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Efficacy of Commercial Produce Sanitizers against Nontoxigenic *Escherichia coli* O157:H7 during Processing of Iceberg Lettuce in a Pilot-Scale Leafy Green Processing Line

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ABSTRACT

Chemical sanitizers are routinely used during commercial flume washing of fresh-cut leafy greens to minimize cross-contamination from the water. This study assessed the efficacy of five commercial sanitizer treatments against *Escherichia coli* O157:H7 on iceberg lettuce, in wash water, and on equipment during simulated commercial production in a pilot-scale processing line. Iceberg lettuce (5.4 kg) was inoculated to contain 10^6 CFU/g of a four-strain cocktail of nontoxigenic, green fluorescent protein–labeled, ampicillin-resistant *E. coli* O157:H7 and processed after 1 h of draining at ~22°C. Lettuce was shredded using a commercial slicer, step-conveyed to a flume tank, washed for 90 s using six different treatments (water alone, 50 ppm of peroxyacetic acid, 50 ppm of mixed peracid, or 50 ppm of available chlorine either alone or acidified to pH 6.5 with citric acid [CA] or T-128), and then dried using a shaker table and centrifugal dryer. Various product (25-g) and water (50-ml) samples collected during processing along with equipment surface samples (100 cm^2) from the flume tank, shaker table, and centrifugal dryer were homogenized in neutralizing buffer and plated on tryptic soy agar. During and after iceberg lettuce processing, none of the sanitizers were significantly more effective ($P \leq 0.05$) than water alone at reducing *E. coli* O157:H7 populations on lettuce, with reductions ranging from 0.75 to 1.4 log CFU/g. Regardless of the sanitizer treatment used, the centrifugal dryer surfaces yielded *E. coli* O157:H7 populations of 3.49 to 4.98 log CFU/100 cm^2. Chlorine, chlorine plus CA, and chlorine plus T-128 were generally more effective ($P \leq 0.05$) than the other treatments, with reductions of 3.79, 5.47, and 5.37 log CFU/ml after 90 s of processing, respectively. This indicates that chlorine-based sanitizers will likely prevent wash water containing low organic loads from becoming a vehicle for cross-contamination.

In 2009, leafy greens were ranked as the riskiest food category regulated by the U.S. Food and Drug Administration, accounting for 363 outbreaks and 13,568 reported cases of illness *(13)*. Between 1995 and 2006, leafy green–associated outbreaks increased by 38.6%, whereas consumption increased by only 9% *(22)*. The nationwide outbreak of *Escherichia coli* O157:H7 that was traced to baby spinach in 2006 resulted in 205 confirmed infections, 103 hospitalizations, and three deaths *(10, 17)*. Following two additional *E. coli* O157:H7 outbreaks in 2006 linked to shredded iceberg lettuce resulting in 150 illnesses *(12),* at least nine more outbreaks responsible for nearly 300 cases of *E. coli* O157:H7 infection have been documented in the United States through 2012 *(14),* heightening continued safety concerns surrounding fresh-cut leafy greens.

Bacterial pathogens can contaminate leafy greens at any point during the farm-to-fork continuum *(31)*. Major on-farm areas of concern now recognized by the U.S. Food and Drug Administration include agricultural water, biological soil amendments (e.g., manure), domesticated and wild animals, field worker health and hygiene, and the cleanliness of harvesting equipment, tools, and buildings *(47)*. However, leafy greens are also prone to contamination during commercial processing, packing *(8),* distribution, marketing *(51),* and in-home preparation *(35)*. Regarding leafy greens, pathogens are most likely to attach to stomata, irregularities on intact surfaces, cut surfaces, or cracks on the external surfaces *(20, 36, 38, 39, 42)* and can be protected from sanitizers by biofilms *(40)*. Because sanitizers in the wash water cannot be relied upon to inactivate attached or internalized pathogens during processing, it is imperative that growers and harvesters follow good agricultural practices and good handling practices to reduce the likelihood of contamination *(19)*.

Washing of leafy greens remains important for removing soil and debris, decreasing the microbial load, improving quality and appearance, and enhancing product shelf life and safety *(21)*. Numerous small-scale laboratory studies have shown that produce sanitizers reduce pathogen populations only 1 to 3 log CFU on lettuce *(4, 18, 20, 36, 38),* with water alone decreasing *E. coli* O157:H7 levels about 1 log CFU on lettuce during pilot-scale processing *(6)*. Recirculation of this wash water during processing can further magnify the spread of contaminants at large, centralized processing facilities *(21, 28)*. Hence, the

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addition of sanitizers to processing water is imperative to minimize cross-contamination during commercial production of fresh-cut leafy greens *(2, 29, 38, 46)*.

Chlorine-based sanitizers are preferred for commercial flume washing systems because of their relatively low cost compared with other sanitizers and minimal negative impact on end-product quality *(11, 21, 29, 33, 36)*. Since the active component of chlorine, hypochlorous acid (HClO), is most abundant at pH 6.5 to 7.0 *(3)*, the pH of the wash water typically needs to be lowered by adding a weak acid, most commonly citric acid *(21)*. A new, generally recognized as safe acidifying agent composed of phosphoric acid and propylene glycol, known as T-128 (SmartWash Solutions, Salinas, CA), has been developed to improve the stability of chlorine *(25, 29, 33, 41)*. However, chlorine use has raised concerns regarding potentially hazardous by-products, worker safety, environmental damage, and most importantly, decreased efficacy in the presence of an increasing organic load in recirculating flume water, which has heightened interest in other alternatives such as peroxyacetic acid–based sanitizers *(38, 43)*.

Numerous small-scale laboratory studies have assessed sanitizer efficacy against pathogens on leafy greens *(1, 4, 23, 24, 27, 30, 34, 44, 52, 53)*. However, these findings are difficult to extrapolate to large-scale commercial production facilities. Previous work completed by our group was performed without chemical sanitizers to quantify *E. coli* O157:H7 transfer during pilot-plant production of fresh-cut leafy greens *(6, 7)*. Since chemical sanitizers remain the sole intervention strategy to prevent cross-contamination during commercial production of fresh-cut leafy greens, it is imperative that these sanitizers be reevaluated under conditions that more closely resemble commercial operations. Consequently, the objective of this study was to assess the efficacy of five commercial sanitizer treatments against *E. coli* O157:H7 during processing of iceberg lettuce in a pilot-scale leafy green processing line.

MATERIALS AND METHODS

Experimental design. The efficacy of five different sanitizing treatments was assessed in triplicate against *E. coli* O157:H7 by processing a 5.4-kg batch of iceberg lettuce inoculated at 10^6 CFU/g, with sanitizer-free water serving as the control. All lettuce was processed by shredding, conveying, fluming, shaker table dewatering, and/or centrifugal drying, during and/or after which various product, water, and equipment surface samples were collected and quantitatively examined for *E. coli* O157:H7.

Iceberg lettuce. Individually wrapped heads of iceberg lettuce (*Lactuca sativa* L.) (24 heads per case) were obtained from a local wholesaler (Stan Setas Produce Co., Lansing, MI), with the product originating from California or Arizona depending on the growing season. All lettuce was stored in a 4°C walk-in cooler and used within 5 days of delivery.

Bacterial strains. Four nontoxigenic (stx_1^- and stx_2^-) strains of *E. coli* O157:H7 (ATCC 43888, CV2b7, 6980-2, and 6982-2) were obtained from Dr. Michael Doyle at the Center for Food Safety, University of Georgia, Griffin. These strains had been previously transformed with a pGFPuv plasmid containing a green fluorescent protein gene and ampicillin-resistance gene. All four strains were stored at −80°C in tryptic soy broth (Difco, BD, Sparks, MD) containing 0.6% (wt/vol) yeast extract (Difco, BD) (TSBYE) and 10% (vol/vol) glycerol (Sigma Chemical Co., St. Louis, MO) until needed. Working cultures were prepared by streaking each stock culture on tryptic soy agar plates (Difco, BD) containing 0.6% (wt/vol) yeast extract and 100 ppm of ampicillin (ampicillin sodium salt, Sigma Life Science, St. Louis, MO) (TSAYE plus amp). After 18 to 24 h of incubation at 37°C, a single colony was transferred to 9 ml of TSBYE containing 100 ppm of ampicillin (TSBYE plus amp) and similarly incubated.

Lettuce inoculation. A 0.2-ml aliquot of each nontoxigenic *E. coli* O157:H7 strain was transferred to 200 ml of TSBYE with amp and incubated for 18 to 20 h at 37°C. Based on similar growth rates as determined previously *(6)*, the four strains were combined in equal volumes to obtain an 800-ml cocktail, which was added to 80 liters of municipal tap water (~15°C, <0.05 ppm of free chlorine) in a 121-liter plastic container (Rubbermaid, Wooster, OH) to achieve a level of $\sim 10^7$ CFU/ml. Hand-cored heads of iceberg lettuce (~12 heads) were immersed in the *E. coli* suspension for 15 min and then drained or air dried for 1 h at 22°C before being spun in a dewatering centrifuge (described below) to remove residual inoculum from the interior of the heads. Duplicate 25-g samples were then aseptically collected to determine the initial inoculation level at the time of processing.

Lettuce processing line. The same small-scale commercial leafy green processing line consisting of a lettuce shredder, step conveyer, flume tank, shaker table, and dewatering centrifuge was used as previously described in detail by Buchholz et al. *(6)*. For this work, a custom-made stainless steel gate with 1.25-cm-diameter holes spaced 0.65 cm apart (Heinzen Manufacturing, Inc., Gilroy, CA) was added at the end of the 3.3-m-long stainless steel flume tank to retain the product during 90 s of washing.

Wash water. Iceberg lettuce (0.5 kg) was homogenized in 500 ml of Michigan State University tap water using a mechanical blender (model BLC10650MB, Black & Decker, New Britain, CT) and then added to 890 liters of processing water at 12 to 15°C to achieve a low organic load. The following five commercial produce sanitizer treatments were assessed: 30 ppm of peroxyacetic acid (Tsunami 100, Ecolab, St. Paul, MN), 30 ppm of mixed peracid (Tsunami 200, Ecolab), 30 ppm of available chlorine (XY-12, Ecolab) at pH 7.85, 30 ppm of available chlorine (XY-12) acidified to pH 6.50 with citric acid (Sigma-Aldrich, St. Louis, MO), and 30 ppm of available chlorine (XY-12) acidified to pH 6.50 with T-128 (SmartWash Solutions) as measured with a pH probe (pHTestr 30, Oakton, Vernon Hills, IL). Peroxyacetic acid test kit 311 (Ecolab) was used to confirm the peroxyacetic acid and mixed peracid sanitizer concentrations, and chlorine test kit 321 (Ecolab) was used to measure available chlorine. Sanitizer-free Michigan State University tap water (<0.05 ppm of free chlorine) served as the control.

Lettuce processing. Inoculated heads of cored iceberg lettuce (5.4 kg) were hand-fed into the shredder at a rate of about 0.5 kg per s, with the shredded product then step-conveyed at a rate of 2.85 m/s to the top of the conveyor. Processing was then halted for ~10 min to aseptically collect and bag five 25-g lettuce samples in red mesh produce bags (5 lb Header Bag, Pacon Inc., Baldwin Park, CA) for subsequent sampling. Thereafter, processing was resumed with the iceberg lettuce conveyed to the flume tank, washed in 890 liters of recirculating wash water with or without a

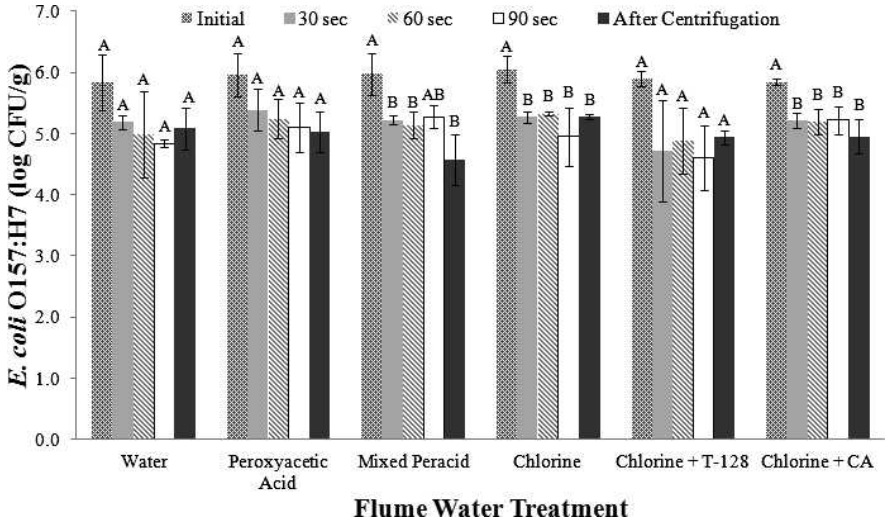

FIGURE 1. *Mean (±SD)* E. coli *O157:H7 populations on the iceberg lettuce inoculated at ~6 log CFU/g during and after processing (*n = *3). Means of the same wash water treatment with different letters are significantly different (*P ≤ *0.05).*

sanitizer for 90 s, partially dewatered on the shaker table, collected in a single centrifugation basket, and centrifugally dried.

Sample collection. During the 90 s of flume washing, three prebagged iceberg lettuce samples (25 g each) were retrieved at the flume gate at 30-s intervals and were immediately added to 100 ml of sterile Difco neutralizing buffer (BD, Franklin Lakes, NJ) in a Whirl-Pak filter bag (Nasco, Fort Atkinson, WI). In addition, nine 50-ml water samples were collected at 10-s intervals in 50-ml centrifuge tubes containing 38× concentrated Difco neutralizing buffer (BD). After shaker table dewatering, product in the basket was dried in the preset 50-lb (110-kg) capacity Spin Dryer (model SD50-LT, Heinzen Manufacturing). During centrifugal drying, four water samples (50 ml each) were similarly collected from the centrifuge drain at 10-s intervals for the first 40 s of the 80-s cycle. After centrifugation, two bagged lettuce samples (25 g each) were also retrieved from the centrifugation basket. Nine product contact areas on the equipment (three flume tank, three shaker table, and three dewatering centrifuge), previously described in detail by Buchholz et al. *(6),* measuring 100 cm^2 as previously identified using Glo Germ (Glo Germ Co., Moab, UT) were sampled immediately after processing as described by Vorst et al. *(48)* using one-ply composite tissues moistened with 1 ml of sterile Difco neutralizing buffer (BD).

Microbiological analyses. All lettuce samples (25 g) were homogenized in a stomacher (Stomacher 400 Circulator, Seward, Worthington, UK) for 1 min at 260 rpm and then either appropriately diluted in sterile 1% (wt/vol) phosphate buffer (8.5 g/liter NaCl, 1.44 g/liter Na_2HPO_4, and 0.24 g/liter KH_2PO_4; J.T. Baker, Mallinckrodt Baker Inc., Phillipsburg, NJ) and plated on TSAYE with amp (calculated minimum detection limit of 40 CFU/g) or processed using 0.45-μm-pore-size membrane filters (Millipore, Millipore Corporation, Billerica, MA) (calculated minimum detection limit of 0.04 CFU/g), which were placed on 60-mm-diameter petri plates containing TSAYE with amp to quantify *E. coli* O157:H7. The one-ply composite tissue samples were added to 15 ml of sterile Difco neutralizing buffer in a Whirl-Pak bag, homogenized for 1 min at 260 rpm, and then plated identically to the lettuce samples, giving a calculated lower detection limit of 1 CFU/100 cm^2. The 50-ml water samples were either appropriately diluted in sterile 1% phosphate buffer and plated on TSAYE with amp or processed by membrane filtration, which gave a calculated minimum detection limit of 0.02 CFU/ml. Following 20 to 24 h of incubation at 37°C, all green fluorescing colonies as seen under UV light (365 nm; Blak-Ray, Ultra-violet Product Inc., San Gabriel, CA) were counted as *E. coli* O157:H7.

Sanitizer neutralization confirmation. Triplicate 1-liter water samples containing 30 ppm of available chlorine (XY-12), 30 ppm of peroxyacetic acid (Tsunami 100), or 30 ppm of mixed peracid (Tsunami 200 ppm) were prepared and confirmed with chlorine test kit 321 or peroxyacetic acid test kit 311. Citric acid (Sigma-Aldrich) and T-128 were used to acidify the chlorine-based sanitizer solution to pH 6.5. A 50-ml centrifuge tube containing 3 ml of 38× concentrated neutralizing buffer (BD) was filled with the sample containing sanitizer, agitated for 5 s, and then immediately assessed for neutralization of the sanitizer as previously described using the appropriate test kit. Preliminary experiments found that a 38× concentration would neutralize various concentrations of the active component of each sanitizing agent used in this study without impacting *E. coli* O157:H7 counts.

Statistical analysis. *E. coli* O157:H7 counts were converted to log CFU per gram, milliliter, or 100 cm^2 and were subjected to analysis of variance using JMP 9.0 (SAS Institute Inc., Cary, NC). Values equaling half the limit of detection were used for samples without *E. coli* O157:H7 counts. The three equipment surface samples from each respective piece of equipment were averaged. A *P* value of ≤0.05 was considered significant for all tests. The Tukey-Kramer honestly significant difference test was used to identify significant differences in *E. coli* O157:H7 populations for individual lettuce, water, and equipment surface samples.

RESULTS

Lettuce. Iceberg lettuce contained an average *E. coli* O157:H7 inoculum of 5.93 log CFU/g at the time of processing (Fig. 1). After shredding, conveying, 90 s of washing, shaker table dewatering, and centrifugal drying, no significant difference ($P > 0.05$) was seen in populations of *E. coli* O157:H7 recovered from the finished product, regardless of sanitizer treatment. Using mixed peracid, *E. coli* O157:H7 populations decreased 1.40 log CFU/g; however, this decrease was not significantly different ($P > 0.05$) compared with the 0.75-log CFU/g reduction seen for water alone. Processing significantly reduced ($P \leq 0.05$) *E. coli* O157:H7 populations on lettuce when mixed peracid, chlorine,

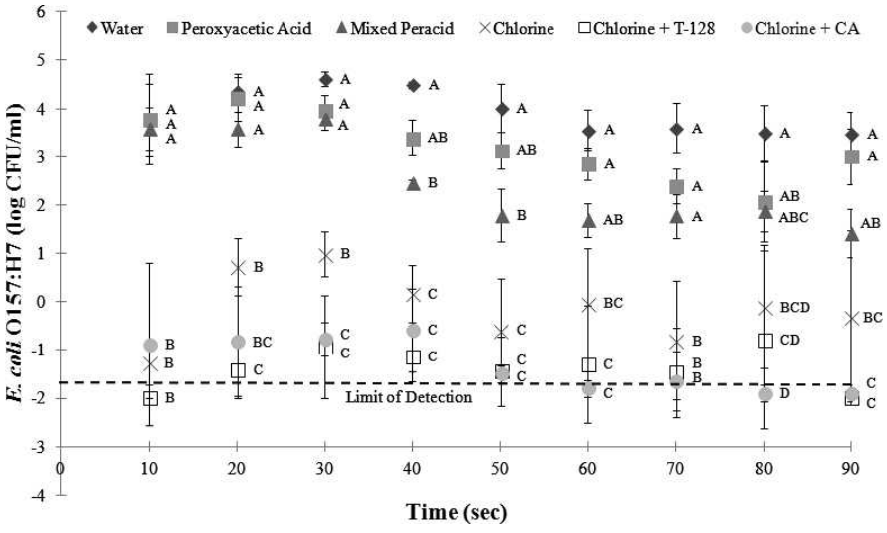

FIGURE 2. *Mean (±SD)* E. coli *O157:H7 populations in flume water during processing iceberg lettuce inoculated at ~6 log CFU/g (n = 3). Half the limit of detection was used to calculate the mean log value when a sample did not yield any colonies by direct plating. Means of the same product type with different letters are significantly different (P ≤ 0.05).*

or chlorine plus CA were used, with reductions of 1.40, 0.77, and 0.89 log CFU/g, respectively. The reductions of 0.75, 0.93, and 0.97 log CFU/g seen for water alone, peroxyacetic acid, and chlorine plus T-128, respectively, were not significant ($P > 0.05$) (Fig. 1).

Flume water. Wash water containing chlorine, chlorine plus T-128, and chlorine plus CA had significantly lower ($P \leq 0.05$) *E. coli* O157:H7 populations at all sampling times (maximum of 0.99 log CFU/ml) compared with 4.61 log CFU/ml in water alone. Using chlorine plus CA and chlorine plus T-128, *E. coli* O157:H7 levels were below the limit of detection of 0.02 log CFU/ml by the end of processing. *E. coli* O157:H7 populations were similar ($P > 0.05$) using water alone and peroxyacetic acid, with respective populations of 3.47 and 3.01 log CFU/ml recovered after 90 s of processing. Similar *E. coli* O157:H7 populations were obtained using mixed peracid ($P > 0.05$) and peroxyacetic acid, with these populations rarely lower ($P \leq 0.05$) than those in water alone (Fig. 2).

Centrifugation water. Using peroxyacetic acid, mixed peracid, or chlorine, wash water exiting the centrifuge drain after spin drying yielded maximum *E. coli* O157:H7 populations of 4.51, 4.36, and 5.48 log CFU/ml, respectively, which were not significantly different ($P > 0.05$) from those in water alone (maximum population of 5.58 log CFU/ml) during the 40-s sampling period. However, chlorine plus CA and chlorine plus T-128 resulted in *E. coli* O157:H7 populations that were lower than those in water alone ($P \leq 0.05$) during the first 20 s of centrifugation. Water samples collected after 40 s of centrifugation yielded *E. coli* O157:H7 populations that were not significantly different for any of the treatments (Fig. 3).

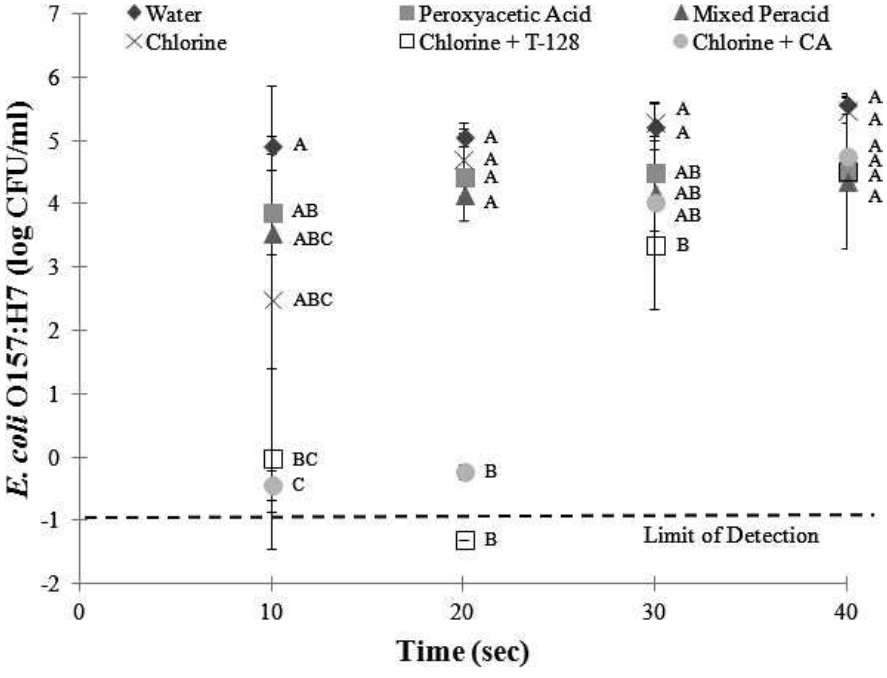

FIGURE 3. *Mean (±SD)* E. coli *O157:H7 populations in spent centrifugation water from iceberg lettuce inoculated at ~6 log CFU/g (n = 3). Half the limit of detection was used to calculate the mean log value when a sample did not yield any colonies by direct plating. Means of the same product type with different letters are significantly different (P ≤ 0.05).*

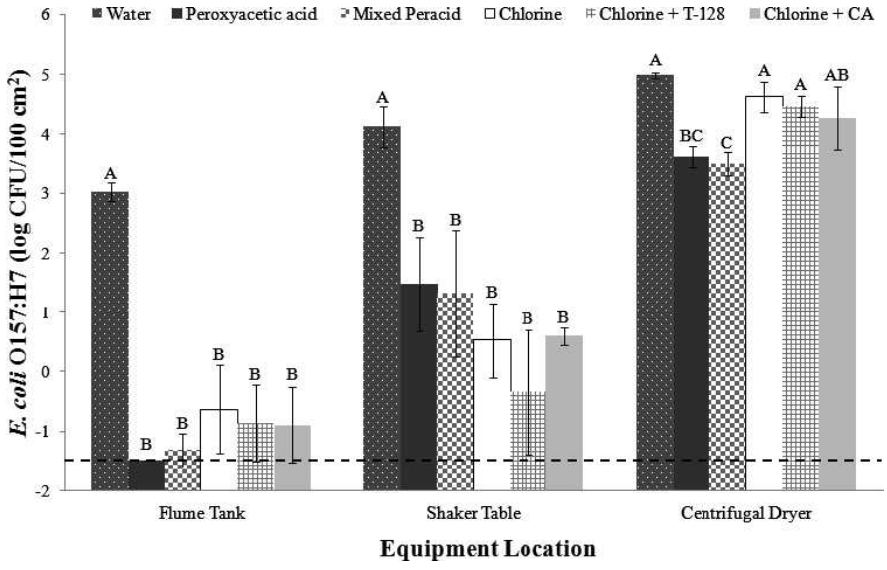

FIGURE 4. *Mean (±SD)* E. coli *O157:H7 populations on equipment surfaces after processing iceberg lettuce inoculated at ~6 log CFU/g* (n = *3). Half the limit of detection was used to calculate the mean log value when a sample did not yield any colonies by direct plating. Means of the same product type with different letters are significantly different (*$P \leq 0.05$*).*

Processing equipment surfaces. After processing iceberg lettuce, all five sanitizer treatments yielded significantly lower ($P \leq 0.05$) *E. coli* O157:H7 populations remaining on the flume tank and shaker table as compared with the water control. Significantly lower ($P \leq 0.05$) *E. coli* O157:H7 populations were recovered on the centrifugal dryer using peroxyacetic acid (3.61 log CFU/100 cm^2) and mixed peracid (3.49 log CFU/100 cm^2) compared with the other treatments, with the highest level (4.98 log CFU/100 cm^2) seen when water alone was used for washing (Fig. 4).

DISCUSSION

Due to the potential production of infectious aerosols during lettuce processing, the same four nontoxigenic strains of *E. coli* O157:H7 were used as in our earlier transfer studies *(6, 7)*. The growth and adherence rates for these four nontoxigenic strains were previously shown to be similar to three strains from the 2006 leafy green outbreaks *(6)*. As previously reported, green fluorescent protein labeling also allowed for easy differentiation of the inoculum from background bacteria *(6, 7, 49)*.

Dip inoculation of the lettuce to contain 6 log CFU/g was crucial to ensure uniform distribution of *E. coli* O157:H7 throughout the heads as well as quantifiable results for subsequent mathematical modeling with this work to be reported elsewhere. Although this inoculation level clearly exceeds levels thought to occur on field-grown lettuce, feces from ''super-shedding'' cows can potentially contain *E. coli* O157:H7 at levels of 6 log CFU/g *(15)*, with such fecal material potentially able to come in contact with lettuce through irrigation water. Preliminary experiments using a mixture of Glo Germ and water showed uniform fluorescence in dipped heads of iceberg lettuce. Additionally, Buchholz and others *(6)* found that *E. coli* O157:H7 populations were statistically similar in iceberg lettuce heads before and after shredding, indicating that the inoculation was homogenous. Dip inoculation of the cored lettuce heads may have allowed internalization of *E. coli* O157:H7 through the damaged tissues, with such cells protected from sanitizers *(37)*. Since all lettuce samples were processed by stomaching, any internalized cells would have gone undetected with only the cells on the surface of the leaves recovered.

Commercial producers of fresh-cut leafy greens use different sanitizers, sanitizer concentrations, and contact times, depending on the design of the processing line. In this study, six different wash treatments were assessed during 90 s of flume washing. Processing inoculated iceberg lettuce resulted in *E. coli* O157:H7 reductions of 0.75 to 1.4 log CFU/g on the finished product. Both during and after processing, no significant differences in sanitizer efficacy ($P > 0.05$) were seen against *E. coli* O157:H7 on iceberg lettuce for any of the treatments, including water alone. However, three wash treatments—mixed peracid, chlorine, and chlorine plus CA—significantly reduced ($P \leq 0.05$) *E. coli* O157:H7 populations after washing. Numerous small-scale laboratory studies have shown similar pathogen reductions (~1 log CFU/g) during washing of various fruits and vegetables with or without sanitizers *(4, 5, 9, 50)*. Using a pilot-scale leafy green processing line, Luo et al. *(29)* also reported an *E. coli* O157:H7 reduction of <1 log after processing inoculated baby spinach *(29)*. Consequently, produce sanitizers cannot be relied upon to ensure end product safety. Chemical sanitizers are routinely added to recirculating wash water to minimize the spread of microbial contaminants during flume washing *(27)*. Regarding their use, peroxyacetic acid–based sanitizers are limited to a maximum of 80 ppm of peroxyacetic acid *(16, 21)*, whereas free chlorine concentrations typically range from 10 to a maximum of 200 ppm *(20, 36, 45)*. However, soil, debris, and vegetable latexes released during shredding of leafy greens will accumulate in the flume water over time *(32)*, decreasing the efficacy of many sanitizers, most notably chlorine *(2, 26, 38, 52)*. The wash water used in this study contained an organic load of ~0.0006% blended iceberg lettuce (wt/vol) to simulate wash water quality during the early stages of processing. Hence, higher *E. coli* O157:H7 populations would have been expected after 90 s of processing if the organic load in the wash water had been

higher, especially for the chlorine-based sanitizer. *E. coli* O157:H7 populations recovered from the wash water were consistently lower ($P \leq 0.05$) using chlorine, chlorine plus CA, and chlorine plus T-128 compared with water alone, peroxyacetic acid, and mixed peracid. Both chlorine plus CA and chlorine plus T-128 treatments yielded *E. coli* O157:H7 levels that were below the limit of detection, which is similar to the findings of López-Gálvez et al. *(27)* using 40 ppm of chlorine.

This study was designed to assess the efficacy of sanitizers during processing, not to assess long-term pathogen persistence in the wash water. Produce sanitizers are primarily used to minimize cross-contamination during flume washing, with their effectiveness dependent on the type of sanitizer, concentration, temperature, and organic load in the wash water. The pilot-scale processing line used in this study was not equipped with a chiller. Therefore, all processing needed to be conducted at our incoming tap water temperature of 12 to 15°C rather than at the targeted commercial temperature of 4°C. Since sanitizer efficacy against *E. coli* O157:H7 is enhanced at temperatures above 4°C *(53),* our *E. coli* O157:H7 reductions likely exceed those that would be expected in commercial operations.

Levels of *E. coli* O157:H7 recovered from spent centrifugation water containing sanitizers were rarely lower than those seen in sanitizer-free water. Similar *E. coli* O157:H7 populations were recovered from centrifugation water containing peroxyacetic acid, mixed peracid, chlorine, or no sanitizer at all four sampling times. The combination of chlorine and citric acid or T-128 was significantly more effective than the other sanitizers ($P \leq 0.05$) against *E. coli* O157:H7 in centrifugation water collected during the first 20 s; however, after 40 s no significant difference was seen compared with the water control ($P > 0.05$). These results indicate that, whereas populations of *E. coli* O157:H7 may be close to or below the limit of detection in flume water, populations in the centrifugation water were not significantly different than the water control by the end of sample collection. Therefore, spent centrifugation water would be best suited for pathogen testing.

E. coli O157:H7 cells recovered from equipment surfaces after processing reflect those that were present in the film of water on the equipment surface. During processing, the flume tank was in continuous contact with the recirculating wash water, with water contact decreasing during shaker table dewatering and centrifugal drying. Numbers of *E. coli* O157:H7 recovered from surfaces in the centrifugal dryer were not significantly different from the water control when any of the three chlorine-based sanitizer treatments were used, indicating that those surfaces may also be well suited for pathogen testing, depending on the particular sanitizer used.

This study was done to assess the efficacy of commercial produce sanitizers against *E. coli* O157:H7 on lettuce, in wash water, and on equipment surfaces during small-scale processing of iceberg lettuce. Whereas none of the sanitizers were more effective than water alone against *E. coli* O157:H7 on iceberg lettuce at any point during or after processing, it is important to reiterate that sanitizers are designed to reduce the microbial load in wash water rather than on the product. Overall, the populations of *E. coli* O157:H7 recovered in wash water containing peroxyacetic acid or mixed peracid were rarely significantly different than those seen in water alone. However, the three chlorine-based treatments were significantly more effective than water alone at reducing *E. coli* O157:H7 populations in wash water during processing. The wash water used in this study replicated a ''best-case'' scenario for processors due to the extremely low organic load and freshly added sanitizers. Similar studies using higher organic loads will be needed to assess sanitizer efficacy against *E. coli* O157:H7 under conditions that more closely simulate commercial processing.

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