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Application of underwater dielectric barrier discharge as a washing method for reduction of *Salmonella* Typhimurium on perilla leaves

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ABSTRACT

To develop an alternative washing method for sanitizing fresh produce, a submerged plasma reactor of dielectric barrier discharge (underwater DBD) was evaluated. *Salmonella* Typhimurium in suspension was reduced by 4.3 log after underwater DBD treatment for 1 min. Membrane damage was apparent in *S.* Typhimurium that was exposed to underwater DBD based on scanning electron microscopic analysis. DNA electrophoresis analysis revealed that the amount of genomic DNA that was purified from *S.* Typhimurium cells decreased after underwater DBD treatment. These results suggest that DNA might be released from the cytoplasm due to the membrane damage induced by underwater DBD treatment. The reduction of *S.* Typhimurium-inoculated perilla leaf was 2.5 log after the underwater DBD treatment, which was similar to chlorine washing (100 ppm). The color parameters did not show significant changes after underwater DBD treatment ($p > 0.05$). In conclusion, underwater DBD could potentially be used as a washing method to reduce the amount of pathogenic bacteria on fresh produce.

1. Introduction

Fresh produce consumption has increased with the recognition of a well-balanced diet for a healthier lifestyle (FAO/WHO, 2008). Among the consumed fresh produce, leafy vegetables are consumed in salads, which show growth trends in the fresh-cut market of the United States (Cook, 2014). In Korea, leafy vegetables such as perilla leaf are widely consumed raw and are frequently used to wrap rice, condiments and meat. Despite its frequent consumption and well-established safety precautions, foodborne outbreaks related to the presence of pathogenic bacteria in produce have increased (Painter et al., 2013). *Salmonella* is the most common pathogenic bacteria responsible for produce-associated outbreak in the United States and the European Union (Callejón et al., 2015). Perilla leaf also has the risk of contamination due to spoilage and pathogenic microorganisms. Therefore, post-harvest sanitation technology is required to control the foodborne pathogens and increase the safety of produce.

For fresh produce, several washing steps are required, and a washing step for sanitation, such as using a sanitizer, should be employed (Olaimat & Holley, 2012; Ruiz-Cruz, Acedo-Féliz, Díaz-Cinco, Islas-Osuna, & González-Aguilar, 2007). Chlorine-based sanitizer (up to 200 ppm) has been routinely applied to reduce microbial contamination in produce processing lines (Wei, Cook, & Kirk, 1985). However, some studies have shown that chlorine is ineffective at inactivating pathogens, and there are concerns that undesirable by-products may be formed (Morris, Audet, Anqelillo, Chalmers, & Mosteller, 1992). Although the test results from Committee on Toxicity did not indicate any cause for concern regarding the presence of chlorination by-products in prepared salads (COT, 2006), the presence of chlorine in prepackaged lettuce was reported as the prompted issue of concern to consumers (McDowell et al., 2007). Organic acid-based sanitizers have been introduced as alternatives to chlorine-based sanitizers. However, the former decrease the water quality by increasing the chemical oxygen demand (COD) and decreasing the pH (López-Gálvez, Allende, Selma, & Gil, 2009). Therefore, requirements of alternative methods for produce sanitation have been increased to fulfil the demand of quality assurance with microbial and environmental safety.

Underwater electrical discharge has been studied in wastewater treatment (Hao, Zhou, Xin, & Lei, 2007; Shi, Bian, & Yin, 2009).

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Underwater electrical discharge is an environmentally friendly technology because it does not need any aids and does not produce harmful by-products (Hao et al., 2007; Shi et al., 2009). It produces physical and chemical factors such as high electric fields, UV, and reactive species such as atomic oxygen, atomic hydrogen and hydroxyl (OH) radical (Shi et al., 2009). The types and intensities of the factors produced may differ, depending on the types of discharge (Shi et al., 2009). The various factors produced by underwater electrical discharge treatment can lead to a synergistic reduction effect and decrease the microbial tolerance, which can easily arise when a single factor is used (Grymonpre, Finney, Clark, & Locke, 2004; Locke, Sato, Sunka, Hoffmann, & Chang, 2006). These properties could be applied in sanitation for fresh produce, and underwater electrical discharge is a potential washing method for fresh produce.

Various types of underwater electrical discharge have been introduced to inactivate bacteria. Zuckerman, Krasik, and Felsteiner (2002) obtained a 7.5 log reduction of *Escherichia coli* using pulsed high-current underwater discharge, Kim et al. (2013) obtained a 6 log reduction of *E. coli* using gliding arc discharge, and Kim, Hong, Lee, and Lee (2012) obtained a 6 log reduction of *E. coli* using capillary discharge in water. Different factors affecting bacterial inactivation were reported for different types of underwater electrical discharge, such as pressure wave and UV light for pulsed high-current underwater discharge, low pH and hydrogen peroxide (H_2O_2) for gliding arc discharge, and atomic oxygen, atomic hydrogen and OH radical for capillary discharge. The reactive species generated by underwater electrical discharge and their inactivation effects on bacteria may differ, depending on the types of discharge and device, which can be designed to adapt for a purpose.

Among plasma reactors, dielectric barrier discharge (DBD) produced ozone, UV light, reactive oxygen species (ROS), and reactive nitrogen species (RNS), which have strong capacities to inactivate bacteria without a residue problem (Kogelschatz, 2003). Underwater electrical discharge using a DBD plasma reactor (underwater DBD) has been originally developed by Mok, Jo, Lee, Ahn, and Kim (2007). For an alternative washing method of produce sanitation, underwater DBD could be applicable to avoid the use of chlorine which is confronted with environmental and health concerns (Ölmez & Kretzschmar, 2009).

Underwater electrical discharge has been commonly studied in relation to the environmental and wastewater (Horikoshi, Sato, Abe, & Serpone, 2011; Joubert et al., 2013; Kim et al., 2013; Locke et al., 2006), but it has rarely been used in bacterial inactivation studies for food safety (Loske, Alvarez, Hernández-Galicia, Castaño-Tostado, & Prieto, 2002; Zuckerman et al., 2002). In this study, we investigated the application of underwater DBD as a washing system for sanitizing perilla leaves. For this purpose, optical emission spectrum (OES) and concentrations of ozone, and H_2O_2 were assessed to analyze the components produced by underwater DBD. Then, the inactivation mechanisms of suspended *S.* Typhimurium in a water bath were investigated. Finally, the reduction effects of *S.* Typhimurium inoculated on perilla leaf were compared between underwater DBD and chlorine treatment.

2. Materials and methods

2.1. Underwater electrical discharge apparatus

A schematic diagram of the experimental apparatus is shown in Fig. 1. The original DBD plasma reactor by Mok et al. (2007) and Kim and Park (2011) was modified in our study to wash leafy vegetables. Underwater DBD was composed of an electrode (DBD reactor), power supply, and a gas supply. Table 1 presents the specific conditions of the apparatus. A quartz tube was used as a dielectric

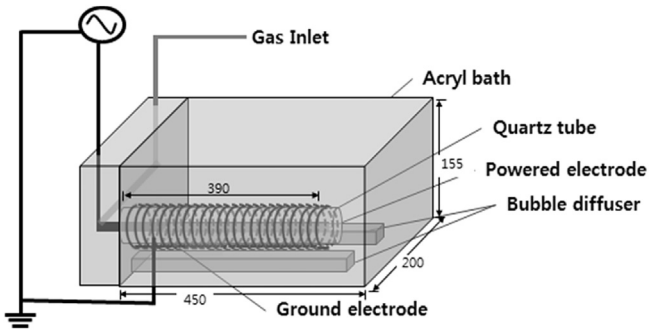

Fig. 1. The schematic diagram of underwater dielectric barrier discharge.

Table 1
Conditions of underwater dielectric barrier discharge.

Parameters	Conditions
Input voltage	220 V (AC power)
Output voltage	18 kV
Gas	Clean dry air
Gas flow rate	5 L/min
Quartz tube diameter	20 mm
Powered electrode diameter	7 mm
Powered electrode length	360 mm
Powered electrode composition	Copper
Ground electrode composition	Copper
Tab water volume	8 L
Dimensions of acryl bath	450 × 200 × 155 mm^3
Tab water temperature	20 °C

(30 mm internal diameter). Electrode was rod type (7 mm diameter, copper), and ground electrode (1 mm diameter, copper) was spring type coiled around the quartz tube. A neon transformer (18 kV, 60 Hz) with an input voltage of 220 VAC/60 Hz was used as the power supply. Clean, dry air flowed at 5 L/min (adjusted with a regulator) to supply to the inside of quartz tube in the DBD where plasma was generated, and the plasma escaped through the diffuser in the form of bubbles. The DBD reactor was submersed in an acryl water bath (450 × 200 × 155 mm^3).

Optical emission spectroscopy (OES) was performed using an optical emission spectrometer (HR4000, Ocean Optics Inc., USA). The ozone concentration in the water was measured using a UV ozone monitor (Model 620, Ebara Jitsugyo, Japan) with water circulation from a bottom drain. H_2O_2 in the water was measured using a hydrogen peroxide assay kit (Amplex® Red hydrogen peroxide assay kit, Molecular Probes, Eugene, OR, USA).

2.2. Underwater DBD treatment of the bacterial suspension

The bacterium used in this study was *Salmonella* Typhimurium ATCC 14028 obtained from the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea). One colony of *S.* Typhimurium was grown on Plate Count Agar (PCA, BD, Sparks, MD, USA) at 37 °C for 1 day and was used to inoculate TSB broth (BD) at 37 °C for 15–16 h. The culture was harvested and washed once by centrifugation at 10,000 × *g* for 10 min. The cell pellet was re-suspended and adjusted to approximately 6–7 log CFU/ml in 8 L of deionized water (DW, 20 °C). The cell suspension (8 L) was poured into the acryl bath with the DBD reactor. The diffuser played a role in mixing the cell suspension to achieve homogeneity. An aliquot (1 ml) of the bacterial suspension was collected and immediately transferred to 9 ml of Dey-Engley Neutralizing (D/E) broth (BD) at 0, 10, 30, 60, 90, and 120 s and the viable counts were conducted by the 10-fold serial dilution of the cell suspension in 0.85% sterile saline and