Non-Equilibrium Short-Pulsed Dielectric Barrier Discharge Treatment of Micrometer-sized Water Droplets for Disinfection of Fresh Produce During Transportation and Storage

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Abstract: Treating fresh produce using short-pulsed dielectric discharge barrier (DBD) plasma activated water droplets (mist) both disinfects and prolongs shelf life effectively and does not alter the nutritional value of produce, nor does it negatively affect its cuticle or pigments. The DBD plasma system effectively inactivates most pathogens responsible for food borne illness outbreaks. Ultrasonic nebulizers were used to create water droplets that, similar to charged droplet deposition in xerography, coat the fresh produce surface uniformly, ensuring treatment of entire product, making it an effective sanitation system for food.

Keywords: plasma mist, plasma agriculture, fresh produce, disinfection, sanitation, dielectric barrier discharge

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Introduction

Many challenges in the food industry are caused by the hurdles of short shelf life combined with the transportation of perishable food items and the processes of keeping them sanitary. Plasma can help prevent food borne illness outbreaks from contaminated food items and thus extend the shelf life of foods as well. Plasma generates reactive oxygen and nitrogen species (RONS) [1], which both have disinfection properties, and do not alter the nutritional value of produce, nor does it negatively affect its cuticle or pigments [2].

Dielectric barrier discharge (DBD) plasma is one of the approaches to effectively inactivate the pathogens known to cause majority of food borne illness. According to the Centers for Disease Control and Prevention (CDC), approximately 90% of all food borne illness known to be caused by pathogens are included in Table 1 [3].

Table 1. List of pathogens and the types of plasma that effectively inactivates them.

Pathogen	**Plasma Type**	**Reference**
Campylobacter	DBD	[4, 5]
Clostridium perfringens	Plasma Jet, Microwave Cold Plasma	[6, 7]
E. Coli	DBD, Afterglow Discharge, Uniform Glow Discharge	[8-10]
Listeria	DBD, Afterglow Discharge, Uniform Glow Discharge	[8-10]
Salmonella	DBD, Afterglow Discharge, Uniform Glow Discharge	[8-10]
Norovirus	DBD (Surface Micro Discharge)	[11-13]

Materials and Methods

DBD system, used in this study, was already reported on by the authors previously [15]. In short, we apply 1–10 µsec positive pulses of 26 kV magnitude to a set of three concentric electrodes. Water droplets are then carried by air through the plasma zone and into the treatment chamber. Our system includes a chamber of the 15 ultrasonic nebulizers that produce the droplets, and the DBD system above the mini-refrigerator on the right of Figure 1. Attached to the ultrasonic nebulizer chamber is air coming in through a tube connected to a mass flow controller. The air going through the mass flow sensor is guiding the droplets from the ultrasonic nebulizer chamber, through DBD system, to the refrigerator where the treatment will take place. The flow rate on the mass flow sensor was is set at 10 slpm.

Escherichia coli (*E. coli*) O157:H7, grown in tryptic soy broth is plated on tryptic soy agar Petri dishes: 100 µl of 10^6 CFU/mL are mixed with 500 µl of sterile distilled water and spread on surface of the dish. Thus-prepared dish is allowed to dry for 15 minutes prior to plasma treatment. The dishes are placed in the plasma treatment system for the designated amount of time, plasma settings, and air flow rate. After the treatment dishes are placed in an incubator at 37 ºC for 24 hours.

Fig. 1. On the left, spinach leaves are inside the refrigerator, where treatment occurs. On the right is an overall view of the system.

When using fresh produce, e.g. spinach leaves, the produce is inoculated and treated using the plasma system for the designated amount of time. Post-plasma treatment, each leaf is put in an individual bag (Nasco Whirl-Pak® B01018WA Bags) along with 10mL of phosphate buffered saline. Each bag is placed in the stomacher (Stomacher® 80 Biomaster) and is stomached at 120 seconds at normal speed. Then, each bag removed from the stomacher, and 500µL of liquid is extracted from the bag and plated on tryptic soy agar Petri dishes. The prepared dishes are allowed to dry for 15 minutes prior to being placed in an incubator at 37 ºC for 24 hours.

Results and Discussion

An experiment was conducted where approximately 10^5 *E. Coli* were plated on each tryptic soy agar Petri dish: 100 µl of 10^6 CFU/mL are mixed with 500 µl of sterile distilled water and spread on surface of the dish. After allowing the Petri dish to dry for 15 minutes, 8 dishes were treated at a time in the refrigerator on the left in Figure 1. All dishes, besides the control dishes, were treated for 15 minutes with an air flow rate of 10 slpm and the plasma at a 9 µs pulse. After the initial 15 minutes of treatment, both the ultrasonic nebulizers and the DBD plasma system were turned off, and the dishes were held in the refrigerator for an additional 30 minutes. All dishes were placed in the 37 ºC incubator for 24 hours. The yielded results are graphed in Figure 2.

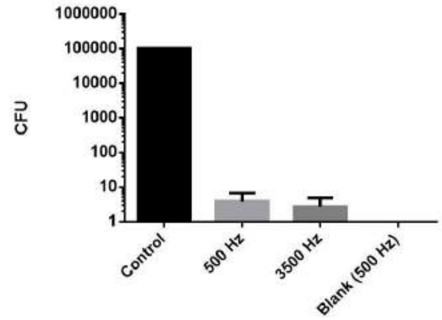

Fig. 2. Average CFU per Petri dish post-designated treatment and incubation

From the results displayed in Figure 2, the Petri dishes treated at both 500 Hz and 3500Hz exhibited 5-log reduction compared to the untreated control dishes, therefore this method of disinfecting produce meets the standards of a sanitizer according to the 2017 Food Code [16]. Figure 2 also shows that there were more *E. Coli* inactivated on the dishes treated at 3500 Hz than 500 Hz.

Conclusion

Treating fresh produce using short-pulsed dielectric discharge barrier (DBD) plasma activated water droplets both prolongs shelf life, and disinfects effectively, which is demonstrated in Figure 2. From Figure 2, we can conclude that the DBD plasma system is capable of a 5-log reduction of *E. Coli* plated on tryptic soy agar plates. However, there are many unanswered questions such as the DBD plasma system's effectiveness inactivating other microorganisms, and the settings that maximize the inactivation of other organisms. The questions of toxicity, carcinogenicity, and market acceptance also arise. Nonetheless, the DBD plasma system poses a solution to the challenges faced in the food industry.

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