

Efficacy of Antimicrobial Agents in Lettuce Leaf Processing Water for Control of *Escherichia coli* O157:H7

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ABSTRACT

The objectives of this research were to study transfer and control of *Escherichia coli* O157:H7 during simultaneous washing of inoculated and uninoculated lettuce pieces and to determine the efficacy of antimicrobial agents (peroxyacetic acid, mixed peracid, and sodium hypochlorite) on reducing the transfer of *E. coli* O157:H7 through processing water with or without organic load. Lettuce leaf pieces (5 by 5 cm) were inoculated with a five-strain mixture of green fluorescent protein–labeled *E. coli* O157:H7 at 5.6 log CFU per piece. One inoculated lettuce piece was added to five uninoculated leaves during washing. Peroxyacetic acid and mixed peracid were tested at 10, 20, and 30 ppm, and chlorine was tested at 30 and 50 ppm. No organic load (liquefied lettuce leaves) and 10% organic load in processing water were compared. Without organic load, peroxyacetic acid at 30 ppm, mixed peracid at 10, 20, and 30 ppm, and chlorine at 30 and 50 ppm all significantly reduced *E. coli* O157:H7 in processing water by 1.83, 1.73, 1.50, 1.83, 1.34, and 1.83 log CFU/ml, respectively, compared with washing with water alone. These antimicrobials at all concentrations tested also significantly reduced transfer of the bacteria from an inoculated leaf to uninoculated leaves in the processing water by 0.96 to 2.57 log CFU per piece. A 10% organic load in the processing water reduced efficacy of antimicrobial agents. In this contaminated water, peroxyacetic acid at 10 and 20 ppm and chlorine at 30 ppm produced effects not significantly different from those of water alone. Therefore, it is important to understand the impact of organic load when validating the effectiveness of antimicrobial treatments.

Lettuce is one of the most commonly consumed leafy greens, with a farm value of over \$1.5 billion in 2005 in the United States (10). Lettuce is perceived by consumers as healthful and nutritious. Contamination of vegetables by human pathogens can occur at many locations in the farm-to-fork continuum, including contamination of seeds and of product during production, harvesting, postharvest handling, transport distribution, storage, processing, and preparation (13). Survival and growth of foodborne human pathogens on fresh and fresh-cut produce has been widely reported (3–5, 12, 14, 15, 21). The efficacy of different antimicrobials used to kill foodborne pathogens on fresh and fresh-cut produce has been studied extensively, and most antimicrobials are minimally effective, reducing pathogen contamination by only 1 to 2 log CFU/g (3, 5, 9, 21).

Antimicrobial agents often are added to water in flumes that convey or wash fresh fruits and vegetables. The addition of these agents reduces the number of microorganisms in fruit and vegetable processing water. Reducing the number of microorganisms in recycled processing water helps prevent the water from becoming a vehicle of cross-contamination (7, 8, 11, 16, 18, 19). Antimicrobial chemicals in processing water also can reduce microorganisms on the surfaces of fruits and vegetables. However, processing water antimicrobials are more effective for reducing microorganisms in water suspensions than on fruit and vegetable surfaces (1, 2, 6, 11, 18–20).

This study was conducted (i) to investigate transfer of *Escherichia coli* O157:H7 from an inoculated lettuce leaf piece to uninoculated lettuce leaf pieces during washing, (ii) to determine the efficacy of peroxyacetic acid, mixed peracid, and chlorine for reducing the transfer of *E. coli* O157:H7 under conditions of high organic load, and (iii) to determine the efficacy of peroxyacetic acid, mixed peracid, and chlorine for reducing *E. coli* O157:H7 in lettuce processing water.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Five strains of *E. coli* O157:H7 were used: ATCC 43888 (human feces), EO122 (cattle isolate), K3995 (spinach isolate), K4492 (lettuce, clinical isolate), and F4546 (alfalfa sprout outbreak isolate). A plasmid (pGFPuv) containing a *gfp* gene was introduced into each strain using a CaCl₂ heat shock method (17). Expression of green fluorescent protein (GFP) in labeled cells was evaluated by epifluorescence microscopic examination of colonies. The five strains were cross-streaked onto tryptic soy agar (Difco, Becton Dickinson, Sparks, MD) to confirm lack of cross-inhibitory activity. All strains were grown at 37°C for 24 h on brain heart infusion agar (BHIA; Difco, Becton Dickinson) or in brain heart infusion broth (BHIB; Difco, Becton Dickinson) supplemented with ampicillin (Roche Diagnostics, Indianapolis, IN) at a concentration of 100 µg/ml (BHIA-amp and BHIB-amp, respectively). Colonies of these GFP-labeled strains were viewed under a 396-nm wavelength UV lamp for enumeration.

All *E. coli* O157:H7 strains were transferred to BHIB-amp three times at 24-h intervals before they were used as inocula. Cells from overnight culture (10 ml) were sedimented by centri-

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fugation at $5,000 \times g$ for 10 min and resuspended in 10 ml of 0.1% sterile peptone water (Difco, Becton Dickinson). Approximately equal populations of each of the five strains were combined. Dilutions were made in 0.1% sterile peptone water to create a culture suspension for inoculation of approximately 10^6 CFU/ml.

Antimicrobial agents. Peroxyacetic acid (Tsunami 100), mixed peracid (Tsunami 200), and sodium hypochlorite (XY-12) were provided by Ecolab, Inc. (St. Paul, MN).

Preparation of lettuce for inoculation. Iceberg lettuce (*Lactuca sativa* L.) was purchased from a grocery store (Griffin, GA). Two or three layers of outer leaves were removed from each head of lettuce, and inner leaves were aseptically cut into pieces (ca. 5 by 5 cm), using as much of the leaf portion as possible and avoiding stem areas.

Inoculation of lettuce leaves. Lettuce leaf pieces were placed on a sterile surface in a laminar flow biosafety cabinet, and 100 μ l of the five-strain mixture of culture suspension was spot inoculated with a micropipettor onto the adaxial side of each leaf piece to achieve an initial *E. coli* O157:H7 population of 5.6 log CFU per inoculated lettuce piece. The inoculated leaf pieces were placed in a sterile plastic container with a lid and held at 4°C for approximately 2 h to allow bacterial attachment before treatment. A minor cut (ca. 2 mm) on one side was made on all inoculated leaf pieces to differentiate these pieces from uninoculated leaf pieces during treatment.

Organic load preparation. Two outer layers of iceberg lettuce leaves were discarded. Green leaves (100 g) were placed in a sterile blender jar with 100 g of sterile water tempered to 4°C. Leaves were blended on high speed until they were liquefied and particulates were small enough to be suctioned through a pipette. This organic load preparation was blended immediately before use.

Antimicrobial use solution: chemistry without organic load. The appropriate amount of test antimicrobial was pipetted into 250 ml of sterile deionized water in a 500-ml volumetric flask, and additional sterile deionized water was added to the 500-ml mark.

Antimicrobial use solution: chemistry with 10% organic load. The appropriate amount of test antimicrobial was pipetted into 250 ml of sterile deionized water as above, 50 ml of the organic load preparation was added, and additional sterile deionized water was added to the 500-ml mark.

Antimicrobial use solution: concentration of antimicrobial agent. Concentrations of free chlorine and total peracid in use solutions were determined by an iodine–sodium thiosulfate redox titration (Oxidizer Kit 322, Ecolab). The following antimicrobial agents were evaluated: water; peroxyacetic acid at 10, 20, and 30 ppm; mixed peracid at 10, 20, and 30 ppm; and sodium hypochlorite at 30 and 50 ppm at pH 6.8. All antimicrobials were evaluated without organic load and with a 10% organic load preparation.

Treatment of lettuce leaves with antimicrobial agents. All testing was conducted in a refrigerated room (4 to 5°C). The use solutions (with or without organic load) were poured into the mixing vessel (modified version of the CDC Biofilm Reactor, Bio-Surface Technologies Corp., Bozeman, MT) and stirred at 125 rpm with a magnetic stir bar on a stir plate. Five uninoculated lettuce pieces and one inoculated lettuce piece were placed in the

mixing vessel and agitated for 1.5 min treatment. Lettuce pieces were then removed aseptically and separately placed into Whirl-Pak bags (Nasco, Fort Atkinson, WI) containing 10 ml of 0.5% sodium thiosulfate neutralizing agent (Fisher Scientific, Fair Lawn, NJ). Lettuce pieces were then individually macerated at 230 rpm for 30 s, and serial dilutions were plated in duplicate on BHIA-amp and incubated at $35 \pm 2^\circ\text{C}$ for 48 h.

One milliliter of each treated use solution from the mixing vessel was pipetted into 9 ml of 0.5% sodium thiosulfate. Serial dilutions were plated in duplicate and incubated under the conditions described above.

Control: test substance neutralization. Triplicate neutralization checks were performed on each type of chemistry. If more than one use solution concentration was used, the most concentrated solution was tested. For control A, an uninoculated lettuce piece was dipped into the test substance use solution for 1.5 min and then removed and placed into a small Whirl-Pak bag containing 10 ml of the neutralizing agent (0.5% sodium thiosulfate). Subsequently, 0.1 ml of *E. coli* O157:H7 test system suspension (10^5 CFU/ml) was added and mixed. For control B, an uninoculated lettuce piece was dipped into the test substance diluent (sterile deionized water) for 1.5 min and then removed and placed into a small Whirl-Pak bag containing 10 ml of the neutralizing agent. Subsequently, 0.1 ml of *E. coli* O157:H7 test system suspension (10^5 CFU/ml) was added and mixed. For control C, 0.1 ml of *E. coli* O157:H7 test system suspension (10^5 CFU/ml) was added to 10 ml of sterile peptone water and mixed. Leaf pieces from controls A, B, and C were held at room temperature for 30 min before the microbiological assay. Portions (0.25 ml in quadruplicate and 0.1 ml in duplicate) of each control were plated on BHIA-amp and incubated at $35 \pm 2^\circ\text{C}$ for 48 h.

The neutralizing agent was considered to have effectively neutralized the test substance when the average plate count from control C equaled that of control A $\pm 10\%$. The neutralizing agent was not detrimental to the culture suspension when the average plate count from control C equaled that of control B $\pm 10\%$.

Control: test substance diluent (sterile deionized water) sterility. Portions (0.25 ml in quadruplicate and 0.1 ml in duplicate) of sterile deionized water were plated on BHIA-amp and incubated at $35 \pm 2^\circ\text{C}$ for 48 h.

Control: *E. coli* O157:H7-free lettuce pieces. An uninoculated lettuce piece was aseptically placed into a Whirl-Pak bag, 10 ml of neutralizing agent was added, and the bag contents were homogenized in a laboratory blender (Stomacher 400, Seward, Worthington, UK) at 230 rpm for 30 s. Portions (0.25 ml in quadruplicate and 0.1 ml in duplicate) of homogenate were plated on BHIA-amp and incubated at $35 \pm 2^\circ\text{C}$ for 48 h.

Control: *E. coli* O157:H7-free organic load. Portions (0.25 ml in quadruplicate and 0.1 ml in duplicate) of organic load were plated on BHIA-amp and incubated at $35 \pm 2^\circ\text{C}$ for 48 h.

All chemical solutions were stored at 4°C 1 day before the experiment. The entire experiment was conducted in a room with temperature set at 4°C.

Statistical analysis. Data were analyzed using the general linear models procedure of SAS (SAS 9.1.3; SAS Institute, Inc., Cary, NC at $\alpha = 0.05$). Duncan's multiple range tests were used to determine significant differences ($\alpha = 0.05$) between mean values. The entire study was repeated three times.

TABLE 1. *E. coli* O157:H7 on lettuce leaves and in processing water with and without antimicrobials and without organic load^a

Antimicrobial agent	Concn (ppm)	Mean (\pm SD) <i>E. coli</i> O157:H7 population ^b		
		Inoculated leaves after treatment (log CFU/piece)	Posttreatment processing water (log CFU/ml)	Uninoculated leaves after treatment (log CFU/piece)
Peroxyacetic acid	10	3.31 \pm 0.11 AB	0.88 \pm 0.84 AB	0.20 \pm 0.34 BC
	20	3.21 \pm 0.36 ABC	0.76 \pm 1.32 AB	0.44 \pm 0.27 B
	30	2.38 \pm 0.18 BC	ND B	ND C
Mixed peracid	10	2.27 \pm 0.55 BC	0.10 \pm 0.17 B	0.07 \pm 0.12 BC
	20	2.10 \pm 1.84 C	0.33 \pm 0.58 B	0.18 \pm 0.18 BC
	30	ND D	ND B	ND C
Chlorine	30	3.42 \pm 0.35 AB	0.49 \pm 0.84 B	0.19 \pm 0.32 BC
	50	2.60 \pm 0.44 ABC	ND B	0.07 \pm 0.11 BC
Water		3.68 \pm 0.23 A	1.83 \pm 0.24 A	2.54 \pm 0.19 A

^a *E. coli* O157:H7 population on inoculated untreated leaves was at 5.6 log CFU per piece.

^b Within a column, means with the same letter are not significantly different at $\alpha = 0.05$. ND, not detected. Detection limits were 1 CFU/ml of processing solution and 10 CFU per leaf piece.

RESULTS

E. coli O157:H7 populations for control A were 2.97, 2.92, and 2.98 log CFU/ml for 30 ppm of peracetic acid, 30 ppm of mixed peracid, and 50 ppm of chlorine, respectively, and 2.98 and 2.96 log CFU/ml for controls B and C, respectively. These values were approximately the same, indicating that the neutralizing agent effectively neutralized the test substance and was not detrimental to *E. coli* O157:H7. The sterile deionized water used for all solutions, the lettuce leaves, and the prepared organic load were all negative for *E. coli* O157:H7.

A single lettuce leaf piece inoculated with *E. coli* O157:H7 at 5.6 log CFU transferred contamination in 500 ml of water at approximately 2 log CFU/ml with or without the presence of organic material. Although the contamination levels were not significantly different, peroxyacetic acid at 10 and 20 ppm held the level of contamination in the solution to 1 log CFU/ml less than that of water when no additional organic material was present. All other antimicrobial solutions had significantly less *E. coli* O157:H7 than did water when no additional organic material was present. In posttreatment solutions without organic load containing mixed peracid at 10 and 20 ppm, *E. coli* O157:H7 levels were 1.5 log CFU/ml less than those in water. *E. coli* O157:H7 was not detected (detection limit of 1 CFU/ml) in posttreatment solutions when mixed peracid and peroxyacetic acid were at 30 ppm or chlorine was at 50 ppm. The average *E. coli* O157:H7 population detected was 0.5 log CFU/ml after chlorine treatment at 30 ppm, which was more than 1 log CFU/ml less than that for water alone (Table 1).

The presence of 10% organic material reduced the effectiveness of several antimicrobial treatments for control of *E. coli* O157:H7 transfer to the washing solutions. There were no significant differences between *E. coli* O157:H7 levels in water and in chlorine at 30 ppm, mixed peracid at 10 ppm, and peroxyacetic acid at 10 and 20 ppm. In posttreatment solutions with 10% organic load, *E. coli* O157:H7 was not detected in mixed peracid at 20 and 30 ppm. Peroxyacetic acid at 30 ppm had *E. coli* O157:H7

levels that were significantly less than those in water ($\alpha = 0.05$) by 1.7 log CFU/ml. Chlorine at 30 ppm and 50 ppm had *E. coli* O157:H7 levels that were 0.8 and 1.3 log CFU/ml, respectively, less than those in water only (Table 2).

In contrast to the results for the posttreatment solutions, the *E. coli* O157:H7 populations transferred to uninoculated leaves were significantly smaller for all antimicrobial treatments than for water only with or without added organic material. When one leaf piece inoculated with *E. coli* O157:H7 at 5.6 log CFU was mixed with five uninoculated leaf pieces in 500 ml of untreated water, the mean population on the uninoculated leaves after treatment was greater than 2.5 log CFU per leaf piece. When no added organic material was present, the mean population on uninoculated leaves in antimicrobial solutions was at least 2 log units less than that for water only, and no *E. coli* O157:H7 was detected on uninoculated leaves treated with peroxyacetic acid or mixed peracid at 30 ppm. There was no significant difference between the results for those treatments and the leaf results for mixed peracid at 10 and 20 ppm and chlorine at 30 or 50 ppm (Table 1).

The presence of 10% organic material added to the antimicrobial solutions reduced the effectiveness of limiting transfer of *E. coli* O157:H7 to uninoculated leaves; however, all antimicrobial treatments resulted in significantly lower cell numbers on uninoculated leaves compared with the numbers on leaves in untreated water. Chlorine at 30 and 50 ppm and peroxyacetic acid at 10 ppm had mean cell numbers 1 log or more lower than those for untreated water. Peroxyacetic acid at 20 and 30 ppm and mixed peracid at 10, 20, and 30 ppm had mean cell numbers >2 log less than those in untreated water (Table 2).

For *E. coli* O157:H7 on inoculated lettuce leaves after treatment without organic load, a significant reduction ($\alpha = 0.05$) of 1.9 log CFU per leaf piece was achieved by washing with water alone. A reduction of 3.2, 3.5, and >4.6 log CFU per leaf piece was achieved by peroxyacetic acid at 30 ppm, mixed peracid at 20 ppm, and mixed peracid at 30 ppm, respectively, and this reduction was significantly different from that achieved with water alone.

TABLE 2. *E. coli* O157:H7 on lettuce leaves and in processing water with and without antimicrobials and in the presence of 10% organic load^a

Antimicrobial agent	Concn (ppm)	Mean (±SD) <i>E. coli</i> O157:H7 population ^b		
		Inoculated leaves after treatment (log CFU/piece)	Posttreatment processing water (log CFU/ml)	Uninoculated leaves after treatment (log CFU/piece)
Peroxyacetic acid	10	3.99 ± 0.45 A	1.61 ± 0.09 AB	1.26 ± 0.70 BC
	20	3.25 ± 0.69 A	1.27 ± 0.63 AB	0.68 ± 0.79 CD
	30	1.66 ± 1.47 BC	0.10 ± 0.17 CD	0.07 ± 0.12 D
Mixed peracid	10	3.42 ± 0.43 A	1.24 ± 0.81 AB	0.57 ± 0.39 CD
	20	2.57 ± 0.46 AB	ND D	0.27 ± 0.31 D
	30	0.90 ± 0.85 C	ND D	0.13 ± 0.12 D
Chlorine	30	2.86 ± 0.41 AB	1.15 ± 1.00 ABC	1.68 ± 1.00 B
	50	2.88 ± 0.29 AB	0.59 ± 1.02 BCD	0.80 ± 1.39 BCD
Water		3.93 ± 0.56 A	1.96 ± 0.26 A	2.64 ± 0.15 A

^a *E. coli* O157:H7 population on inoculated untreated leaves was at 5.6 log CFU per piece.

^b Within a column, means with the same letter are not significantly different at α = 0.05. ND, not detected. Detection limit was 1 CFU/ml of processing water.

The 2.18-log reduction achieved by washing with chlorine at 30 ppm and the 3.0-log reduction with 50 ppm of chlorine was not significantly different than that achieved with water alone. The reduction of *E. coli* O157:H7 on inoculated leaves was significantly greater for the mixed peracid solution at 30 ppm than for any other treatment. When no added organic material was present, *E. coli* O157:H7 was not detected, representing a >5-log reduction from the initial level of 5.56 log CFU per leaf piece. A similar trend was observed for treatments with 10% organic load, with slightly lower efficacy of all antimicrobial agents (Table 2).

DISCUSSION

Compared with water without antimicrobial agents, peroxyacetic acid and mixed peracid at 30 ppm were more effective for reducing the numbers of *E. coli* O157:H7 cells in processing water, with or without 10% organic load, and

on inoculated lettuce leaves. However, peracid agents at 10 and 20 ppm (which are below the specified label use concentration) were much less effective than 30 ppm for reducing *E. coli* O157:H7 in processing water and on inoculated lettuce leaves (Tables 1 and 2). According to the Federal Insecticide, Fungicide and Rodenticide Act (<http://www.epa.gov/oecaagct/lfra.html>), it is a violation of Federal Law to use an Environmental Protection Agency-registered product in a manner that is inconsistent with its labeling. The results of this study demonstrate that improper use of antimicrobial agents (e.g., reduced concentration) under produce processing conditions will not achieve the intended purpose of controlling pathogenic microorganisms in processing water.

E. coli O157:H7 on inoculated leaves contaminated processing water and was transferred to uninoculated leaves in the processing water for all treatments except 30 ppm of mixed peracid and 30 ppm of peroxyacetic acid. *E. coli* O157:H7 contamination reached 2.5 and 2.6 log CFU per leaf piece on uninoculated leaf pieces when they were washed with leaf pieces inoculated at 5.6 log CFU per leaf piece in water without and with 10% organic load, respectively (Tables 1 and 2). In comparison with washing with water only, peroxyacetic acid, mixed peracid, and chlorine treatments at all concentrations resulted in significantly lower numbers of *E. coli* O157:H7 cells on uninoculated leaves (Tables 1 and 2). Proper levels of antimicrobials in processing water are necessary to prevent transfer of pathogens from contaminated leaves to uncontaminated leaves during washing.

Treatments with 30 ppm of peroxyacetic acid and mixed peracid reduced the population of *E. coli* O157:H7 on inoculated leaves by ≥1 log CFU per leaf piece more than did treatment with chlorine at 30 ppm with or without 10% organic load; however, only the 30-ppm mixed peracid treatment result was significantly different from that of chlorine (Figs. 1 and 2). In the postwash water containing 10% organic load, only peroxyacetic acid at 30 ppm, mixed peracid at 20 and 30 ppm, and chlorine at 50 ppm were

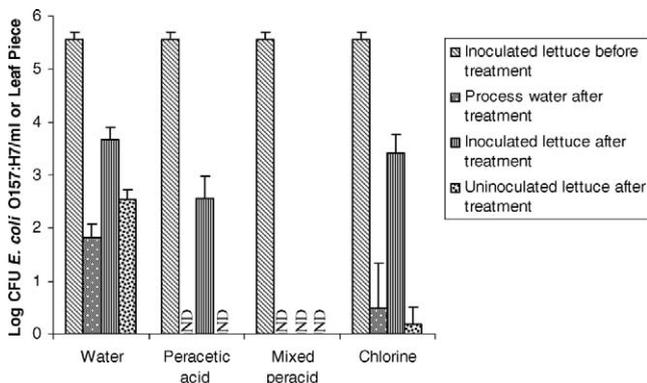


FIGURE 1. Comparison of antimicrobial agents at 30 ppm in processing water without organic load for their effect on *E. coli* O157:H7 in processing water and on inoculated and uninoculated lettuce leaves. ND, not detected. The experiment was repeated three times. One sample was evaluated for inoculated lettuce before treatment, processing water after treatment, and inoculated lettuce after treatment in each replicate. Five samples were evaluated for uninoculated lettuce after treatment in each replicate. Error bars represent the standard deviation.

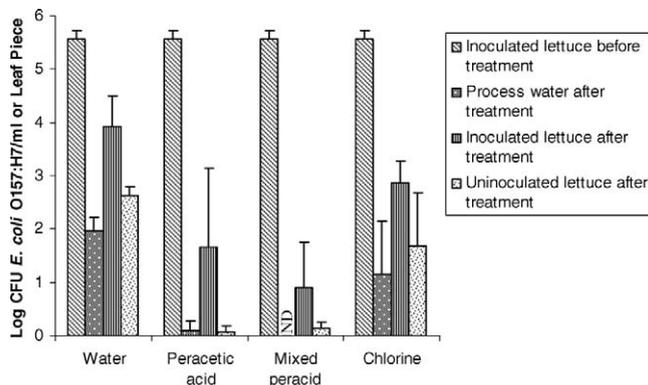


FIGURE 2. Comparison of antimicrobial agents at 30 ppm in processing water in the presence of 10% organic load for their effect on *E. coli* O157:H7 in processing water and on inoculated and uninoculated lettuce leaves. ND, not detected. The experiment was repeated three times. One sample was evaluated for inoculated lettuce before treatment, processing water after treatment, and inoculated lettuce after treatment in each replicate. Five samples were evaluated uninoculated lettuce after treatment in each replicate. Error bars represent the standard deviation.

significantly more effective than water for reducing *E. coli* O157:H7. In the presence of 10% organic load in processing water, peroxyacetic acid and mixed peracid at 30 ppm significantly reduced the contamination of uninoculated leaves by *E. coli* O157:H7 (ca. 0.1 log CFU per leaf piece), whereas chlorine at 30 ppm left 1.68 log CFU per leaf piece on uninoculated leaves (Tables 1 and 2). Results of this study revealed that mixed peracid at 30 ppm in the presence of organic load was more effective for inactivating *E. coli* O157:H7 in processing water and preventing contamination of uninoculated leaves than was chlorine at 30 ppm.

The organic load had a greater effect on the efficacy of chlorine than on that of peroxyacetic acid and mixed peracid. The 10% organic load in the processing water reduced the efficacy of chlorine at 30 ppm but had only minor effects on the mixed peracid and peroxyacetic acid treatments at 30 ppm. For example, *E. coli* O157:H7 counts in posttreatment water with 30 ppm of chlorine, peroxyacetic acid, or mixed peracid but without organic load were 0.49 log CFU/ml, not detected, and not detected, respectively, but with 10% organic load were 1.15 and 0.1 log CFU/ml and not detected, respectively (Tables 1 and 2). The organic load also negatively impacted the effectiveness of chlorine at 30 ppm but not the effectiveness of mixed peracid or peroxyacetic acid for preventing the transfer of *E. coli* O157:H7 to the uninoculated leaves. *E. coli* O157:H7 was not detected on uninoculated leaves after treatment with 30 ppm of peroxyacetic acid or mixed peracid without organic load, but the pathogen counts increased by approximately 0.1 log CFU per leaf piece in the presence of 10% organic load. In contrast, treatment with 30 ppm of chlorine resulted in an increase of *E. coli* O157:H7 on uninoculated leaves from 0.19 log CFU per leaf piece without organic load to 1.68 log CFU per leaf piece with 10% organic load (Tables 1 and 2). Thus, the reuse of processing water and subsequent buildup of organic matter both influence the effectiveness of antimicrobial treatments.

The results of this work revealed the potential impact of organic load on the effectiveness of antimicrobial treatment used to reduce the transfer of *E. coli* O157:H7 from contaminated leaves to the processing water and to uncontaminated leaves. Although this study did not replicate conditions that exist during processing, it illustrates the need to evaluate more than just the antimicrobial concentration when validating the effectiveness of produce processing controls. Factors such as organic load, fluid/produce ratio, antimicrobial type and concentration, and other variables during processing can have a profound effect on the potential for spreading contamination throughout a production lot. Additional research on the critical factors beyond antimicrobial type and concentration is needed to enhance pathogen control during produce processing.

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