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RESEARCH NOTE

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Effects of Aerosolized Sanitizers of Different Droplet Sizes on Foodborne Pathogen Reduction

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Abstract The diffusivity of aerosol sanitizers may be determined by the weight and droplet size of the aerosol. To test the effects of droplet size, 2 types of aerosol sanitizers were prepared using different ultrasonic nebulizer frequencies (1.6 and 2.4 MHz) and their reduction activities were determined against *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium*. A sodium hypochlorite aerosol was treated for 10, 30, or 60 min in a model aerosol cabinet. When the aerosol prepared by nebulizing at 1.6 MHz was treated for 30 min, a 0.2 log reduction was observed in *E. coli* O157:H7 and 0.3 log reductions were exhibited in *L. monocytogenes* and *S. typhimurium*, respectively. After 60 min, the 3 pathogens were reduced by 1.7, 0.6, and 0.8 log units, respectively. However, when the aerosol prepared by nubulizing at 2.4 MHz was treated, the microbes presented 1.6, 0.5, and 0.6 log reductions at 30 min, and 1.8, 0.9, and 1.1 log reductions at 60 min of treatment, respectively.

Key words: aerosol, sanitizer, reduction, pathogen, droplet size

Introduction

The sanitation of raw foodstuffs is an important intervention in order to reduce the occurrence of foodborne outbreaks. It is common to apply sanitizing agents directly to produce, which is accomplished by spraying or dipping into an aqueous sanitizer. These techniques are reasonably effective in reducing pathogens, but they are limited due to interferences from surface features such as cracks and biofilms, which impede the sanitizer's contact with pathogens.

Charkowski *et al.* (1) found that wrinkled alfalfa seeds harbor more aerobic bacteria and are more difficult to sanitize than smooth seeds. Itoh *et al.* (2) reported the presence of viable *Escherichia coli* O157:H7 in the inner tissues and stomata of cotyledons in radish sprouts grown from artificially inoculated seeds. Han *et al.* (3) determined that significant *E. coli* O157:H7 growth and multiplication occurred on injured surfaces of green peppers. Seo and Frank (4) found many live *E. coli* O157:H7 cells in the stomata and on the cut edges of lettuce after treating with aqueous sanitizer.

Aqueous sanitizers can fail to reach and kill pathogens located in the aforementioned sites. One potential way to overcome this disadvantage is to use gaseous sanitizers. A number of reports have been published on the efficacy of gaseous sanitization (3,5-7), and this method is an effective tool for pathogen reduction on foods having surface hindrances. However, gaseous sanitizers have several disadvantages: a sophisticated apparatus is needed for gas generation and the number of applicable sanitizers is limited.

Aerosol sanitization was recently reported as an alternative method to gaseous sanitization (8,9). When peroxyacetic acid ($C_2H_4O_3$) and hydrogen peroxide (H_2O_2) were atomized to aerosol sanitizer and treated on foodborne pathogens in laboratory media within a large semi-trailer cabinet (14.6×2.6×2.8 m), the sanitizer offered 7.69, 6.93, and 8.18 log reductions against *Listeria innocua*, *Staphylococcus aureus*, and *Salmonella typhimurium*, respectively. And interestingly, their reduction patterns were not statistically different ($p \geq 0.05$) relative to different heights (0-2.8 m) and orientations (face-up, vertical, face-down). As a food application test, artificially inoculated lettuce leaves were treated with an aerosol sanitizer for 60 min in a model chamber, presenting 3.4, 4.5, and 3.8 log reductions in *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes*, respectively. Thus, it was concluded that aerosol sanitizers have higher diffusivities and can be effective in interventions against foodborne pathogens.

An aerosol is defined as a dispersion of a liquid material or solution in air in the form of a fine mist. A smaller sized aerosol is expected to have better diffusivity and can overcome food surface hindrances.

This study was conducted to determine the effect of droplet size on foodborne pathogen reduction. Aerosols of 2 different particle sizes were generated with sodium hypochlorite solution and tested on artificially inoculated lettuce leaves in a model chamber system.

Materials and Methods

Bacterial strains Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890), *S. typhimurium* (ATCC 19585, ATCC 43174, and ATCC 363755), and *L. monocytogenes* (ATCC 19114, ATCC 19113, and ATCC 7644) were obtained from the Food Safety Division at the Korea Food Research Institute (Seongnam, Korea). These strains were then used to inoculate the surfaces of lettuce leaves. The *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes* strains

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were cultured in tryptic soy broth (TSB: Difco, Becton Dickinson, Sparks, MD, USA) at 37°C for 24 hr, harvested by centrifugation at 4,000×g for 20 min at 4°C, and then washed 3 times with buffered peptone water (Difco, Becton Dickinson). The final pellets were resuspended in buffered peptone water, corresponding to approximately 10^8 to 10^9 CFU/mL. Next, the strains from each foodborne pathogen group were combined to construct culture cocktails. These culture cocktails were used in subsequent experiments.

Sample preparation and inoculation Lettuce was purchased at a local grocery store (Seongnam, Korea). The lettuce leaves were trimmed to make 25 g samples, and were separated and placed on sterile aluminum foil in a laminar flow biosafety hood. For inoculation, 100 mL of each pathogen cocktail was applied to the surface of the leaves by depositing droplets at 10 locations with a micropipettor. The leaves were dried in a hood for 30 min with the fan running.

Preparation of different droplet-sized aerosol sanitizers A commercial sodium hypochlorite product was purchased from a local market. The sodium hypochlorite solution was diluted to 200 ppm of total chlorine content. The total chlorine content was measured by the iodometric titration method (10). The aerosol sanitizers were prepared using 1.6 and 2.4 MHz nebulizer frequencies. The prepared aerosol sanitizers were delivered to the model chamber with an 8 cm diameter hose.

Droplet size determination The droplet sizes of the aerosol sanitizers were determined by the laser diffraction method. A CILAS 1064 particle size analyzer (Marcoussis, France) was used for the analysis. The prepared aerosols were introduced between detection and 2 sequenced laser sources, which were positioned at 0 and 45°C using the 8 cm diameter hose.

Antimicrobial aerosol treatment A model cabinet (68×50×50 cm) was used to test the efficacies of the antimicrobial aerosols (Fig. 1). The aerosols were routed to the sealed cabinet from an aerosol generator, to an interface located in the upper cover. During treatment, samples were taken at 10, 30, and 60 min. All tests were performed at room temperature (22±2°C).

Enumeration of healthy cells After the 10, 30, and 60 min treatments, the lettuce leaves (25 g) were placed in a stomacher bag containing 50 mL of buffered peptone water and homogenized for 2 min with a Seward stomacher (400 Circulator; Seward, London, UK). After homogenization, sample aliquots (1 mL) were serially diluted in 9 mL of sterile buffered peptone water and 0.1 mL of sample or diluent was spread-plated onto each selective agar. Sorbitol MacConkey agar (SMAC; Difco), xylose lysine desoxycholate agar (XLD; Difco), and Oxford agar base (OAB; Difco) with an antimicrobic supplement (Bacto™ Oxford Antimicrobic Supplement, Difco) were used as selective media for the enumeration of *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes*, respectively (Table 1). All agar media were incubated at 37°C for 24 to 48 hr as appropriate, and then the colonies were enumerated.

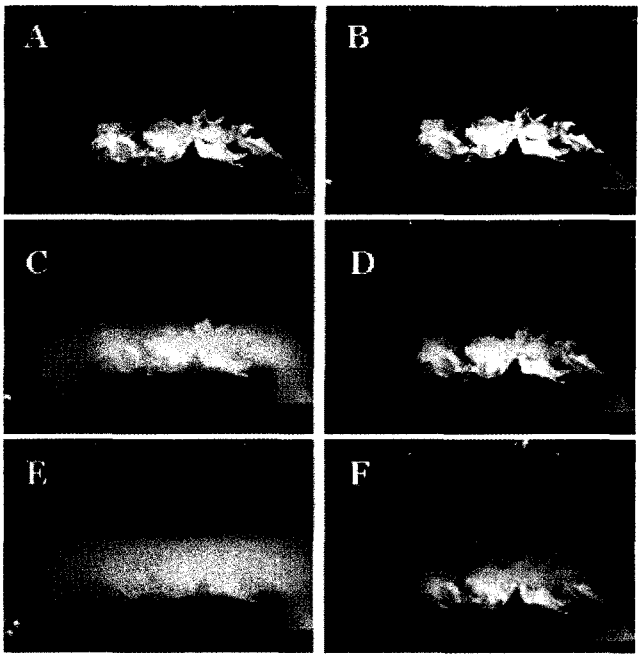

Fig. 1. Diffusion patterns of aerosol sanitizers prepared by nebulization at 1.6 and 2.4 MHz according to time. A, B: 0 min; C, D: 1 min; E, F: 2 min.

Table 1. Particle size distribution of aerosol sanitizers

Aerosol cumulative values	Aerosol produced by 1.6 MHz nebulizer (μm)	Aerosol produced by 2.4 MHz nebulizer (μm)
Diameter at 10%	2.08	1.24
Diameter at 50%	9.48	7.54
Diameter at 90%	27.54	16.07
Mean diameter	12.77	8.21

Statistical analysis The data are presented as means± SEMs of at least 3 separate experiments. Statistical comparisons were performed using the ANOVA procedure of SAS, and significant differences among the various treatments were compared with Duncan's multiple range tests.

Results and Discussion

Aerosol diffusion dynamics Figure 1 shows the diffusion dynamics of the aerosol sanitizers in the model cabinet at room temperature. The aerosols made from sodium hypochlorite dispersed evenly in the model system and showed the expected gaseous behavior. After 1 min of treatment, the aerosol made by nebulization at 2.4 MHz was evenly dispersed around the model cabinet. However, the aerosol made by nebulization at 1.6 MHz settled closer to the bottom of the cabinet. The aerosol made at 2.4 MHz was almost saturated after 2 min of treatment. These results could be attributed to the droplet sizes of the aerosols.

Particle size distribution patterns of the aerosol sanitizers The droplet sizes of the aerosol sanitizers were determined by the laser diffraction method (Fig. 2). The distribution

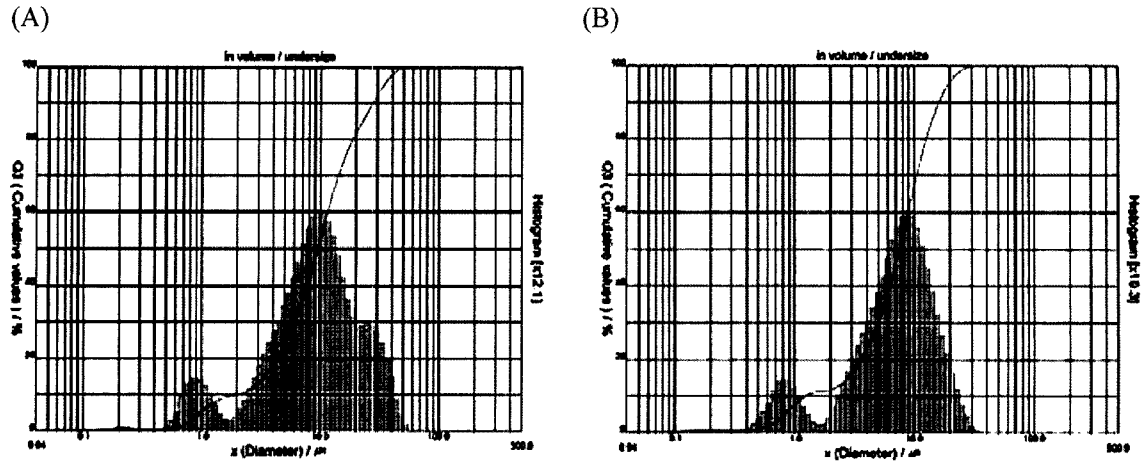

Fig. 2. Particle size distribution patterns of aerosol sanitizers as measured by a CILAS 1064 analyzer. (A) Aerosol prepared by nebulization at 1.6 MHz, (B) aerosol prepared by nebulization at 2.4 MHz.

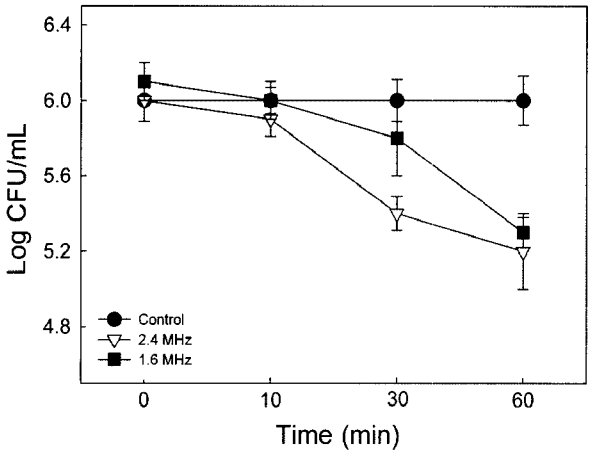

Fig. 3. Survival curves for *E. coli* O157:H7 on iceberg lettuce leaves exposed to aerosolized sodium hypochlorite at room temperature (22±2°C). Sorbitol MacConkey agar was used as selective media. Values are the means of 3 determinations. Error bars indicate 95% confidence intervals.

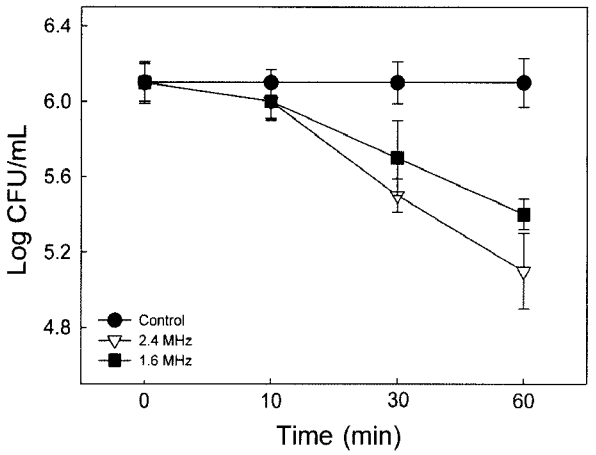

Fig. 4. Survival curves for *L. monocytogenes* on iceberg lettuce leaves exposed to aerosolized sodium hypochlorite at room temperature (22±2°C). Oxford agar base was used as selective media. Values are the means of 3 determinations. Error bars indicate 95% confidence intervals.

peak of the aerosol made by nebulizing at 2.4 MHz was located further to the left than that of the aerosol made at 1.6 MHz. Thus, this aerosol was thought to have a much smaller diameter. The droplet size of the aerosol made at 1.6 MHz was determined to be 2.08 μm at 10% cumulative value, 9.48 μm at 50% cumulative value, and 27.54 μm at 90% cumulative value. The total mean diameter was calculated as 12.77 μm. The droplet size of the aerosol prepared by nebulization at 2.4 MHz was determined as 1.24 μm at 10% cumulative value, 7.54 μm at 50% cumulative value, and 16.07 μm at 90% cumulative value. The total mean diameter was 8.21 μm. Thus, it was concluded that nebulizing at a higher intensity can reduce the aerosol droplet size and increase diffusivity, which may have greater efficacy for microbial reduction.

Microbial reduction of foodborne pathogens The efficacies of the different droplet size aerosols were determined against artificially inoculated *E. coli* O157:H7 on lettuce leaves in a model cabinet at room temperature. The inoculated lettuce leaves treated with the 1.6 MHz nebulized aerosol presented *E. coli* O157:H7 reductions of 0.1, 0.2, and 1.7 log units after 10, 30, and 60 min, respectively. However, treating with the aerosol sanitizer produced by nebulization at 2.4 MHz resulted in *E. coli* O157:H7 reductions of 0.1, 1.6, and 1.8 log units after the same treatment times, respectively, (Fig. 3). In the case of *L. monocytogens*, reductions of 0.1, 0.3, and 0.6 log units were observed with the aerosol prepared at 1.6 MHz, and 0.1, 0.5, and 0.9 log unit reductions occurred with the aerosol produced at 2.4 MHz (Fig. 4). *S. typhimurium* levels were reduced by 0, 0.3, and 0.8 log units after 10, 30, and 60 min of treatment when treated with the 1.6 MHz nebulized aerosol, respectively. However, *S. typhimurium* was reduced by 0.1, 0.6, and 1.1 log units with the 2.4 MHz nebulized aerosol after the same respective treatment times (Fig. 5). Thus, it was conclude that smaller droplet size improves the diffusing activity of an aerosol and can lead to more efficient microbial reductions.

Microorganisms that are attached to fruit and vegetable surfaces are especially inaccessible to aqueous treatments when they are concealed within injured sites or present in

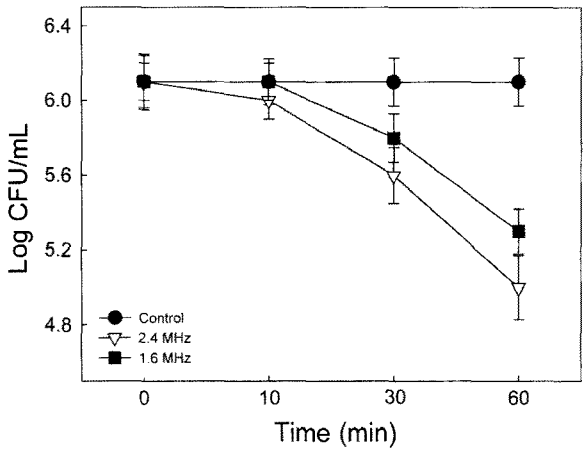

Fig. 5. Survival curves for *S. typhimurium* on iceberg lettuce leaves exposed to aerosolized sodium hypochlorite at room temperature (22±2°C). Xylose desoxycholate agar was used as selective media. Values are the means of 3 determinations. Error bars indicate 95% confidence intervals.

hydrophobic pockets or the folds of leaf surfaces (11). Numerous researchers have investigated how microorganisms attach to food surfaces, along with their responses to various sanitizer treatments (12-15). These studies have concluded that microbial attachment on food surfaces enhances resistance to sanitization. And most of these studies indicate that a less than 2 log pathogen reduction results when using aqueous sanitizers on fruits and vegetables. However, the use of gaseous sanitizers can overcome the limitations inherent to aqueous sanitizers. Han *et al.* (3) studied the attachment of *E. coli* O157:H7 to injured and uninjured green pepper surfaces and the effect of chlorine dioxide gas treatment. They observed approximate 3.3 and 6.5 log reductions following treatments with 0.62 and 1.24 mg/L of chlorine dioxide gas, respectively. Lee *et al.* (16) reported a 5.4 log unit reduction in *S. typhimurium* on inoculated lettuce when using chlorine dioxide gas as a sanitizer. Although gaseous sanitizers have advantages over aqueous sanitizers, they also have several disadvantages such as the need for a sophisticated apparatus for gas generation and a limited number of applicable sanitizers.

Aerosols have a gaseous nature with a higher penetrating activity than aqueous sanitizers, and sanitizers that dissolve in water can easily be applied to target sites by aerosolization. Aerosolization is a powerful delivery tool for diverse antimicrobial agents. In therapeutic applications, various antibiotics are delivered by aerosol ventilation (17-20), and in room disinfection applications, organic acids such as lactic acid and acetic acid are delivered by aerosolization. Aerosolization combines the advantages of both aqueous and gaseous sanitizers: a wide selection of applicable sanitizers and a high penetration activity suitable for a wide variety of foods.

Oh *et al.* (8) reported that foodborne pathogens did not show statistically different ($p>0.05$) reduction patterns relative to height and orientation in a large model semi-trailer system (14.6×2.6×2.8 m). Thus, it is thought that aerosols have powerful diffusiveness characteristics and the ability to penetrate all surface irregularities much like gas sanitizers. In addition, this research team demonstrated that aerosol sanitizer treatments caused strong reduction patterns against artificially inoculated foodborne pathogens on lettuce leaves (9), suggesting that aerosolization may be a new and convenient method for sanitizing produce for storage and transport.

The physical characteristics of aerosols such as droplet size can influence diffusiveness and reduction efficiency. It is thought that smaller droplet size aerosols have greater diffusivity. This was indeed demonstrated with the different droplet sized aerosols examined in this study. Thus, if researchers can determine the most effective particle size for aerosol sanitizers as well as the optimum sanitizer components suitable for foods, aerosol sanitizers could be widely used for foodborne pathogen reduction. However, various treatment parameters such as relative humidity, temperature, the composition of the treatment container, etc., can impact the effectiveness of antimicrobial aerosol treatments. Therefore, additional studies on the environmental effects need to be performed before this method can be applied commercially.

Acknowledgments

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