Efficacy of Aerosolized Peroxyacetic Acid as a Sanitizer of Lettuce Leaves

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ABSTRACT

Aerosolized sanitizer was investigated as a potential alternative to aqueous and gaseous sanitizers for produce. Peroxyacetic acid was aerosolized (5.42 to 11.42 μm particle diameter) by a commercially available nebulizer into a model cabinet. Iceberg lettuce leaves were inoculated with three strains each of Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella Typhimurium and then treated with aerosolized peroxyacetic acid for 10, 30, or 60 min in a model aerosol cabinet at room temperature (22 ± 2°C). After treatment, surviving healthy and injured bacterial cells were enumerated on appropriate selective agars or using the overlay agar method. Inoculated iceberg lettuce leaves exposed to aerosolized peroxyacetic acid for 10 min exhibited a 0.8-log reduction in E. coli O157:H7, a 0.3-log reduction in Salmonella Typhimurium, and a 2.5-log reduction in L. monocytogenes when compared with the control. After 30 min of treatment, the three pathogens were reduced by 2.2, 3.3, and 2.7 log, and after 60 min, the reductions were 3.4, 4.5, and 3.8 log, respectively. Aerosolization may be a new and convenient method for sanitizing produce for storage or shipping.

Foodborne pathogens cause millions of illnesses and thousands of deaths every year in the United States (41). As consumption of raw or minimally processed fruits and vegetables has increased, so has the frequency of outbreaks of illness associated with these foods (2, 43). Sanitization of raw produce is an important intervention for reducing the occurrence of foodborne illness. Direct application of sanitization agents to produce is common and is accomplished by spraying with or dipping into aqueous sanitizer. These techniques are reasonably effective in reducing pathogens, but they are limited because of interference from such surface features as cracks and biofilms, which impede contact of sanitizer with pathogens. Charkowski et al. (4) found that wrinkled alfalfa seeds harbor more aerobic bacteria and are more difficult to sanitize than are smooth seeds. Ito et al. (19) found viable Escherichia coli O157:H7 in the inner tissues and stomata of cotyledons of radish sprouts grown from artificially inoculated seeds. Han et al. (12) observed significant growth and multiplication of E. coli O157:H7 on injured surfaces of green peppers. Seo and Frank (36) found many live E. coli O157:H7 cells in stomata and on cut edges of lettuce after treatment with aqueous sanitizer.

Aqueous sanitizers often fail to reach and thus kill pathogens located in inaccessible sites (36). Gaseous sanitizer can overcome this limitation. A number of reports have been published on the efficacy of gaseous sanitizer (11–13, 20, 21), and it is an effective tool for sanitization of foods with surface hindrance. However, gaseous sanitizers have their own disadvantages: a sophisticated apparatus is needed for gas generation, and the number of applicable sanitizers is limited.

Aerosolization is the dispersion of liquid as a fine mist in air. Antimicrobial applications of aerosols include respiratory medical treatments (32, 35, 42) and room disinfection (8, 16). Diseases that involve infection of the respiratory system can be treated using aerosol therapy (1, 5, 14, 23, 27, 28). Pseudomonas aeruginosa (7, 15), Staphylococcus aureus (25), Alcaligenes xylosoxidans (33), and Aspergillus fumigatus (30) have been targets of clinical treatment with aerosolized antibiotics (10, 26, 30, 31). Aerosol disinfection of poultry houses has been an effective tool for increasing poultry production (29, 38–40). Fišer (8) reported that continual disinfection by lactic acid aerosolization resulted in an improved state of health of chickens. Hiom et al. (16) found that fine aerosol mists have better penetrating ability than trigger spray for reduction of surface bioburden during hospital aseptic processing.

With its penetrating activity and broad spectrum of applicable sanitizers, aerosolization may be an alternative sanitizer delivery system for the food industry. However, the use of aerosolized sanitizer on foods has not been studied. The present study was conducted to evaluate the efficacy of aerosolized peroxyacetic acid against Listeria monocytogenes, E. coli O157:H7, and Salmonella Typhimurium on the surface of iceberg lettuce (Lactuca sativa) leaves.

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FIGURE 1. Aerosolization of peroxyacetic acid (Tsunami 200) in a model cabinet system: (A) 0 s, (B) 5 s, (C) 10 s, (D) 15 s, (E) 30 s, and (F) 40 s.

MATERIALS AND METHODS

Cultures and cell suspensions. Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890), *Salmonella Typhimurium* (ATCC 19585, ATCC 43174, and ATCC 363755), and *L. monocytogenes* (ATCC 19114, ATCC 19113, and ATCC 7644) were obtained from the Food Science and Human Nutrition culture collection at Washington State University (Pullman, Wash.) and then inoculated onto iceberg lettuce leaves. Each strain of *E. coli* O157:H7, *Salmonella* Typhimurium, or *L. monocytogenes* was cultured in tryptic soy broth (Difco, Becton Dickinson) at 37°C for 24 h, harvested by centrifugation at 4,000 × g for 20 min at 4°C, and washed three times with buffered peptone water (Difco, Becton Dickinson). Pellets were resuspended in buffered peptone water to obtain approximately 10⁸ to 10⁹ CFU/ml. The nine strains were combined to construct culture cocktails, which were used to inoculate lettuce leaves.

Sample preparation and inoculation. Iceberg lettuce was purchased at a local grocery store (Pullman, Wash.). Leaves were trimmed to weigh 25 g and were placed on sterile aluminum foil in a laminar flow hood. For inoculation, 100 μl of pathogen cocktail was applied to lettuce leaves by depositing droplets at 10 locations with a micropipette. The lettuce was dried in the hood for 30 min.

Sanitizer preparation and residual peroxyacetic acid measurement. Peroxyacetic acid (Tsunami 200, EcoLab, St. Paul, Minn.) was diluted according to the manufacturer’s instructions with 1 liter of distilled water to 40 ppm, as determined using a peracid/peroxide test kit (EcoLab) according to the manufacturer’s instructions. All experiments were performed at this concentration.

Antimicrobial aerosol treatment. A model glass cabinet (50 by 25 by 30 cm) was used to test the efficacy of aerosolized peroxyacetic acid (Fig. 1). The cabinet was sealed, and aerosol was routed from a commercially fabricated nebulizer (Royal G Enterprise, ShenZhen, China) using a flexible plastic tube. The tube entered the lid of the cabinet through a rubber stopper, which was sealed around the edges with silicone caulk. Aqueous peroxyacetic acid was atomized into approximately 5.42- to 11.42-μm particles. During treatment, lettuce samples were removed at 10, 30, and 60 min. The aerosol was not visibly disturbed by sample removal. All tests were performed at room temperature (22 ± 2°C).

Enumeration of healthy cells. After 10, 30, and 60 min of treatment, lettuce leaves (25 g) were placed in a stomacher bag (Pulse, Washington, Wash.) and then homogenized for 2 min with a Seward stomacher (400 Circulator, Seward, London, UK). After homogenization, 1 ml aliquots were serially diluted in 9 ml of sterile peptone water, and 0.1 ml of sample or diluent was spread plated onto each selective agar. Sorbitol MacConkey agar (SMAC; Difco, Becton Dickinson), xylose lysine desoxycholate agar (XLD; Difco, Becton Dickinson), and Oxford agar base (OAB; Difco, Becton Dickinson) with antimicrobial supplement (Bacto Oxford antimicrobial supplement, Difco, Becton Dickinson) were used as selective media for the enumeration of *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes*, respectively. All agar media were incubated at 37°C for 24 to 48 h as appropriate before colonies were enumerated.

Enumeration of injured cells. Phenol red agar base (SPRAB; Difco, Becton Dickinson) with 1% sorbitol (3%) was used to enumerate chemically injured *E. coli* O157:H7 cells. Ten percent of isolates from SPRAB plates were randomly selected...
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RESULTS AND DISCUSSION

Figure 1 shows diffusion of aerosolized peroxyacetic acid in the model cabinet. The cabinet filled rapidly and was almost visually saturated after 40 s. The aerosol dispersed evenly in the model cabinet and was not visibly disturbed by sample removal. Figure 2A shows survival of E. coli O157:H7 enumerated on SMAC and OV-SPRAB from iceberg lettuce leaves treated with aerosolized peroxyacetic acid. Lettuce leaves were inoculated with E. coli O157:H7 at 10^6 to 10^7 CFU/g, and no reduction was observed after 60 min in the model aerosol cabinet at room temperature. When inoculated lettuce leaves were treated with aerosolized peroxyacetic acid, E. coli O157:H7 concentrations were reduced by 0.8, 2.2, and 3.4 log after 10, 30, and 60 min, respectively. However, there was no significant difference between the concentrations on SMAC and SPRAB (P > 0.05). Treatment with aerosolized peroxyacetic acid reduces E. coli O157:H7 on lettuce leaves without producing chemically injured cells.

For Salmonella Typhimurium and L. monocytogenes, lettuce leaves were inoculated with 10^6 to 10^7 CFU/g. Concentrations of Salmonella Typhimurium were reduced by 0.3, 3.3, and 4.5 log after 10, 30 and 60 min of treatment, and L. monocytogenes was reduced by 2.5, 2.7, and 3.8 log, respectively (Fig. 2B and 2C). There was no significant difference between numbers of injured and healthy cells (P > 0.05) for either Salmonella Typhimurium or L. monocytogenes.

Other researchers have reported the antimicrobial activity of peroxyacetic acid on produce. Lukasik et al. (24) found that treatment with 100 ppm peroxyacetic acid produced a 96.8% reduction of E. coli O157:H7 and 97.3% reduction of Salmonella Montevideo on strawberries. Treatment of seeds for 10 min at 23°C with 40 or 60 ppm peroxyacetic acid resulted in E. coli O157:H7 concentrations of 1.12 and 1.30 log CFU/g (3). Treatment for 5 min at 58°C with 40 and 160 ppm resulted in reductions of 2.88 and 2.46 log CFU/g, respectively (3).

For sanitizers to be effective, there must be direct contact between the sanitizer and the target microorganism. Microorganisms are especially inaccessible to aqueous treatments when concealed in injured sites, hydrophobic pockets, or folds in leaf surfaces (34). Numerous researchers have investigated attachment of microorganisms to food surfaces and the efficacy of subsequent sanitizer treatments (9, 22, 37, 45) and have found that attachment of microorganisms to food surfaces enhances resistance to aqueous sanitization. In several studies, the ineffectiveness of aqueous sanitizers on fruits and vegetables has been demonstrated. Beuchat (2) observed less than 1-log reductions in Salmonella on iceberg lettuce treated with aqueous chlorine. Zhang and Farber (44) reported that treatment with aqueous chlorine and chlorine dioxide for 10 min at 22°C reduced L. monocytogenes on lettuce by 1.7 and 0.8 log, respectively. Most investigators found that a less than 2-log reduction was achieved when using aqueous sanitizers at the manufacturer-recommended concentration on fruits and vegetables.

Use of gaseous sanitizer may overcome the limitations associated with aqueous sanitizers. Chlorine dioxide can be used in gaseous or aqueous forms. Han et al. (13) studied the attachment of E. coli O157:H7 to uninjured and injured green pepper surfaces and compared the efficacy of aqueous versus gaseous chlorine dioxide for reduction in pathogen numbers. For gaseous chlorine dioxide at 3 mg/liter, they observed reductions of 6 and 3.5 log on uninjured and injured surfaces, respectively. In contrast, when 3 mg/liter aqueous chlorine dioxide was used, the reductions were only 3.7 and 0.4 log for uninjured and injured surfaces, respectively. However, the use of gaseous sanitizers is limi-
ited because of the sophisticated apparatus needed for gas generation and the scarcity of applicable sanitizers.

Aerosolized sanitizers may have higher penetrating activity than aqueous sanitizers because of their gaseous behavior. Sanitizers that dissolve in water can be easily applied to target sites by aerosolization. Various antibiotics are routinely delivered by aerosolization for therapeutic applications (6, 10, 25, 26) and room disinfection, and organic acids such as lactic acid and acetic acid have been delivered by aerosolization (29, 38–40). Aerosolization combines the advantages of aqueous and gaseous sanitizers: a wide selection of applicable sanitizers and high penetration activity suitable for a wide variety of foods. With the continued development of new aerosolization systems (17, 18) with smaller mist droplets, the penetration capacity and diffusivity of aerosolized sanitizers will be increased.

Aerosolized peroxycetic acid is an effective sanitizer of lettuce leaves and has potential application in the food industry. Aerosolized sanitizers may be useful for preventing regrowth of pathogens and spoilage microorganisms during shipping or extended storage of produce when dipping or spraying with aqueous sanitizers is not feasible.

Various treatment parameters such as relative humidity, temperature, and the composition of the treatment container may impact the effectiveness of aerosolized sanitizer. All surfaces of the treated food must be reached by the sanitizer, so produce must be shifted during treatment. Before this method can be applied commercially, studies should be performed to determine ideal treatment parameters for aerosolized sanitizers.

REFERENCES


3. Beuchat, L. R., and A. J. Scouten. 2002. Combined effects of water activity, temperature, and the composition of the treatment container may impact the effectiveness of aerosolized sanitizer. All surfaces of the treated food must be reached by the sanitizer, so produce must be shifted during treatment. Before this method can be applied commercially, studies should be performed to determine ideal treatment parameters for aerosolized sanitizers.


