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Combination treatment of chlorine dioxide gas and aerosolized sanitizer for inactivating foodborne pathogens on spinach leaves and tomatoes

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ABSTRACT

The objective of this study was to evaluate the antimicrobial effect of chlorine dioxide (ClO_2) gas and aerosolized sanitizer, when applied alone or in combination, on the survival of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* inoculated onto spinach leaves and tomato surfaces. Spinach leaves and tomatoes were inoculated with a cocktail of three strains each of the three foodborne pathogens. ClO_2 gas (5 or 10 ppmv) and aerosolized peracetic acid (PAA) (80 ppm) were applied alone or in combination for 20 min. Exposure to 10 ppmv of ClO_2 gas for 20 min resulted in 3.4, 3.3, and 3.4 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on spinach leaves, respectively. Treatment with 80 ppm of aerosolized PAA for 20 min caused 2.3, 1.9, and 0.8 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. Combined treatment of ClO_2 gas (10 ppmv) and aerosolized PAA (80 ppm) for 20 min caused 5.4, 5.1, and 4.1 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on tomatoes experienced similar reduction patterns to those on spinach leaves. As treatment time increased, most combinations of ClO_2 gas and aerosolized PAA showed additive effects in the inactivation of the three pathogens. Combined treatment of ClO_2 gas and aerosolized PAA produced injured cells of three pathogens on spinach leaves while generally did not produce injured cells of these pathogens on tomatoes. Combined treatment of ClO_2 gas (10 ppmv) and aerosolized PAA (80 ppm) did not significantly ($p > 0.05$) affect the color and texture of samples during 7 days of storage.

1. Introduction

Consumption of fresh produce has increased because of its health benefits (Perni et al., 2008). However, several foodborne outbreaks related to the presence of pathogenic bacteria in fresh produce have been reported in recent years (Fernández and Thompson, 2012). Fresh spinach and spinach-containing products were implicated in an outbreak of *Escherichia coli* O157:H7 (CDC, 2006; Maki, 2006) which infected a total of 205 persons and resulted in 4 deaths (Wendel et al., 2009). In 2012, a total of 33 persons infected with *E. coli* O157:H7 traced to organic spinach and spring mix blend was reported from 5 US states (CDC, 2012). Tomatoes were associated with more than 14 outbreaks of foodborne illness between 1996 and 2008, and accounted for 17% of all produce-associated outbreaks in the United States during that period (Gravani, 2009).

Chlorine dioxide (ClO_2) has emerged as a promising non-thermal sanitizing technology for fresh produce in recent years (Bhagat et al., 2010). Several factors such as gas concentration, relative humidity (RH), treatment time, and temperature could affect the antimicrobial effect of ClO_2 gas. Especially, the combination of gas concentration and RH shows a synergistic effect (Han et al, 2001a; Park and Kang, 2015). The antimicrobial effect of ClO_2 gas has been evaluated on fresh produce such as spinach (Neal et al., 2012), potatoes (Wu and Rioux, 2010), mung bean sprouts (Prodduk et al., 2014), lettuce (Mahmoud and Linton, 2008), onions, cabbage (Sy et al., 2005), cantaloupe (Mahmoud et al., 2008), and strawberries (Han et al., 2004). However, the concentration of ClO_2 gas used in previous studies was excessive (Morino et al., 2011).

Combinations of different technologies, known as hurdle technology, could be an alternative to the use of high ClO_2 gas concentrations. Combined treatments could achieve required levels of food safety and the maintenance of organoleptic qualities of foods, while decreasing the intensity of each hurdle, that is, the antimicrobial concentration (Leistner and Gorris, 1995). Studies which evaluated sanitizer–sanitizer or sanitizer–novel technique combinations have both drawn great attention (Huang and Chen, 2011; Singh et al., 2002).

Aerosolization, another non-thermal technology, is the dispersion of a liquid material as a fine mist in air (Oh et al., 2005a, 2005b). Some studies have investigated the efficacy of aerosolized sanitizers for inhibiting foodborne pathogens on fresh produce. Aerosolized peroxyacetic acid,

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hydrogen peroxide, and malic acid were effective for controlling *E. coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on lettuce and spinach leaves (Choi et al., 2012; Huang et al., 2012; Oh et al., 2005a, 2005b). Not only the antimicrobial effect, but also the ability to control humidity is an advantage of aerosolized sanitizers for combination with ClO_2 gas. Since aerosolized sanitizer exists as a fine mist dispersed in air, it can be used to control RH of the ClO_2 gas treatment chamber. Thus, aerosolized sanitizers in combination with ClO_2 gas could enhance the inactivation efficacy of ClO_2 gas by maintaining conditions of high RH.

The objective of this study was to evaluate the antimicrobial effect of ClO_2 gas and aerosolized sanitizer, when applied alone or in combination, on the survival of inoculated *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on spinach leaves and tomato surfaces. Also, any changes in color and texture of samples were assessed.

2. Materials and methods

2.1. Bacterial strains and cell suspension

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), *S.* Typhimurium (ATCC 19586, ATCC 43174, DT 104), and *L. monocytogenes* (ATCC 7644, ATCC 19114, ATCC 19115) were provided by the bacterial culture collection of the Food Hygiene Laboratory at Seoul National University (SNCC; Seoul, Korea), for this study. All strains of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were cultured individually in 5 ml of tryptic soy broth (TSB; Difco, Sparks, MD, USA) at 37 °C for 24 h, followed by centrifugation (4000 ×*g* for 20 min at 4 °C) and washed three times with buffered peptone water (BPW; Difco). The final pellets were resuspended in sterile BPW, corresponding to approximately 7–8 log CFU/ml. *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* strains were combined to make culture cocktails for use in experiments.

2.2. Sample preparation and inoculation

Spinach and whole tomatoes were purchased from a local market (Seoul, South Korea). Spinach leaves were trimmed to approximately 5 cm × 3 cm in size, and the outer surface of tomatoes was cut into 5 cm × 2 cm size pieces. Prepared spinach leaves and tomato surface samples were placed on aluminum foil in a laminar flow biosafety hood, and 0.1 ml of culture cocktail was inoculated onto one side of each prepared sample by depositing droplets with a micropipettor at 15–20 locations. Samples were dried in the hood for 1 h at 22 ± 2 °C with the fan running.

2.3. Combined treatment system of ClO_2 gas and aerosolized sanitizer

The combined treatment of ClO_2 gas and aerosolized sanitizer was conducted in the treatment apparatus described previously (Park and Kang, 2015). ClO_2 gas produced by the ClO_2 gas generator (Daehan E&B, Goyang-si, South Korea) was introduced into the polyvinyl chloride treatment chamber (length × width × height, 0.7 m × 0.5 m × 0.6 m). A ClO_2 gas transmitter (ATi F12, Analytical Technology, UK) was used to monitor and control the concentration of ClO_2 gas in the treatment chamber. A ring blower (HRB-101, Hwanghae electronic, Incheon, South Korea) was used to continuously circulate ClO_2 gas in the treatment chamber. A commercial ultrasonic nebulizer (H-C976, Osungsa, Changwon-si, South Korea) was used to control RH in the treatment chamber by generating aerosolized sanitizer or distilled water. RH and temperature in the treatment chamber were monitored with a thermohygrometer (YTH-600, Uins, Seoul, South Korea).

2.4. Procedures for treating samples

Peracetic acid (PAA) (Omega Chemical, Gyeongbuk, Korea) was used as an aqueous sanitizer and diluted with distilled water to a concentration of 80 ppm. The U.S. Food and Drug Administration (FDA) approved the use of PAA for sanitizing fruits and vegetables at concentrations that do not exceed 80 ppm in wash water (Anonymous, 2000). Inoculated spinach leaves and tomatoes were placed in the treatment chamber and covered with a plastic lid. For treatments with ClO_2 gas alone, samples were subjected to 5 or 10 ppmv ClO_2 gas for 20 min. The RH of the treatment chamber was adjusted with distilled water to 90% with an accuracy of ± 2%. For treatment with only aerosolized PAA, samples were exposed to 80 ppm of aerosolized PAA for 20 min. During treatment, the RH of the treatment chamber was adjusted with aerosolized PAA to 90 ± 2%. For combined treatments, samples were subjected to ClO_2 gas (5 or 10 ppmv) and 80 ppm of aerosolized PAA for 20 min. The RH of the treatment chamber was adjusted with aerosolized PAA to 90 ± 2% during treatment. All experiments were performed at 22 ± 2 °C. When the desired ClO_2 gas concentration and RH were achieved, the plastic lid was removed and the inoculated side of spinach leaves and tomatoes were exposed to ClO_2 gas. Samples were withdrawn after 5, 10, 15, and 20 min exposure to each treatment, and treated samples were used to determine surviving bacterial populations. These experiments were repeated three times.

2.5. Bacterial enumeration

Treated and untreated (control) spinach leaves (10 ± 0.2 g) and one piece of tomato were transferred into sterile stomacher bags (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 90 or 30 ml of neutralizing buffer (Difco), respectively. Stomacher bags were homogenized for 2 min with a stomacher (EASY MIX, AES Chemunex, Rennes, France). After homogenization, 1 ml sample aliquots were tenfold serially diluted in 9 ml of BPW, and 0.1 ml of sample or diluent was spread-plated onto selective media. Sorbitol MacConkey agar (SMAC; Difco), Xylose Lysine Desoxycholate agar (XLD; Difco), and Modified Oxford Medium (MOX; Difco) were used as selective media for the enumeration of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. Where low numbers of surviving cells were anticipated, 250 μl of undiluted sample was plated onto each of four plates to lower the detection limit. The plates were incubated at 37 °C for 24–48 h, and colonies were counted after incubation.

For the resuscitation of injured *E. coli* O157:H7, phenol red agar base (Difco) with 1% sorbitol (SPRAB) was used (Rhee et al., 2003). One hundred microliter of sample or diluent was spread-plated onto SPRAB and incubated at 37 °C for 24 h. Injured cells of *S.* typhimurium and *L. monocytogenes* were enumerated using the overlay (OV) method proposed by Kang and Fung (1999, 2000). One hundred microliter of sample or diluent was spread-plated onto TSA and incubated at 37 °C for 2 h to allow injured cells to resuscitate before overlaying with 7 mL of XLD (OV-XLD) or MOX (OV-MOX) for *S.* Typhimurium and *L. monocytogenes*, respectively. The plates were incubated at 37 °C for 22 h after the overlay solidified. Where low numbers of surviving cells were anticipated, 250 μl of undiluted cell suspension was plated onto four plates of each respective medium.

2.6. Measurement of color and texture of samples

Treated spinach leaves and tomatoes (uninoculated) were stored at 7 °C for 7 days to identify quality changes during storage following each treatment. Color values (Hunter's L, a, b) of spinach leaves and tomatoes were measured with a Minolta colorimeter (model CR300, Minolta Co., Osaka, Japan) at 3 locations on each sample. The texture of spinach leaves and tomatoes was evaluated with a texture analyzer (TA-CT3, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) with a blade set and cylinder probe with a 4 mm diameter, respectively. Twenty grams of spinach leaves was placed onto the press holder with the stems positioned perpendicular to the path of the blade, and a blade was moved down at 2 mm/s (path length 10 mm). For tomatoes, the loading rate and path length were set at 2 mm/s and 10 mm. Three