica oleracea*) leaves

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ABSTRACT

The efficacy of non-equilibrium atmospheric pressure-pulsed dielectric barrier discharge (cold plasma)-treated mist in disinfecting baby kale leaves, and its effect on color values and cuticle composition were evaluated. UV-treated baby kale leaves were spot-inoculated (0.04-mL of 10^6 CFU/mL) with rifampicin-resistant strain *E. coli* O157:H7 ATCC 700728 incubated (0–4 °C) overnight and treated with plasma mist at 60, 120, 180, 240 or 300 s. Treated and untreated leaves were analyzed for change in color values (L*, a*, b*, chroma, hue, and browning index (BI)) using a Chroma meter. Functional groups (alcohols, esters, aldehydes, and ketones) were determined in the cuticle using FTIR. Color stability of treated leaves was also evaluated after refrigerated storage (4 °C) for 12 days. Levels of *E. coli* O157:H7 on the kale leaves were reduced below the detection limit of 5.5×10^3 CFU/mL after plasma treatment for 300 s with no significant change in color values. Visible change in color (browning or leaf damage) was observed after 600 s of plasma treatment. Color stability of plasma-treated leaves was enhanced during refrigerated storage (4 °C), indicated by a lower BI value of 34.4 ± 5.4 at 120-s plasma treatment compared to untreated leaves after 12 days of storage. Fourier transform infrared (FTIR) spectroscopy analysis concluded that plasma treatment of kale did not negatively affect functional groups in the cuticle. This study demonstrated that cold plasma mist has the potential to reduce *E. coli* on the surface of baby kale leaves with no significant change in the color values.

Industrial relevance: Kale leaves are consumed worldwide owing to their health-promoting and nutritional benefits. However, in the past two decades there has been an increase in foodborne diseases outbreaks caused by microorganisms associated with leafy vegetables. The food industry faces challenges in improving food safety while maintaining quality of the food. Chemicals in wash water or heat treatment (70–121 °C), commonly used methods for killing microorganisms, can have negative effect on the aesthetic (color) and nutritional quality of the product. Cold plasma treatment of fresh produce, an emerging food preservation technology, is an efficient, quick, and environmentally-friendly method of disinfecting the product at low temperature (30–40 °C). This study demonstrated that treatment of baby kale with cold plasma mist not only inactivated *E. coli*, but also enhanced its color stability during refrigerated storage. Hence, cold plasma mist has a potential application as an efficient disinfection technology, thus enhancing shelf-life of fresh leafy greens.

1. Introduction

Fresh produce and leafy greens in particular have been implicated in foodborne disease outbreaks on a global level (Gajraj, Pooransingh, Hawker, & Olowokure, 2012). The number of leafy vegetable-associated outbreaks reported to Centers for Disease Control and Prevention (CDC) between 1973 and 2012 was higher than that of outbreaks caused by other food types (Herman, Hall, & Gould, 2015). Currently,

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antimicrobial interventions on fresh produce consist primarily of washes with a few approved chemical agents such as sodium hypochlorite and peroxyacetic acid/hydrogen peroxide. With these agents, it is necessary to find concentrations that are effective in inactivating undesirable microorganisms, but do not have negative effects on the quality of treated produce.

Cold plasma treatment is an emerging green process technology for decontamination of fresh fruit and vegetables (Pankaj et al., 2014). Plasma is a partially ionized gas consisting of ions, electrons, uncharged atoms and molecules, as well as radicals (Hoffmann, Berganza, & Zhang, 2013). Non-thermal plasma discharges can be generated under ambient temperatures. Therefore, cold plasma technology has the potential of reducing costs on an industrial scale compared to various non-thermal disinfection treatments such as ionizing radiation, high hydrostatic pressure, pulsed electric field, oscillating magnetic field and high power ultrasound (Kim et al., 2011).

Various studies have been carried out to evaluate the ability of cold plasma treatment in inactivating foodborne pathogens including gram-negative, gram-positive, and biofilm-forming bacteria. Liu et al. (2010) reported complete inactivation of *Staphylococcus aureus* suspended in aqueous solution after 16 min of treatment with a cold atmospheric pressure air plasma microjet. Their study also indicated that acidity of the liquid solution and interaction of plasma-activated species (radicals) resulted in peroxidation of fatty acids in the cell membrane that was responsible for inactivation of *S. aureus* in aqueous suspension. Decrease in microbial load was observed for apples inoculated with *E. coli* O157:H7, cantaloupes inoculated with *Salmonella*, and lettuce inoculated with L. *monocytogenes* when treated with a uniform glow discharge plasma (Critzer, Kelly-Wintenberg, South, & Golden, 2007). Biofilms produced by *Bacillus subtilis* spores on polycarbonate membranes supported by a layer of agar in a petri dish were inactivated by dielectric barrier discharge (DBD) with a helium plasma plume (Deng, Shi, & Kong, 2006). Their results showed that while reactive oxygen species (ROS) generated by plasma caused leakage of cytoplasmic components and complete rupture of the bacterial spore membrane, UV photons and the electric field made little or no contribution towards spore inactivation. In the present study, we evaluated the efficacy of cold plasma (non-equilibrium atmospheric pressure-pulsed dielectric barrier discharge)-treated mist in disinfecting baby kale leaves inoculated with *E. coli* O157:H7.

The aim of a disinfection technology is not only the inactivation of pathogens, but also to prolong shelf-life while maintaining nutritional and organoleptic food quality (Misra, Oliver, & PJ, 2016). Previous studies have reported both positive and negative effects of cold plasma on the quality of fresh produce. No change in quality was observed when fresh lettuce (color, antioxidant activity, and ascorbic acid) and strawberry (texture, firmness, color and pH) were treated with atmospheric plasma jets and DBD in-package plasma, respectively (Smeu et al., 2012; Misra et al., 2014). While carrots and tomatoes treated with a cold argon plasma needle showed insignificant change in color (0.5 to 10 min treatment), lettuce exhibited a change in color under the same treatment conditions beyond 7 min (Bermúdez-Aguirre, Wemlinger, Pedrow, Barbosa-Cánovas, & Garcia-Perez, 2013). Song et al. (2015) reported an increase in yellowness of lettuce after refrigerated storage of microwave-powered nitrogen cold plasma treatment. Another study reported loss of freshness, color, odor, texture, and overall acceptability of DBD plasma-treated radicchio leaves (Pasquali et al., 2016). Changes in food quality following cold plasma treatment can occur because reactive species catalyze oxidation and peroxidation processes inside the cell membrane not only of adhering microorganisms, but also the cells of produce being treated. Plants generally have cuticles covering the epidermal surfaces of leaves as a protective barrier (Chaumat & Chamel, 1991; Heredia-Guerrero et al., 2014). The wax components present in the cuticle are responsible for maximum resistance to moisture loss, insects, and pests (Lamikanra, Imam, & Ukuku, 2005; Znidarcic, Valič, & Trdan, 2008). The cuticle and its components (fatty acids, alcohols, aldehydes, esters, and alkanes) are critical in maintaining freshness and aesthetic quality of produce (Domínguez, Heredia-Guerrero, & Heredia, 2010). No study till date has investigated the effect of cold plasma treatment on cuticles of fresh leafy vegetables.

For the present study, a non-equilibrium atmospheric pressure-pulsed DBD with a single-dielectric layer on the inner electrode was designed. The effect of micrometer-sized water droplets (mist) passing through this system was investigated with respect to inactivation of *E. coli* O157:H7, color values and cuticle composition of baby kale leaves. Furthermore, the effect of DBD cold plasma mist treatment on color stability of baby kale leaves during storage at 4 °C for 12 days was determined.

2. Materials and methods

2.1. Plant material

Baby kale leaves (Simple Beginnings, MA, USA) were procured from a local supermarket and stored at 4 °C until use. The samples were treated and analyzed within one week of storage.

2.2. DBD cold plasma system

A non-equilibrium atmospheric pressure-pulsed dielectric barrier discharge system (Nyheim Plasma Institute, Camden, NJ) was used as described by Patel et al. (2016).

2.2.1. System setup

A single-dielectric cylindrical DBD system was used for this study. Basic configuration and design of the system are shown in Fig. 1a, b, and c. The system consisted of an inner electrode made of copper covered with quartz (1-mm thick) dielectric layer, and an outer electrode made of stainless steel pipe (3-mm thick). The inner electrode was connected to a high voltage supply and the outer electrode was grounded. The gap between the two electrodes was 1.5-mm. Teflon spacers were used to keep the electrodes parallel while allowing for adequate mist and air passage. For the treatment of baby kale leaves the plasma system was set up at 26 kV, 2500 Hz, and mist flow rate of four SLPM (standard liters per minute).

2.2.2. Plasma mist activation

Mist was generated in a reservoir constituting of four nebulizers, an input tube for controlling mass flow rate of air, and an output tube for mist to reach the electrode. The mist reservoir was filled with tap water to a level below the airflow input tube connected to the mass flow controller. It was kept in an ice bath to prevent an increase in temperature. The output tube from the reservoir was connected to the inlet of the electrode. To produce plasma, electrodes were connected to a high voltage continuous wave power supply. A green laser pointer beam was used to validate generation of plasma from the electrode (Fig. 1b). Two controls were used to evaluate the effect of plasma on kale leaves (a) “tap water mist” when electrode power supply was turned off and plasma was not generated; the output from the electrode was only mist, and (b) “untreated” when samples were subjected to no treatment (neither plasma mist nor tap water mist). Tap water mist was used as a control for microbial load analysis, and tap water and untreated were used as controls for color and cuticle analysis.

2.2.3. Treatment of samples

The treatment of baby kale leaves with plasma mist was performed by placing the leaf in a petri dish under the electrode such that the center of the leaf was directly below (25.4-mm) the electrode. The leaves were treated for 60, 120, 180, 240 or 300 s. Two separate leaves were used for plasma mist and control treatments. Control treatment was performed immediately after plasma mist treatment at each

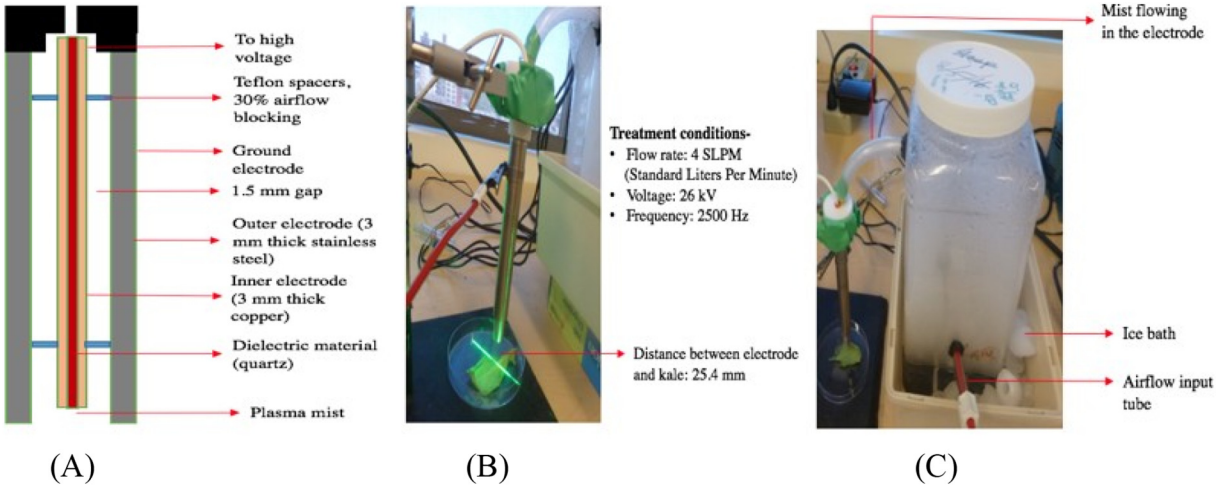


Fig. 1. Cold plasma system setup: (a) DBD electrode (b) Plasma mist (observed with laser), (c) Mist box.
Nebulizers in the mist box were used to generate mist from tap water. Once generated, mist was passed through the dielectric barrier discharge (DBD) electrode on the leaf to be treated. A green laser pointer beam was used to validate generation of plasma from the electrode. The leaf was placed in a petri dish and positioned under the electrode such that the center of the leaf was directly below (25.4-mm) the electrode.

treatment time. Care was taken that before starting a tap water mist treatment, the mist from the electrode was allowed to run for 30 s to purge leftover plasma species from the plasma mist treatment. All treatments were performed in at least duplicates. Leaf surface temperature (measured using infrared contactless thermometer), throughout our experiments, did not exceed 31 °C, except for the 600-s treatment time when the temperature reached 37 °C.

2.3. Analyses

2.3.1. Microbial analysis

2.3.1.1. Preparation of inoculum. E. coli O157:H7 ATCC 700728, a rifampicin-resistant strain, was a gift from Dr. Nitin Nitin (Department of Food Science and Technology, University of California at Davis). The strain was cultured on tryptic soy agar (TSA) plates with rifampicin (concentration: 25-μL of 10 mg/mL). One loop full of the bacteria from the TSA plates were cultured in 5-mL tryptic soy broth containing rifampicin (25-μL of 10 mg/mL). This culture was incubated overnight (18 h) at 37 °C. This stock culture was used to prepare the working bacterial culture (~10^8 CFU/mL) in phosphate-buffered saline by comparison with McFarland standards (10^8 CFU/mL, latex particles; Hardy Diagnostics, Springboro, USA). *E. coli* culture of 75-μL to 100-μL was added to 1-mL of phosphate-buffered saline. The culture was further diluted to 10^6 CFU/mL and used for inoculation on the leaf.

2.3.1.2. Inoculation of leaves. Leaf samples were prepared and inoculated using a modified protocol of Mansur and Oh (2015). Before inoculation, petri dishes containing one baby kale leaf each were placed in a laminar flow hood and treated with UV light (50 min–25 min for each side of the leaf) to kill natural microbiota. The UV-treated leaves were then spot-inoculated (at four locations, 0.01-mL each around the center of the leaf) with the prepared *E. coli* inoculum. In order to allow bacterial attachment to the leaf surface, the samples were air-dried in a laminar flow hood (30 min with no UV light), the petri dishes were then covered and stored in a refrigerator for 24 h. The next day, the leaves were treated individually, with plasma mist or tap water mist for 60, 120, 180, 240 or 300 s.

2.3.1.3. Estimation of E. coli O157:H7 spot-inoculated on leaves. A plasma-treated (plasma mist) or control (tap water mist) leaf was placed in a sterile stomacher bag (7 × 12 in., LabPlas, Hardy Diagnostics, USA) containing peptone water (5-mL of 0.1% (w/v)). The contents of the bag were blended using a stomacher (Stomacher 80 Biomaster, Seward, UK) for 2 min at high speed (300 rpm). A 100-μL aliquot from the bag was spread-plated on TSA plates. Before spread-plating, each TSA plate was plated with 100-μL of rifampicin-dimethyl sulfoxide (DMSO) (rifampicin concentration: 50 μg/mL) solution and allowed to dry for 24 h in the refrigerator away from light (rifampicin is light-sensitive). After spread-plating, the plates were incubated (37 °C for 18 h) and colonies counted using a colony counter and reported as colony-forming units/mL (CFU/mL).

2.3.2. Color analysis

Color (CIE L*, a*, b*) of baby kale leaves was measured using a chroma meter CR-300 (Minolta Inc., Osaka, Japan). The light source for color measurements was D65 with a 2° standard observer. Each time the system was turned on, it was standardized with a white calibration plate (CR-A43). Color values (L*, a*, b*) were measured by placing the measuring head of the chroma meter on the leaf and recording the reading. L*, a*, and b* values were further used to calculate hue angle, chroma and BI according to Sekhon, Maness, and Jones (2015), using Eqs. (1), (2), (3), and (4).

$$\text{Hue angle} = \tan - 1\,(b^*/a^*) \quad (1)$$

$$\text{Chroma} = \sqrt{(a^*2 + b^*2)} \quad (2)$$

$$x = (a^* + 1.75\,L^*)/(5.645\,L^* + a^* - 3.012b^*) \quad (3)$$

$$BI = (100\,(x - 0.31)/0.17) \quad (4)$$

L* indicates lightness (0 = black; 100 = white), a* indicates degree of greenness (negative = greenness; positive = redness), b* indicates degree of yellowness (negative = blueness; positive = yellowness). Hue angle is used to specify the color of the food products such as an angle of 0 to 360 represents red hue, and angles 90°, 180°, and 270 indicate yellow, green, and blue hues, respectively. Chroma is the degree of saturation of the perceived color. BI indicates purity of brown color. Color analysis was performed on whole leaves and cut leaves. For whole leaves, the leaves were treated either with plasma mist, tap water mist or kept untreated for 120, 180, 240 or 300 s. Color values of an entire leaf were measured (six color readings per leaf including three from the center). The area of the leaf directly below the electrode received more mist compared to other areas. For each experiment, the leaves were arranged under the electrode such that the center of the leaf was directly below the electrode. Therefore, three color readings from the center of each leaf were recorded and considered separately for

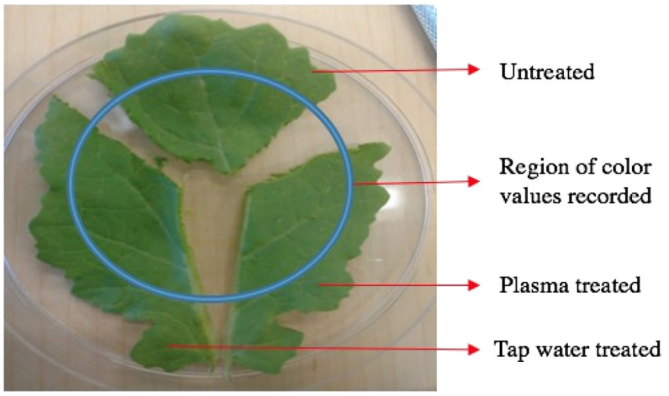


Fig. 2. Cut segments of a baby kale leaf. Encircled region represents the area of color measurement.

To evaluate the variability in color values of different leaves, color values of cut portions of the same leaf subjected to plasma mist, tap water mist or untreated were measured. Three color readings were recorded for each segment such that combined it would form center of the whole leaf.

statistical analysis to observe the difference in the pattern of readings between the center and whole leaf. Each treatment (plasma mist and tap water mist) and treatment time were performed in triplicates. In addition, whole kale leaves were treated with plasma mist longer than 300 s to observe the time at which damage can take place.

To evaluate the variability in color values of different leaves (used for plasma and control treatments), color values of cut portions of the same leaf subjected to plasma mist or tap water mist or untreated were measured. A leaf was cut into three segments from the center as shown in Fig. 2. Each cut segment was treated with either plasma mist (right segment), tap water mist (left segment) or kept untreated (top segment) for 60, 120, 180, 240 or 300 s. Three color readings were recorded for each segment such that combined it would form center of the whole leaf (Fig. 2). Duplicates were performed for each type of treatment and at each treatment time.

2.3.3. Cuticle analysis

Fourier transform infrared (FTIR) spectroscopy was used to determine changes in the primary and secondary alcohols, ester, aldehyde, and ketones present in the cuticles of baby kale leaves according to Villena, DomõÂnguez, Stewart, and Heredia (1999) and Merk, Blume, and Riederer (1997). Whole leaves were treated for 240 s with tap water mist or plasma mist. After treatment, the leaves were immediately placed on top of an ATR (Attenuated Total Reflection) crystal of a Nicolet 8700 instrument (Thermo Scientific, WI, USA). The spectra (40 scans/sample) were recorded with a resolution of 4 cm^{-1}. After analysis of each sample, the system ran a background of air to minimize noise from oxygen and water. The readings were recorded in absorbance mode and the peaks were compared to observe changes in functional groups in cuticle due to different treatments. The graphs were categorized into primary and secondary alcohols (wavelength regions of 900–1400 cm^{-1}), and aldehydes, ketones, and esters (wavelength regions of 1400–1800 cm^{-1}). Triplicates were performed and a representative result is reported. FTIR provides information on the chemical functional groups of the entire leaf and any changes the leaf experiences. The penetration depth into the sample is typically between 0.5 and 2 μm so both surface and internal changes are measured.

2.4. Storage study

Color values of ten untreated whole leaves were recorded on Day 0. From the same package (procured from supermarket), whole leaves were treated with plasma mist or tap water mist for 60, 120, 180, 240 or 300 s. Triplicates were performed for both types of treatments and at each treatment time. The leaves treated with plasma mist and tap water mist and untreated leaves were then individually wrapped in a paper towel, sealed in a Ziploc® bag and stored in the refrigerator at 4 °C for 12 days. All samples from each treatment were placed in the same Ziploc® bag. At the end of the storage period, three color readings (L*, a*, b*) from the entire leaf were recorded. The color values were compared with the controls (untreated and tap water mist leaves) stored under the same conditions.

2.5. Statistical analysis

The data was analyzed using Analysis of Variance (ANOVA) in SPSS version 24 (IBM, 2016), means were compared using *t*-test and Duncan's test at $p = 0.05$, level of significance.

3. Results and discussion

3.1. Effect of DBD cold plasma treatment on E. coli O157:H7 spot-inoculated on leaves

A statistically significant decrease to 0 CFU/mL in the number of *E. coli* colonies from contaminated whole kale leaves was observed with increase in plasma mist treatment time (from 60 to 300 s). To determine the recoverable yield from the inoculum under control conditions for comparison with plasma treated samples, an experiment was conducted by inoculating each leaf with 10^6, 10^7, 10^8 CFU/mL spot inoculation with 0.1 mL of bacterial suspension at four locations. While 10^8 CFU/mL had too many colonies grown on TSA plate and were marked as "too numerous to count" and 10^7 CFU/mL had a growth of $8 \times 10^3 \pm 1004.3$ CFU/mL on TSA plate; 10^6 CFU/mL gave countable colonies of 4.1×10^3 with lower standard deviation of 512.6, therefore 10^6 CFU/mL was chosen as the inoculum concentration for our experiments. The recovery of this inoculum concentration from TSA plates alone (without inoculating on leaves) was $5.2 \times 10^3 \pm 295$ CFU/mL. Based on the above values the recovery from the untreated leaves was between 3.7×10^3 to 5.5×10^3CFU/mL with minimum detectable number of 100 CFU/mL.

At all treatment times, except at 120 s, the colonial count for tap water mist treatment was statistically higher compared to that of plasma-treated leaves (Table 1). Plasma treatment for 240 s exhibited almost negligible number of colonies (0 − 10) and a treatment for 300 s reduced *E. coli* counts to 0 CFU/mL. The difference in microbial load on kale leaves between plasma treatment times 120, 240, and 300 s was statistically insignificant (Table 1). These results indicated that cold plasma mist has the potential to inactivate *E. coli* even at lower treatment times (~120 s). On the commercial scale, rapid disinfection of fresh produce is desired to process high volume of produce efficiently in minimum time.

Table 1
Effect of treatment type on inactivation of *E. coli* spot-inoculated on whole baby kale leaves.

Time (s)	Average CFU/mL of *E. coli* O157:H7	
	Plasma	Tap water
60	$3.3 \times 10^2 \pm 330$a	$7.6 \times 10^2 \pm 358$a
120	$1.4 \times 10^2 \pm 147$ab	$1.3 \times 10^2 \pm 855$a
180	$0.4 \times 10^2 \pm 43$ab	$5.0 \times 10^2 \pm 386$a
240	$0.03 \times 10^2 \pm 3$b	$5.7 \times 10^2 \pm 272$a
300	0.0 ± 0.0b	$8.9 \times 10^2 \pm 837$a

Triplicates were performed at each treatment type and treatment time. Values with different letters (a, b) within the same column are statistically significant (P = 0.05).

Table 2
Effect of treatment type (plasma mist, tap water mist or untreated) on color values of cut baby kale leaves.

Time(s)	Treatment	L*	a* (−ve)	b*	Chroma	Hue angle	BI
	Plasma	40.6 ± 0.5a	13.3 ± 0.2a	19.9 ± 0.3a	23.9 ± 0.4a	179.0 ± 0.0a	35.8 ± 0.6a
60	Tap water	39.8 ± 1.1a	14.2 ± 0.3a	20.9 ± 0.6a	25.3 ± 0.7a	179.0 ± 0.0a	40.6 ± 3.2a
	No treatment	40.8 ± 1.5a	13.1 ± 0.1a	19.7 ± 0.3a	23.7 ± 0.2a	179.0 ± 0.0a	34.9 ± 1.0a
	Plasma	42.2 ± 0.9a	12.3 ± 0.2a	20.5 ± 0.4a	24.0 ± 0.4a	178.9 ± 0.0a	38.8 ± 1.1a
120	Tap water	42.2 ± 0.9a	12.3 ± 0.2a	20.5 ± 0.4a	24.0 ± 0.4a	178.9 ± 0.0a	38.8 ± 1.1a
	No treatment	41.9 ± 0.5a	13.4 ± 0.1a	21.7 ± 0.4a	25.5 ± 0.4a	178.9 ± 0.0a	41.6 ± 1.6a
	Plasma	39.4 ± 0.8a	11.6 ± 0.1a	19.5 ± 0.2a	22.8 ± 0.2a	178.9 ± 0.0a	39.6 ± 1.1a
180	Tap water	39.4 ± 0.8a	11.6 ± 0.1a	19.5 ± 0.2a	22.8 ± 0.2a	178.9 ± 0.0a	39.6 ± 1.1a
	No treatment	40.4 ± 0.3a	13.7 ± 0.2a	21.9 ± 0.4a	25.9 ± 0.5a	178.9 ± 0.0a	44.5 ± 2.1a
	Plasma	37.2 ± 0.8b	11.8 ± 0.3a	17.4 ± 0.4a	21.1 ± 0.6a	179.0 ± 0.0a	33.2 ± 0.5a
240	Tap water	38.4 ± 0.1b	14.0 ± 0.1a	20.5 ± 0.2a	24.9 ± 0.2a	179.0 ± 0.0a	40.9 ± 0.8a
	No treatment	40.9 ± 1.4a	12.5 ± 0.5a	19.2 ± 0.6a	22.9 ± 0.8a	179.0 ± 0.0a	34.7 ± 2.5a
	Plasma	39.3 ± 0.4a	12.0 ± 0.2a	17.7 ± 0.3b	21.4 ± 0.4b	179.0 ± 0.0a	31.0 ± 1.0a
300	Tap water	39.4 ± 0.4a	13.7 ± 0.1a	19.0 ± 0.2a	23.4 ± 0.3a	179.0 ± 0.0a	32.6 ± 1.3a
	No treatment	40.1 ± 0.2a	13.4 ± 0.1a	19.0 ± 0.3a	23.2 ± 0.3a	179.0 ± 0.0a	32.3 ± 1.1a

The leaves were cut into three segments from the center (Fig. 2). Three readings were obtained from the edge (that contributes center of the leaf) of each segment and the average was recorded. Duplicates were performed at each treatment time and for each treatment type.
Color values (L*, a*, b*, chroma, hue angle and BI) with different letters (a, b) within each treatment (plasma, tap water and no treatment) time are statistically significant (P = 0.05).
L* = Lightness, a* = +ve redness and –ve greenness, b* = +ve yellowness and –ve blueness, BI = Browning index.

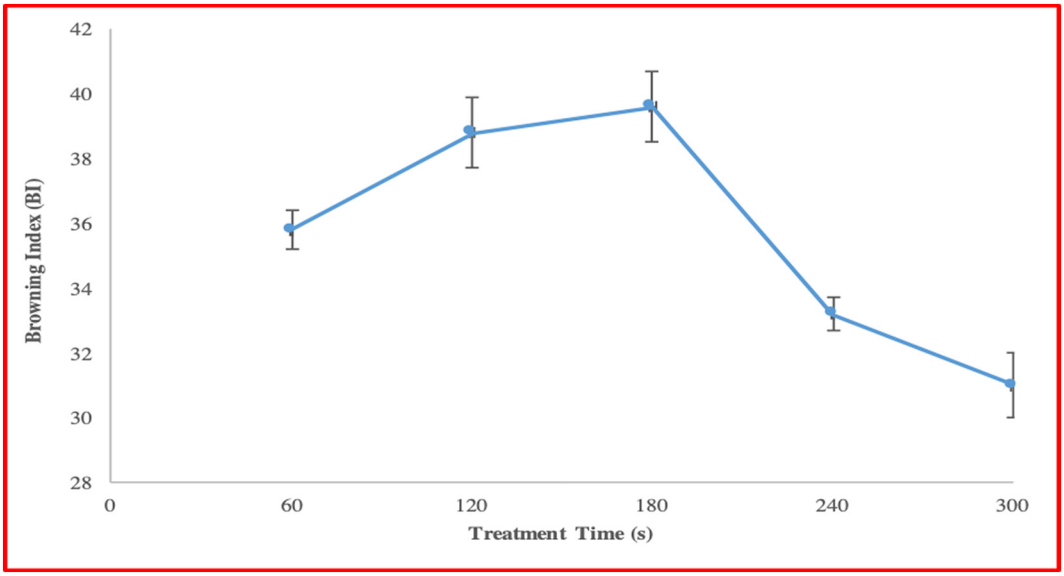


Fig. 3. Effect of plasma treatment time on browning index (BI) values of cut baby kale leaves.
Browning index (BI) values of plasma mist treated leaves increased with plasma treatment time until 180-s and then decreased with further increase in plasma treatment time. However, there was no significant difference between BI values at all treatment times. The decrease in BI values of leaves with plasma treatment time could be due to inactivation of oxidative enzymes.

3.2. Effect of DBD cold plasma treatment on color values of baby kale leaves

Color values of whole and cut leaves were analyzed separately to evaluate if plasma treatment may affect whole and cut leaves differently. It is important to note that each leaf is a biological sample and color values may be different for two leaves from the same batch of produce. Therefore, two different leaves subjected to same treatment may show quality changes to different degrees. In addition, salad mix products also contain pre-cut leafy greens.

3.2.1. Effect of DBD cold plasma treatment on color values of cut leaves

Cut leaves were used to compare the effect of treatment type on color values of different segments from the same leaf. At each treatment time, BI values of plasma mist-treated leaves were either equal or less than that of tap water mist-treated or untreated leaves. This indicated that plasma treatment (at 300 s) did not negatively affect the color values of cut leaves. The difference in hue angle and a* values was not statistically significant between treatment types (tap water mist, plasma mist, and untreated) within each treatment time (Table 2). A statistical insignificant difference in L* values of leaves between plasma mist-treated, tap water mist-treated, and untreated leaf at 60, 120, 180 and 300 s was observed (Table 2). At 300 s, the differences in values of a* and BI values between different treatment types was statistically insignificant. Hence, kale leaves may be treated with cold plasma without change in color values for 300 s compared to control samples (tap water or untreated leaves). Browning of cut leaves is dependent on the activity of oxidative enzymes (polyphenol oxidase and peroxidase) present in the leaves when exposed to air. Our results are in agreement with Bubler (2017) who reported complete prevention of browning in plasma-treated apples and potatoes for 19 days. One reason could be that plasma-inactivated oxidative enzymes involved in enzymatic

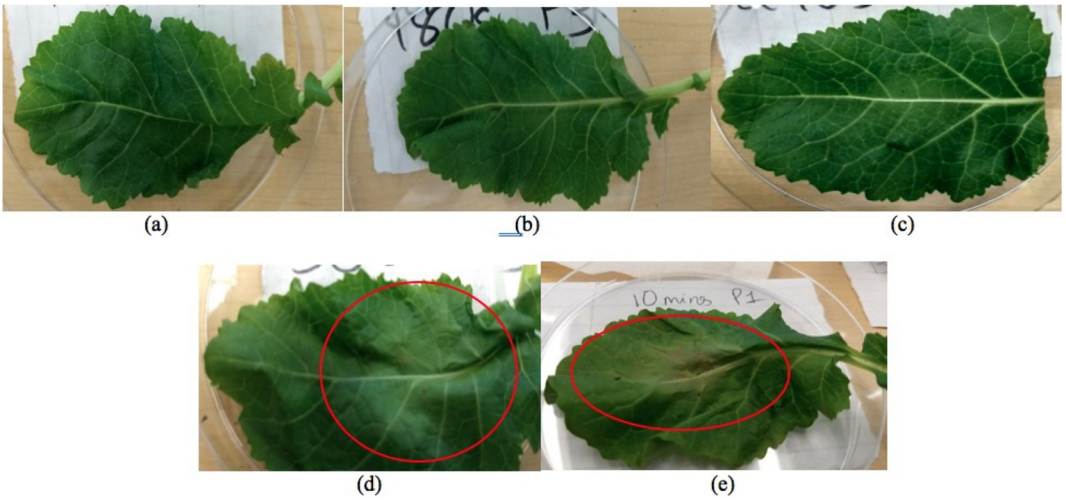


Fig. 4. Effect of plasma treatment time (a) 120 s (b) 180 s (c) 240 s (d) 300 s and (e) 600 s on color of whole baby kale leaves.
A 300 s plasma treatment led to color bleaching at the center of the leaf. However, when leaf was exposed to cold plasma treatment for more than 300 s (for 600 s), extreme browning and damage was observed. Browning may be due to oxidation products from plasma mist formed on the leaf.

Table 3
Effect of treatment type (plasma, tap water or untreated) on color values of whole baby kale leaves.

Time (s)	Treatment	L*	a* (−ve)	b*	Chroma	Hue angle	BI
	Plasma	43.6 ± 1.1a	15.3 ± 0.3a	22.8 ± 0.8a	27.6 ± 0.8a	179.0 ± 0.0a	39.0 ± 2.3a
120	Tap water	44.2 ± 1.2a	15.2 ± 0.3a	23.3 ± 0.7a	27.9 ± 0.7a	179.0 ± 0.0a	40.2 ± 1.2a
	No treatment	45.5 ± 1.3a	15.4 ± 0.3a	24.7 ± 1.3a	29.3 ± 1.0a	179.0 ± 0.0a	43.8 ± 3.5a
	Plasma	45.7 ± 1.6a	15.3 ± 0.3a	24.1 ± 0.9a	28.7 ± 0.8a	179.0 ± 0.0a	41.4 ± 2.5a
180	Tap water	45.0 ± 1.3a	15.9 ± 0.3ab	24.6 ± 0.7a	29.4 ± 0.7a	179.0 ± 0.0a	42.8 ± 1.8a
	No treatment	46.9 ± 1.2a	17.0 ± 0.2b	27.6 ± 0.7a	32.5 ± 0.6a	178.9 ± 0.0a	50.1 ± 1.8a
	Plasma	46.0 ± 1.3a	15.3 ± 0.2a	23.9 ± 0.7a	28.5 ± 0.6a	179.0 ± 0.0a	39.8 ± 1.7a
240	Tap water	45.8 ± 1.3a	13.8 ± 2.0a	25.5 ± 1.0a	30.1 ± 0.8a	179.1 ± 0.1a	48.5 ± 5.0a
	No treatment	43.4 ± 1.0a	15.5 ± 0.3a	23.4 ± 0.7a	28.2 ± 0.7a	179.0 ± 0.0a	41.5 ± 1.4a
	Plasma	47.0 ± 1.1a	14.5 ± 0.6a	25.9 ± 0.8a	29.8 ± 0.8a	178.9 ± 0.0a	48.3 ± 2.2a
300	Tap water	45.1 ± 1.0a	16.4 ± 0.2a	25.2 ± 0.7a	30.1 ± 0.7a	179.0 ± 0.0a	44.1 ± 1.9a
	No treatment	43.4 ± 0.9a	15.0 ± 0.2a	22.2 ± 0.6a	26.8 ± 0.6a	179.0 ± 0.0a	37.5 ± 1.5a

Six readings were recorded for each leaf covering the entire leaf. Triplicates were performed at each treatment time and for each treatment type. Color values (L*, a*, b*, chroma, hue angle and BI) with different letters (a, b) within each treatment (plasma, tap water and no treatment) time are statistically significant (P = 0.05).
L* = Lightness, a* =.
+ve redness and –ve greenness, b* = +ve yellowness and –ve blueness, BI = Browning index.

browning reactions (Simões, Moreira, Mosquim, Soares, & Puschmann, 2015; Surowsky, Fischer, Schlueter, & Knorr, 2013). Surowsky et al. (2013) reported inactivation of peroxidase and polyphenol oxidase in food model system. Fig. 3 shows that the BI values of plasma mist-treated leaves increased with plasma treatment time until 180 s and then decreased with further increase in plasma treatment time. Although BI value increased from 60 to 180 s (Fig. 3), the values were lower compared to tap water treated and untreated leaves (Table 2). Therefore, it is possible that the decrease in BI values of leaves with plasma treatment time could be due to inactivation of these enzymes.

3.2.2. Effect of DBD cold plasma treatment on color values of whole leaves

Though L* values of whole kale leaves treated with plasma mist increased with treatment time, the difference was statistically insignificant. At 240 and 300 s, the L* values of plasma-treated leaves were higher, and a* values (at 300 s) were lower compared to untreated and tap water treated leaves, which could be due to color bleaching because the leaf after plasma treatment for 300 s had turned lighter (Fig. 4d). However, the differences between L* and a* values for different treatment times was statistically insignificant. BI values of plasma-treated whole leaves increased with increase in treatment time (Table 3). At 300 s, the BI values of plasma-treated leaves were higher compared to that of tap water and untreated leaves, but the difference was statistically insignificant. Hence, overall cold plasma treatment did not statistically affect color of whole leaves even at higher treatment time (300 s), but when the leaf was exposed to cold plasma treatment for more than 300 s (for 600 s), extreme browning was observed compared to tap water mist-treated leaves (Fig. 4e). The results from Table 3 and Fig. 4, are in agreement with the hypothesis that BI increased with increase in plasma treatment time. Browning may be due to oxidation products formed on the leaf. However, the difference in BI values between untreated leaves and leaves treated with plasma mist and tap water mist for 300 s were not statistically significant. Hence, from the aesthetic quality perspective it may be concluded that kale leaves can be treated with the tested DBD cold plasma system for up to 300 s without interferences with the color of baby kale whole leaves. In addition, the trend in color readings of center portion was similar to that of color readings from whole leaf. There was no statistic significant difference observed between different plasma treatment times. These results indicated that DBD cold plasma treatment did not cause major color changes in baby kale whole leaves, specifically in the center of the leaf where the mist was highly concentrated.

3.3. Effect of DBD cold plasma treatment on cuticle of baby kale leaves

Fig. 5 shows the effect of plasma treatment on the cuticle

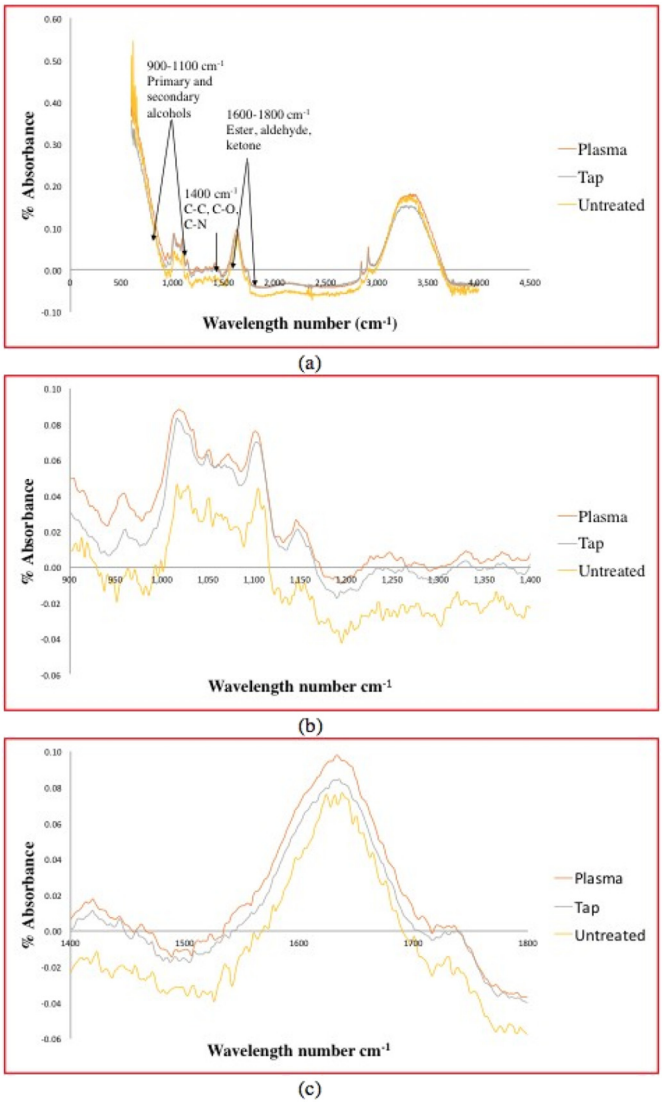


Fig. 5. Fourier transform infrared spectroscopy (FTIR) analysis of untreated leaf, plasma mist treated leaf (240 s) and tap water treated leaf (240 s); (a) Wavelength range: 800 cm^{-1}–4000 cm^{-1}; (b) Wavelength range: 900 cm^{-1}–1400 cm^{-1} (primary and secondary alcohols); (c) Wavelength range: 1400 cm^{-1}–1800 cm^{-1} (ester, aldehydes and ketones).

Plasma treatment did not negatively affect cuticle of kale leaves, this was evident from no abnormal peaks in the Fourier transform infrared spectroscopy (FTIR) results of untreated leaves and leaves treated with plasma mist and tap water mist. On further comparison with untreated leaf, the FTIR results of untreated leaf had more number of peaks than plasma and tap water treated leaves, however, the extent and cause of change was not determined.

composition. It was expected that significant changes in primary and secondary alcohols (wavelength regions of 900–1400 cm^{-1}) as well as aldehydes, ketones, and esters (wavelength regions of 1400–1800 cm^{-1}) in kale cuticle will be observed after contact of cuticle components with reactive species from plasma mist leading to various oxidation reactions (Misra et al., 2016). However, as can be seen in Fig. 5a, b, and c, no abnormal peaks were observed in the Fourier transform infrared spectroscopy (FTIR) results of untreated leaves and leaves treated with plasma mist and tap water mist. On further comparison with untreated leaf, the FTIR results of untreated leaf had more number of peaks than plasma and tap water-treated leaves. Plasma mist did cause changes in the functional groups such as alcohols, aldehydes, ketones, and esters, but it is difficult to conclude the extent and cause of this change. Other parameters such as moisture

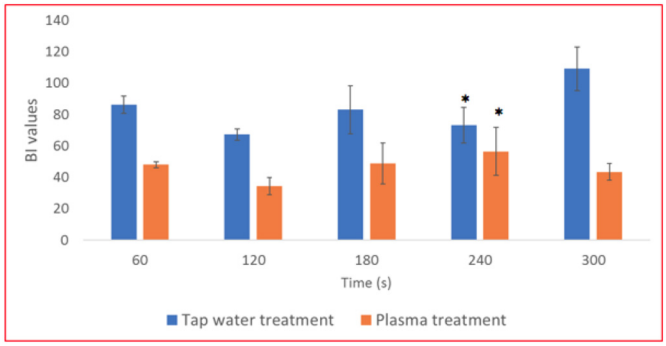


Fig. 6. Comparison of browning index (BI) values of baby kale leaves treated with plasma mist and tap water mist after storage in refrigerator for 12 days. * indicates insignificant difference between values at $P = 0.05$.

When subjected to refrigerated storage, irrespective of treatment time, plasma treated kale leaves showed lower browning index (BI) values compared to tap water mist treated leaves.

content of individual leaf may be responsible in changing the properties of leaf cuticle. Further analyses need to be undertaken to verify these results.

3.4. Storage study

After refrigerated storage (4 °C) for 12 days, irrespective of the treatment time BI values of plasma-treated kale leaves were lower compared to tap water treated leaves (Fig. 6). The BI, chroma, and b* values of plasma-treated leaves after storage for all treatment times were statistically lower than the respective color values of untreated leaves after storage (Table 4). Difference in hue angle values of plasma-treated leaves at all treatment times is statistically insignificant compared to untreated leaves. Values of a* of plasma-treated leaves showed no statistical significant difference at treatment times of 60, 180, and 300 s when compared to untreated leaves. Values of L* of plasma-treated leaves are statistically different compared to L* values of untreated leaves except at 180 s. However, plasma-treated leaves at all treatment times showed lower L* and a* values compared to untreated leaves after storage for 12 days in the refrigerator. Therefore, it may be concluded that cold plasma treatment may better preserve the color and appearance of kale leaves, compared to untreated kale leaves, in refrigerated storage for 12 days.

4. Conclusion

This study demonstrated the potential of cold plasma mist in disinfection of baby kale leaves without affecting leaf color. This study showed complete inactivation of *E. coli* O157:H7 inoculated on baby kale leaves after treatment with plasma mist for 300 s. The effect of plasma treatment time on the color values is insignificant for up to certain time (300 s in this study). BI values of plasma-treated cut leaves are lower compared to whole leaves, which could be due to the inactivation of oxidative enzymes in cut leaves. However, prolonged exposure to cold plasma mist may cause browning or bleaching, as indicated by browning of kale leaves after 600-s cold plasma treatment. Further, color stability of baby kale during storage (4 °C/12 days) can be enhanced after plasma treatment. Furthermore, cold plasma may not negatively affect the leaves, indicated by no change in cuticle composition of kale leaves after plasma treatment. DBD cold plasma, an efficient, quick, and environmental-friendly method has a potential in the food industry to not only disinfect fresh produce, but also enhance shelf-life during refrigerated storage.

Table 4
Effect of cold plasma treatment on color values of kale leaves stored for 12 days under refrigerated conditions (4 °C).

Type of treatment	Time (s)	L*	a*(−ve)	b*	Chroma	Hue	BI
Untreated	0	54.4 ± 6.7b	14.5 ± 0.2a	36.4 ± 10.8a	39.4 ± 9.9a	178.8 ± 0.1a	92.8 ± 11.7c
	60	46.6 ± 1.4a	13.7 ± 0.2ab	25.3 ± 0.0b	28.8 ± 0.1b	178.9 ± 0.0a	47.8 ± 1.7ab
	120	45.9 ± 4.2a	12.4 ± 1.2b	20.8 ± 2.2b	24.3 ± 1.5b	178.9 ± 0.0a	34.4 ± 5.4a
Plasma	180	48.0 ± 2.8ab	12.8 ± 0.7ab	25.2 ± 2.0b	28.4 ± 1.4b	178.9 ± 0.05a	48.6 ± 13.0ab
	240	47.1 ± 2.9a	12.2 ± 0.4b	26.8 ± 4.9b	29.5 ± 4.5b	178.8 ± 0.0a	56.4 ± 15.3b
	300	47.3 ± 1.7a	13.2 ± 1.5ab	24.0 ± 2.0b	27.5 ± 2.4b	178.9 ± 0.0a	43.3 ± 5.2ab

Color values with different letters (a, b, c) within each column are statistically significant (P = 0.05). L* = Lightness, a* = +ve redness and –ve greenness, b* = +ve yellowness and –ve blueness, BI = Browning index.

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