In-package inhibition of *E. coli* O157:H7 on bulk Romaine lettuce using cold plasma

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Short version of title: Bulk lettuce decontamination using in-package cold plasma

ABSTRACT

Dielectric barrier discharge atmospheric cold plasma (DACP) treatment was evaluated for the inactivation of *Escherichia coli* O157:H7, surface morphology, color, carbon dioxide generation, and weight loss of bulk Romaine lettuce in a commercial plastic clamshell container. The lettuce samples were packed in a model bulk packaging configuration (three rows with either 1, 3, 5, or 7 layers) in the container and treated by DACP (42.6 kV, 10 min). DACP treatment reduced the number of *E. coli* O157:H7 in the leaf samples in the 1-, 3-, and 5-layer configurations by 0.4 to 0.8 log CFU/g lettuce, with no significant correlation to the sample location ($P > 0.05$). In the largest bulk stacking with 7 layers, a greater degree of reduction (1.1 log CFU/g lettuce) was observed at the top layer, but shaking the container increased the uniformity of the inhibition. DACP did not significantly change the surface morphology, color, respiration rate, or weight loss of the samples, nor did these properties differ significantly according to their location in the bulk stack. DACP treatment inhibited *E. coli* O157:H7 on bulk lettuce in clamshell containers in a uniform manner, without affecting the physical and biological properties and thus holds promise as a post-packaging process for fresh and fresh-cut fruits and vegetables.

Keywords: cold plasma, nonthermal treatment, *E. coli* O157:H7, lettuce

1. Introduction

The number of foodborne illness outbreaks associated with the consumption of fresh fruits and vegetables has risen along with the increased sale of fresh produce and pre-packaged ready-to-eat produce products (Cevallos-Cevallos et al., 2012; Ziuzina et al., 2014). Conventional post-harvest washing and sanitizing treatments are incompletely effective for the decontamination of foodborne pathogens from these products and, thus, novel technologies should be investigated as alternative methods of intervention.

Recently, cold plasma (CP) treatment has come under the spotlight in food science and technology as an alternative non-thermal processing method for microbial decontamination of fresh fruits and vegetables (Lee et al., 2015; Niemira, 2012). The advantages of CP treatment over conventional techniques include its non-thermal and non-toxic nature, short treatment time, and significant reduction in water usage during the chemical decontamination and post-treatment rinsing (Niemira, 2012; Ziuzina et al., 2012; Ziuzina et al., 2014). CP comprises UV and various kinds of reactive species, including electrons, photons, free radicals, ions, and molecules and atoms with or without excitation, which can inhibit microorganisms (Han et al., 2016). The composition of cold plasma varies with many factors, such as plasma-forming gas, electrical parameters, exposure time, and relative humidity (RH) (Moiseev et al., 2014), which results in the complexity of the species generated and the variability of the treatment. A longer treatment duration of atmospheric CP treatment increased the concentrations of ozone and nitrogen oxides while the increase in RH of air lead to increase in the concentration of nitric oxide and decrease in ozone concentration (Moiseev et al., 2014).

The various CP treatment techniques are categorized by the method of CP generation, and of these techniques, dielectric barrier discharge atmospheric CP (DACP) treatment has been investigated as an innovative method for the in-package decontamination of fresh produce, preventing potential post-processing contamination (Min et al., 2016; Misra et al., 2014a). DACP treatment is of great commercial interest to the food industry as an economic treatment occurring inside sealed packages at atmospheric conditions using atmospheric air as a plasma-forming gas (Fernández et al., 2013; Misra et al., 2014b; Yong et al., 2015; Ziuzina et al., 2014). However, few studies have investigated the effects of in-package DACP decontamination of fresh produce against foodborne pathogens. Although data are available regarding the treatment of single leaves, there is a lack of information on the effects of DACP treatment on the microbial decontamination of bulk leafy produce, which is the normal state of this product in commercial packages. The scaling-up of CP treatment has been proposed as a future topic of interest in food CP research (Fernández et al., 2013), but the development of a large-scale system for produce treatment requires information about the effects of CP on microbial inhibition and the food quality of bulk leafy produce. To date, no studies have investigated the influence of the manner in which the produce samples are stacked on the effectiveness of CP treatment in terms of microbial decontamination. Thus, the objective of the present study was to evaluate the uniformity of DACP treatment applied to bulk Romaine lettuce leaves stacked in a commercial plastic clamshell container, which was selected as the model leafy produce in this study. This process was evaluated for antimicrobial effects against inoculated *Escherichia coli* O157:H7, which is frequently found in lettuce (CDC, 2016). The impact of the DACP treatment on the surface morphology, color, carbon dioxide (CO₂) generation, and weight loss of the leaves were also determined for leaves stacked in different positions within the container.

2. Materials and methods

2.1. Lettuce sample preparation

Pre-washed Romaine lettuce (*Lactuca sativa* L.) was purchased from a local supermarket and stored at 4 °C for up to 3 days until use. The outer lettuce leaves were removed and the intact inner mature leaves were cut into pieces in a laminar flow biohazard hood (Type A/B3, NuAire, Inc., Plymouth, MN, USA) using sterile scissors. The cut lettuce samples measured approximately 4 × 7 cm (2.0 g), 4 × 7 cm (2.0 g), 2 × 7 cm (1.0 g), and 4 × 7 cm (2.0 g) for the analyses of *E. coli* O157:H7 inactivation, leaf color, CO₂ generation, and weight loss, respectively.

For quality analysis, the cut lettuce leaves were washed with running deionized water and then dried in the biohazard hood for 30 min. Without any further preparation, the samples were evaluated to determine the effects of DACP treatment on the color, CO₂ generation, weight loss, and surface morphology of the lettuce samples. For those studies, untreated (control) and treated sample pairs were prepared from one leaf for more effective comparisons.

2.2. Microbial inoculation

The cut lettuce leaves were immersed in a 300 mg/L sodium hypochlorite solution for 3 min to allow for decontamination of the leaves (Hertwig et al., 2015), which reduced the number of the natural mesophilic aerobes on the leaves below the level of detection (> 3 log CFU/g reduction). Next, the decontaminated lettuce samples were rinsed in sterilized deionized water, dewetted using a salad spinner, and dried in the biohazard hood for 30 min at 23 ± 2 °C.

Three strains of *E. coli* O157:H7 (C9490, ATCC 35150, and ATCC 43894) were obtained from the Eastern Regional Research Center (ERRC) Culture Collection (U.S. Department of Agriculture, ERRC; Wyndmoor, PA, USA) and grown in tryptic soy broth (BD; Franklin Lakes, NJ, USA) in an orbital shaker at 37 °C and 150 rpm for 18 h. The cells were washed three times in sterile 0.1% peptone water (BD, Franklin Lakes, NJ, USA) using centrifugation at 5,000 rpm for 10 min; the cocktail was then prepared by combining equal portions of each strain. The cut lettuce leaves were submerged in the inoculum bath ($\sim 10^7$ CFU/mL) with total volume of 1 L for 5 min for inoculation. The inoculated samples were dried in the hood for approximately 1 h prior to DACP treatment. The inoculation level was ~ 5.4 log CFU/g.

2.3. DACP treatment

The DACP treatment was performed using the DACP system described in detail by Min et al. (2016). Briefly, the electrical components of the system consist of two inductor coils, DC power supply, and a function generator. Two inductive coils are used to generate the bipolar pulse high voltage necessary to create a stable plasma field. The frequency range can be varied between 0 and 2,400 Hz using the function generator, and the peak-to-peak voltage measured ranges from 0 to 76 kV.

The cut lettuce samples, either with or without inoculation with *E. coli* O157:H7, were packed in a commercial clamshell container ($14.8 \times 12.8 \times 2.7$ cm; AD16S, Genpak®; Glens Falls, NY, USA), composed of polyethylene terephthalate (PET; 70%) and post-consumer recycled plastics (30%), in a model bulk packaging configuration (Fig. 1). The positions of the lettuce samples were designated as left and center and top, middle, and bottom such that the top, middle, and bottom layers were the first, second, and third layers, the first, third, and fifth layers, and the first, fourth, and seventh layers in the 3-, 5-, and 7-layer configurations, respectively. The

numbers of lettuce pieces were 9, 15, and 21, and the heights of the stacked samples were approximately 0.8, 1.3, and 1.8 cm for the 3-, 5-, and 7-layer configurations, respectively.

The containers were sterilized with a 70% ethanol solution, dried in the biohazard hood prior to being filled and closed. The clamshell packages containing the lettuce samples were treated with DACP in a DACP reactor at 42.6 kV (1.5 A) for 10 min. The gap distance between high voltage and low voltage electrodes was 5.0 cm for all sample configurations. The treatment conditions did not cause any dielectric breakdown (arc) on the lettuce samples in the DACP reactor, and the treatment time (10 min) was the longest duration for which the DACP current was stable throughout. The treatment was conducted at atmospheric pressure using atmospheric air as a CP-forming gas under atmospheric conditions of 24.5 ± 1.4 °C and $22 \pm 2\%$ RH, as measured by a hygrometer (Dickson Temperature/Humidity/Dew Point Recorder, Model THDx, Dickson; Addison, IL, USA). The temperatures of the lettuce samples before and after DACP treatment were measured in non-inoculated samples using an infrared camera (Fluke Ti32, Fluke Corporation; Everett, WA, USA).

A subset of the samples in the 7-layer configuration was shaken to investigate the effectiveness of shaking in terms of microbial inactivation. The shaking was conducted manually for 15 s every 45 s using an insulated bar at two turns/s and changing the direction of shaking from a horizontal direction to a vertical direction, and vice versa every six turns. To maintain sample height during shaking, a pair of nonconductive wooden dowels was placed on top of the lettuce samples when the 7-layer configuration was evaluated. Following treatment, the treated samples were removed in order and then assessed to determine the degree of inhibition of *E. coli* O157:H7 and the surface morphology, color, CO₂ generation, and weight loss of the samples. The results were correlated with leaf position within the bulk stack.

2.4. Microbiological analysis

The untreated and DACP-treated samples (one piece each) were transferred aseptically into separate sterile stomacher bags (384 ml, Nasco Whirl-Pak; Fort Atkinson, WI, USA) with 38 mL sterile 0.1% peptone water, blended for 4 min at 260 rpm using a stomacher (Stomacher 400 Circulator, Seward; London, UK), and then hand-rubbed for 1 min for additional blending. The resulting suspension was serially diluted in 0.1% peptone water, and the diluted samples were plated on MacConkey II with sorbitol (BD). Subsequently, the agar plates were incubated for 24 h at 37 °C prior to enumeration.

2.5. Surface morphology observations

The effects of DACP treatment on the lettuce surface were analyzed by scanning electron microscopy (SEM) according to the method of Keskinen et al. (2009) using a scanning electron microscope (Quanta 200 F, FEI Co.; Hillsboro, OR, USA). Lettuce samples (1 × 1 cm) prepared from the cut lettuce (24 × 7 cm, 2.0 g) with and without DACP treatment were fixed with 1.0 ml 2.5% glutaraldehyde (Electron Microscopy Sciences; Hatfield, PA, USA). The lettuce samples were then rinsed twice with 3 mL 0.1 M imidazole (Electron Microscopy Sciences), dehydrated in 50%, 80%, and 100% ethanol solutions, further dried in a CO₂ critical point drying apparatus (Denton Vacuum, Inc.; Cherry Hill, NJ, USA), and sputter-coated with a thin layer of gold (EMS 150R ES, Electron Microscopy Sciences). The microscope was run at an accelerating voltage of 5-10 kV in the high vacuum and the secondary electron imaging mode.

2.6. Analyses of color, CO₂ generation, and weight loss

The color of the lettuce samples was quantified via the CIELAB L^* , a^* , and b^* values using a colorimeter (Hunter UltraScan VIS, Hunter Associates Lab; Reston, VA, USA) and a 1.3 cm measuring aperture. D65/10° was used as the illuminant-viewing geometry. The treated and untreated lettuce samples were moved carefully into a 40 mL gas vial, and the CO₂ and O₂ levels in the headspace of the vials were analyzed following equilibrium for 4 h using a portable headspace analyzer (Dual Trak 902 D, Quantek Instruments; Grafton, MA, USA). The volume collected from the vial headspace was approximately 20 cm³.

To determine weight loss, the lettuce samples were weighed using a precise three-digit balance (PB 303, Mettler Toledo, Inc.; Columbus, OH, USA). The weight loss values are expressed as a percentage of the initial sample weight.

2.7. Statistical analysis

Entire experiments were performed in triplicate. Five measurements were made for each observation to determine the changes in surface color and weight loss, while three measurements were made to determine CO₂ generation. Analysis of variance (ANOVA) was used to evaluate the differences among the means. If significant differences were detected, Duncan's multiple range test was used to evaluate the means to estimate the significance of differences ($\alpha = 0.05$) using PASW Statistics software (ver. 22.0.0, IBM SPSS Inc.; New York, NY, USA).

3. Results and discussion

3.1. Effects on the inactivation of E. coli O157:H7

DACP treatment, without shaking, significantly inactivated *E. coli* O157:H7 on the lettuce samples ($P < 0.05$), such that the microbial reductions due to DACP treatment were 0.5-0.8 log CFU/g lettuce for the 1-, 3-, and 5-layer configurations (initial concentration: ~ 5.4 log CFU/g). These reductions were consistent in all locations ($P > 0.05$; Table 1), demonstrating uniform microbial reduction at all leaf sample positions in each stacked configuration. On the other hand, without shaking, a greater reduction (0.9-1.1 log CFU/g lettuce) was observed on the top layer in the 7-layer configuration ($P < 0.05$), while the reductions in the middle and bottom layers of the 7-layer configuration did not significantly differ from each other or from the reductions in the other configurations ($P > 0.05$; Table 1). This non-uniform inhibition of *E. coli* O157:H7 in the 7-layer configuration may likely be attributed to the potentially inefficient circulation of CP reactive species in the package. The headspace was narrowest in the 7-layer configuration, while the amount of reactive species formed might have been highest. The accumulation of reactive species in the limited headspace at the top of the 7-layer configuration was assumed to enhance microbial inhibition by increasing the contact efficiency of the CP reactive species with the food. DACP contains reactive oxygen and nitrogen species including atomic oxygen, ozone, singlet oxygen, metastable oxygen molecules, nitric oxide, nitrogen dioxide, and peroxide, superoxide, and hydroxyl radicals (Kim et al., 2015; Yong et al., 2015b). The present findings suggest that the container headspace and position of the food product with respect to the discharge electrodes are important variables to consider when designing packaging for foods to be treated by CP. A location in the center ('center') or not ('left') did not affect microbial reductions in any of the configurations ($P > 0.05$; Table 1), which suggests that there is a uniform inhibition of DACP treatment in a horizontal direction.

Shaking resulted in uniform microbial reductions in the 7-layer configuration, including the

top layer ($P > 0.05$; Table 1), which implies that the accumulation of CP reactive species at the top was avoided by shaking. Thus, shaking improved the microbial reduction at the bottom of the packaging ($P > 0.05$). This prototype is static and unmoving. Modified prototypes which incorporate moving clamshells, as in a conveyor belt-type application, are under investigation. Taken together, these results suggest that DACP treatment will be uniform if the package has sufficient space to facilitate the diffusion of the CP reactive species throughout the package. In contrast, when space is limited, forced diffusion that is assisted by the physical movement of the packaged food would be beneficial for uniform microbial inhibition.

3.2. Effects on morphology, color, CO₂ generation, and weight loss

Based on visual inspections, the DACP-treated samples showed no signs of gross physical changes, including color and apparent crispiness, and were indistinguishable from the untreated control samples. Additionally, there were no significant changes in temperature after DACP treatment in any of the configurations ($P > 0.05$); the changes did not exceed 27°C (ΔT : $1.1 \pm 0.9^\circ\text{C}$). These findings verify that the DACP treatment was a non-thermal process.

The surface morphologies of all the samples in the different positions in the different stacking configurations were not affected by DACP treatment, exhibiting the typical characteristics of the roughened structure of a lettuce leaf, including the stomata (Table 3). Pankaj et al. (2014) reported that polylactic acid film, which was in direct contact with the high-voltage electrode, increased the roughness and formed periodic cone-like structures on the film samples and they explained that these changes were due to etching effect of applied plasma (Pankaj et al., 2014). The lack of changes in the roughness of the lettuce samples and the absence of signs of etching on the samples in the present study confirmed that the treatment conditions, including electrode gap distance, applied voltage, and treatment time, were not strong enough to influence the surface morphology of the lettuce.

Furthermore, the DACP treatment of this study did not significantly influence or induce differences in leaf color changes (ΔE^* ; Table 2), CO₂ generation, or weight loss (Table 4; $P > 0.05$) compared with the untreated controls. Leaf color, respiration, and weight did not differ significantly ($P > 0.05$) among any position in the 1-layer configuration or any bulk stack. These non-significant changes in the physical and biological properties of the lettuce samples can be partially supported by the findings of the SEM analysis, which revealed no changes in surface

morphology after DACP treatment (Table 3). Insignificant changes in lettuce surface morphology after CP treatment were also reported previously (Min et al., 2017).

The evaluation of the quality of CP-treated foods is a key factor in the development of this technology. The color of lettuce can change during conventional food processing due to chlorophyll breakdown (Bolin and Huxsoll, 1991), and the respiration rate of lettuce changes when it undergoes physiological stress or tissue injury due to food processing (Rico et al., 2007; Guan et al., 2010). The weight loss can be accounted for by gas diffusion and the loss of water vapor (Javanmardi and cuvota, 2006; Kader and saltveit, 2003). The present findings demonstrated that the interaction of the CP reactive species with the components of the lettuce did not result in physicochemical or physiological damage to lettuce or alter its quality properties. The insignificant effect on leaf weight also indirectly showed that DACP treatment did not involve temperature elevations during treatment because transpiration, which increases weight loss, increases as temperatures rise (Downs, 1969).

4. Conclusions

In the present study, DACP treatment inhibited *E. coli* O157:H7 on the lettuce samples in a variety of layer configurations. The inhibition rates of *E. coli* O157:H7 were consistent in all locations for the 1-layer configuration and the 3- and 5-leaf stacks but were non-uniform in the 7-leaf stacks. These findings suggest that the container headspace volume and orientation of the lettuce with respect to the discharge electrodes are key variables to be investigated further to develop this technology. Shaking the package, however, led to uniform reductions in the 7-layer configuration, which indicates that this non-uniformity can be improved by the forced diffusion

of CP reactive species. Additionally, DACP treatment did not result in any significant differences in surface morphology, color, CO₂ generation, or weight loss between the treated and untreated samples or among the samples that were in different positions in all configurations.

The reductions in microorganism in a commercial plastic package observed in the present study demonstrated the potential for DACP treatment to be used as a post-processing technology to decontaminate packaged lettuce and possibly other produce commodities. Even though the uniform treatment effects of DACP, which can be improved by the physical movement of the package, were demonstrated in the present study, future studies that scale-up the CP treatment system are required to increase the microbial reduction rates to a level appropriate for commercial microbial decontamination.

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References

- Bolin, H.R., Huxsoll, C.C., 1991. Effect of preparation procedures and storage parameters on quality retention of salad-cut lettuce. *J. Food Sci.* 56, 60–62.
- Centers for Disease Control and Prevention (CDC) USA, 2016. Foodborne outbreak online database (FOOD). <http://wwwn.cdc.gov/foodborneoutbreaks/#> (accessed 16.04.11).
- Cevallos-Cevallos, J.M., Gu, G., Danyluk, M.D., Dufault, N.S., Ariena van Bruggen, H.C., 2012. *Salmonella* can reach tomato fruits on plants exposed to aerosols formed by rain. *Int. J. Food Microbiol.* 158, 140–146.
- Downs, R.W., 1969. Differences in transpiration rates between tropical and temperate grasses under controlled conditions. *Planta* 88, 261–273.
- Fernández, A., Noriega, E., Thompson, A., 2013. Inactivation of *Salmonella enterica* serovar Typhimurium on fresh produce by cold atmospheric gas plasma technology. *Food Microbiol.* 33, 24–29.
- Guan, W., Huang, L., Fan, X., 2010. Acids in combination with sodium dodecyl sulfate caused quality deterioration of fresh-cut iceberg lettuce during storage in modified atmosphere package. *J. Food Sci.* 75, S435–S440.
- Han, S.H., Suh, H.J., Hong, K.B., Kim, S.Y., Min, S.C., 2016. Oral toxicity of cold plasma-treated edible films for food coating. *J. Food Sci.* 81, T3052-T3057.
- Hertwig, C., Reineke, K., Ehlbeck, J., Knorr, D., Schlüter, O., 2015. Decontamination of whole black pepper using different cold atmospheric pressure plasma applications. *Food Control* 55, 221–229.
- Javanmardi, J., Kubota, C., 2006. Variation of lycopene, antioxidant activity, total soluble solid

- and weight loss of tomato during postharvest storage. *Postharvest Biol. Technol.* 41, 151–155.
- Kader, A.A. Saltveit, M.E., 2003. Postharvest physiology and pathology of vegetables. in: Bartz, J.A., and Brecht, J.K. (Eds.), *Atmosphere modification*. Marcel Dekker, Inc., New York, pp. 229–246.
- Keskinen, L.A., Burke, A., Annous. B.A., 2009. Efficacy of chlorine, acidic electrolyzed water and aqueous chlorine dioxide solutions to decontaminate *Escherichia coli* O157:H7 from lettuce leaves. *Int. J. Food Microbiol.* 132, 134–140.
- Kim, H.J., Yong, H.I., Park, S., Kim, K., Choe, W., Jo, C., 2015. Microbial safety and quality attributes of milk following treatment with atmospheric pressure encapsulated dielectric barrier discharge plasma. *Food Control* 47, 451–456.
- Lee, H., Kim, J.E., Chung, M.-S., Min, S.C., 2015. Cold plasma treatment for the microbiological safety of cabbage, lettuce, and dried figs. *Food Microbiol.* 51, 74–80.
- Min, S.C., Roh, S.H., Boyd, G., Sites, J.E., Uknalis, J., Fan, X., Niemira, B.A., 2017. Inactivation of *Escherichia coli* O157:H7 and aerobic microorganisms in Romaine lettuce packaged in a commercial polyethylene terephthalate container using atmospheric cold plasma. *J. Food Prot.* 80, 35–43.
- Min, S.C., Roh, S.H., Niemira, B.A., Sites, J.E., Boyd, G., Lacombe, A., 2016. Dielectric barrier discharge atmospheric cold plasma inhibits *Escherichia coli* O157:H7, *Salmonella*, *Listeria monocytogenes*, and Tulane virus in Romaine lettuce. *Int. J. Food Microbiol.* 237, 114–120.
- Misra, N.N., Keener, K.M., Bourke, P., Mosnier, J.P., Cullen, P.J., 2014a. In-package atmospheric pressure cold plasma treatment of cherry tomatoes. *J. Biosci. Bioeng.* 118, 117–182.

- Misra, N.N., Patil, S., Moiseev, T., Bourke, P., Mosnier, J.P., Keener, K.M., Cullen, P.J., 2014b. In-package atmospheric pressure cold plasma treatment of strawberries. *J. Food Eng.* 125, 131–138.
- Moiseev, T., Misra, N.N., Patil, S., Cullen, P.J., Bourke, P., Keener, K.M., Mosnier, J.P., 2014. Post-discharge gas composition of a large-gas DBD in humid air by UV-Vis absorption spectroscopy. *Plasma Sources Sci. Technol.* 23, 065033.
- Niemira, B.A., 2012. Cold plasma decontamination of foods. *Annu. Rev. Food Sci. Technol.* 3, 125–142.
- Pankaj, S.K., Bueno-Ferrer, C., Misra, N.N., O'Neill, L., Jiménez, A., Bourke, P., Cullen, P.J., 2014. Characterization of polylactic acid films for food packaging as affected by dielectric barrier discharge atmospheric plasma. *Innov. Food Sci. Emerg.* 21, 107–113.
- Rico, D., Martin-Diana, A.B., Barat, J.M., Barry-Ryan, C., 2007. Extending and measuring the quality of fresh-cut fruit and vegetables: a review. *Trends Food Sci. Technol.* 18, 373–386.
- Yong, H.I., Kim, H.J., Park, S., Kim, K., Choe, W., Yoo, S.J., Jo, C., 2015b. Pathogen inactivation and quality changes in sliced cheddar cheese treated using flexible thin-layer dielectric barrier discharge plasma. *Food Res. Int.* 69, 57–63.
- Ziuzina, D., Patil, S., Cullen, P.J., Keener, K.M., Bourke, P., 2012. Atmospheric cold plasma inactivation of *Escherichia coli* in liquid media inside a sealed package. *J. Appl. Microbiol.* 114, 778–787.
- Ziuzina, D., Patil, S., Cullen, P.J., Keener, K.M., Bourke, P., 2014. Atmospheric cold plasma inactivation of *Escherichia coli*, *Salmonella enterica* serovar Typhimurium and *Listeria monocytogenes* inoculated on fresh produce. *Food Microbiol.* 42, 109–116.

Figure Caption

Fig. 1. Schematic diagram of the cold plasma reactor holding a clamshell package treatment sample containing seven layers of lettuce leaves in a three-column configuration.

Table 1

Dielectric barrier discharge atmospheric cold plasma (DACP) treatment^a inactivation of *E. coli* O157:H7 inoculated on Romaine lettuce samples at different positions in the 1-, 3-, 5-, or 7-layer packaging configurations.

		Microbial reduction (log CFU/g lettuce)			
Position		1-layer	3-layer	5-layer	7-layer configuration
		configuration	configuration	configuration	No shaking Shaking
Top	Left		0.6 ± 0.4 ab	0.6 ± 0.4 ab	0.9 ± 0.2 a 0.7 ± 0.2 ab
	Center		0.6 ± 0.1 ab	0.6 ± 0.3 ab	1.1 ± 0.1 a 0.7 ± 0.2 ab
Middle	Left		0.6 ± 0.3 ab	0.5 ± 0.2 ab	0.6 ± 0.1 ab 0.8 ± 0.2 ab
	Center		0.7 ± 0.4 ab	0.8 ± 0.3 ab	0.6 ± 0.2 ab 0.6 ± 0.1 ab
Bottom	Left	0.6 ± 0.2 ab ^b	0.5 ± 0.3 ab	0.5 ± 0.3 ab	0.4 ± 0.1 b 0.7 ± 0.1 ab
	Center	0.7 ± 0.2 ab	0.6 ± 0.1 ab	0.5 ± 0.3 ab	0.4 ± 0.1 b 0.7 ± 0.1 ab

^a The treatment voltage applied to the electrode, current through the cold plasma reactor, and treatment time were 42.6 kV, 1.5 A, and 10 min, respectively.

^b Values followed by different letters within the same columns significantly differed according to Duncan's multiple range test ($P < 0.05$).

Table 2

Dielectric barrier discharge atmospheric cold plasma (DACP) treatment^a effects on the color of Romaine lettuce samples at different positions in the 1-, 3-, 5-, or 7-layer packaging configurations.

		Total color difference (ΔE^*) ^b			
Position		1-layer	3-layer	5-layer	7-layer
		configuration	configuration	configuration	configuration
Top	Left		2.79 ± 1.31 a	2.49 ± 1.78 a	2.88 ± 1.38 a
	Center		2.82 ± 1.41 a	2.32 ± 0.86 a	2.54 ± 1.84 a
Middle	Left		2.94 ± 1.06 a	2.92 ± 1.66 a	2.87 ± 1.23 a
	Center		2.94 ± 1.28 a	2.99 ± 1.78 a	3.00 ± 1.09 a
Bottom	Left	2.95 ± 1.57 a ^c	2.84 ± 1.24 a	2.63 ± 1.09 a	2.62 ± 0.94 a
	Center	2.92 ± 1.53 a	2.85 ± 1.59 a	2.72 ± 1.29 a	2.72 ± 1.34 a

^a The treatment voltage applied to the electrode, current through the cold plasma reactor, and treatment time were 42.6 kV, 1.5 A, and 10 min, respectively.

^b Total color difference (ΔE^*) = $\sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$

^c Values followed by different letters are significantly different according to Duncan's multiple range test ($P < 0.05$).

Table 3

Scanning electron micrographs^a of the surfaces of Romaine lettuce leaves in the 7-layer packaging configuration in the packaged treatment containers that were prepared either with or without dielectric barrier discharge atmospheric cold plasma (DACP) treatment^b.

Sample location	Top		Middle		Bottom	
	Untreated	DACP-treated	Untreated	DACP-treated	Untreated	DACP-treated
Top
Center

^aThe magnification was $\times 2,500$.

^bThe treatment voltage applied to the electrode, current through the cold plasma reactor, and treatment time were 42.6 kV, 1.5 A, and 10 min, respectively.

Table 4

Dielectric barrier discharge atmospheric cold plasma (DACP) treatment^a effects on the CO₂ generation and weight loss of Romaine lettuce samples at different positions in the 1-, 3-, 5-, or 7-layer packaging configurations.

		1-layer configuration		3-layer configuration		5-layer configuration		7-layer configuration		
Position	Sample	CO ₂ generation	Weight loss	CO ₂ generation	Weight loss	CO ₂ generation	Weight loss	CO ₂ generation	Weight loss	
		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	
Top	Left	Untreated		1.80 ± 0.10 a ^b	0.82 ± 0.28 a	1.77 ± 0.40 a	0.76 ± 0.23 a	1.87 ± 0.21 a	1.08 ± 0.28 a	
		DACP-treated		1.80 ± 0.20 a	0.96 ± 0.29 a	1.73 ± 0.42 a	1.11 ± 0.42 a	1.80 ± 0.40 a	1.12 ± 0.52 a	
	Center	Untreated		1.87 ± 0.42 a	0.72 ± 0.39 a	1.60 ± 0.10 a	1.04 ± 0.23 a	1.80 ± 0.36 a	1.06 ± 0.37 a	
		DACP-treated		1.97 ± 0.49 a	0.93 ± 0.16 a	1.83 ± 0.42 a	1.15 ± 0.26 a	1.83 ± 0.25 a	1.14 ± 0.49 a	
Middle	Left	Untreated		1.95 ± 0.35 a	0.87 ± 0.30 a	1.50 ± 0.20 a	0.71 ± 0.28 a	1.63 ± 0.21 a	0.96 ± 0.18 a	
		DACP-treated		1.70 ± 0.20 a	0.85 ± 0.22 a	1.60 ± 0.20 a	0.91 ± 0.46 a	1.72 ± 0.16 a	1.05 ± 0.48 a	
	Center	Untreated		1.80 ± 0.30 a	0.85 ± 0.32 a	1.60 ± 0.17 a	0.98 ± 0.35 a	1.67 ± 0.38 a	1.22 ± 0.43 a	
		DACP-treated		1.77 ± 0.15 a	0.79 ± 0.20 a	1.50 ± 0.10 a	0.76 ± 0.19 a	1.80 ± 0.26 a	1.08 ± 0.26 a	
Bottom	Left	Untreated	1.40 ± 0.20 a	0.73 ± 0.23 a	1.90 ± 0.14 a	0.77 ± 0.16 a	1.50 ± 0.20 a	0.78 ± 0.23 a	1.63 ± 0.61 a	0.96 ± 0.21 a
		DACP-treated	1.40 ± 0.14 a	0.71 ± 0.17 a	1.80 ± 0.26 a	0.89 ± 0.16 a	1.47 ± 0.31 a	1.00 ± 0.33 a	1.97 ± 0.40 a	0.76 ± 0.23 a
	Center	Untreated	1.40 ± 0.30 a	0.76 ± 0.31 a	1.97 ± 0.25 a	0.71 ± 0.15 a	1.57 ± 0.15 a	0.97 ± 0.54 a	1.90 ± 0.00 a	1.02 ± 0.26 a
		DACP-treated	1.45 ± 0.49 a	0.65 ± 0.14 a	1.83 ± 0.15 a	0.70 ± 0.09 a	1.63 ± 0.06 a	0.82 ± 0.33 a	1.80 ± 0.36 a	0.83 ± 0.19 a

^aThe treatment voltage applied to the electrode, current through the cold plasma reactor, and treatment time were 42.6 kV, 1.5 A, and 10 min, respectively.

^bValues followed by different letters within the same parameter are significantly different according to Duncan's multiple range test ($P < 0.05$).