

Efficacy of Sanitizers To Inactivate *Escherichia coli* O157:H7 on Fresh-Cut Carrot Shreds under Simulated Process Water Conditions†

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ABSTRACT

Chlorine is widely used as a sanitizer to maintain the microbial quality and safety of fresh-cut produce; however, chlorine treatment lacks efficacy on pathogen reduction, especially when the fresh-cut processing water contains heavy organic loads. A more efficacious sanitizer that can tolerate the commercial processing conditions is needed to maintain microbial safety of fresh-cut produce. This study evaluated the efficacy of *Escherichia coli* O157:H7 reduction on fresh-cut carrots using new and traditional sanitizers with tap water and fresh-cut processing water scenarios. Fresh-cut carrot shreds inoculated with *E. coli* O157:H7 were washed in sanitizer solutions including 200 ppm chlorine, citric acid–based sanitizer (Pro-San), 80 ppm peroxyacetic acid–based sanitizer (Tsunami 100), and 1,000 ppm acidified sodium chlorite (SANOVA) prepared in fresh tap water or simulated processing water with a chemical oxygen demand level of approximately 3,500 mg/liter. Samples were packaged and stored at 5°C. Microbial analyses performed at days 0, 7, and 14 indicate that the organic load in the process water significantly affected the efficacy of chlorine on pathogen removal and was especially evident on samples tested during storage. Acidified sodium chlorite provided a strong pathogen reduction even under process water conditions with up to a 5.25-log reduction when compared with the no-wash control. *E. coli* O157:H7 was not recovered on acidified sodium chlorite–treated samples during the entire 14 days of storage, even following an enrichment step. These results suggest that acidified sodium chlorite holds considerable promise as an alternative sanitizer of fresh-cut produce.

Fresh-cut produce is one of the hottest growing convenience foods in history because it offers freshness, nutrition, and convenience (14). However, contamination of fresh produce with human pathogens can occur anywhere in their journey from farm to table (6). Given that fresh-cut products are marketed as prewashed and ready to eat, and not subject to further microbial killing steps, the need for effective disinfecting agents during produce washing is clearly evident to ensure product safety.

Chlorine has been used widely in the fresh produce industry as a sanitizer in wash, spray, and flume waters at concentrations of 50 to 200 ppm (as sodium hypochlorite, NaOCl) and a contact time of up to 2 min (11, 26). However, the efficacy of chlorine on pathogens is limited (7, 11, 27). Chlorine also loses its activity rapidly on contact with organic matter, increased temperature, exposure to light, and contact with metals (12).

The U.S. Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) have recently approved the use of acidified sodium chlorite for spray or dip sanitizing certain food products, including fruits and vegetables, at levels of 500 to 1,200 ppm (3). Acidified sodium

chlorite is a combination of citric acid and sodium chlorite in aqueous solution, with a mostly oxidative mode of action. In a recent study, Lukasik et al. (17) found that acidified sodium chlorite produced the greatest reduction in the numbers of *Escherichia coli* O157:H7 and *Salmonella* Montevideo from inoculated strawberries compared with chlorine, peroxyacetic acid, and stabilized chlorine dioxide.

Peroxyacetic (peracetic) acid is a strong oxidizing agent that has been used extensively to disinfect food processing equipment and has been approved by the FDA as a disinfectant for fruits and vegetables (2). Pilot-scale trials undertaken to determine the suitability of peracetic acid for washing minimally processed vegetables showed it to be effective against *Listeria monocytogenes*, *Salmonella* spp., and *E. coli* O157:H7, achieving reductions of 97.2, 92.4, and 87.8%, respectively, at 10 ppm and as little as 30 s of immersion in a batch washing scheme with chilled water (22).

A citric acid–based sanitizer also has been approved recently by both the EPA and FDA as a no-rinse sanitizer. No published data is available on its performance.

Most sanitizer studies are conducted with pure lab water. Given that fresh-cut process water contains a large organic load as a result of water reutilization, comparisons of sanitizers in water containing organic matter is needed in order to select the most efficacious sanitizers that can tolerate commercial processing conditions and maintain microbial safety of fresh-cut produce. The main objective of

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this study was to evaluate the efficacy of chlorine, citric acid, peracetic acid, and acidified sodium chlorite in reducing populations of *E. coli* O157:H7, total aerobic bacteria, and yeasts and molds from shredded carrots under tap water and simulated fresh-cut wash water conditions. The role of these sanitizers in maintaining the microbial safety of wash water was also evaluated.

MATERIALS AND METHODS

Bacterial strains and growth conditions. A nalidixic acid-resistant (Nal^r) derivative of an outbreak *E. coli* O157:H7 strain (F6460) was used throughout this study. F6460 was isolated from patient fecal samples during a 1999 Nebraska lettuce outbreak. The nalidixic acid-resistant derivative strain, F6460 Nal^r, was isolated as described previously (25) and stored at -80°C in Luria-Bertani broth (Difco, Becton Dickinson, Sparks, Md.) containing 25% (vol/vol) glycerol. *E. coli* O157:H7 was grown overnight in Luria-Bertani-Nal broth at 37°C with constant agitation at 175 rpm. Cultures were mixed, washed by centrifugation ($4,000 \times g$, 15 min, 4°C) with 0.1% peptone saline water, and added proportionally to tap water to obtain a dip inoculum solution with a population of *E. coli* O157:H7 Nal^r high enough to result in about 10^5 to 10^6 CFU/g on carrot shreds after inoculation, as determined from unpublished preliminary studies.

Sample preparation. Fresh carrots (*Daucus carota*, L.) were purchased from a produce wholesale market (Jessup, Md.) and used within 24 h following storage at 5°C . Carrots were shredded with a food processor (Cuisinart, East Windsor, N.J.) and divided into individual 120-g portions contained in nylon mesh bags (Liners N' Things, Clifton, N.J.).

Procedure for inoculation. Dip inoculation, a commonly used method in sanitizer challenge studies, was chosen to simulate the immersion process of commercial produce washing operations (9). Samples were immersed in the *E. coli* O157:H7 Nal^r inoculum solution (sample-inoculum = 1:10, wt/vol) and kept under constant agitation for 30 min, followed by drainage for 1 min and spin-drying in a manually actuated salad spinner (OXO Good Grips, Elmira, N.Y.). The salad spinner was operated by pushing the actuator 15 times at about one push per second. Samples were removed from the spinner after it came to a full stop.

Treatment procedure. New sanitizers, including acidified sodium chlorite (1,000 ppm SANOVA, Alcide Corp., Redmond, Wash.), citric acid-based sanitizer (1% Pro-San, Microcide Corp., Detroit, Mich.), and peroxyacetic acid (80 ppm Tsunami 100, Ecolab, St. Paul, Minn.), were obtained directly from the manufacturers; the concentrations of the solutions tested were based on each manufacturer's recommendation. Sodium hypochlorite (200 ppm free chlorine, pH 6.5) was also evaluated, along with water and a no-wash treatment, both used as controls. The chemical oxygen demand (COD) was chosen as an estimate of the organic load of the process water, and a level close to that of commercial fresh-cut reused process water (3,500 mg/liter, as determined by our previous survey) was obtained by repeatedly dipping a known mass of shredded carrots in a fixed volume of tap water. COD levels were determined by the reactor digestion method (Chemical Oxygen Demand, Method 8000, HACH, Loveland, Colo. (15, 16)) approved by the EPA (EPA Method 410.4 (23)). All sanitizers were then tested under regular tap water and simulated process water conditions.

The inoculated shredded carrots were dipped into each sanitizer solution at a sample/wash water ratio of 1:20 (wt/vol), and

kept submerged for 2 min, followed by drainage for 30 s and spin-drying in a sanitized salad spinner. The salad spinner was operated by pushing the actuator 30 times for every sample at about one push per second. Wash water samples were taken after each treatment for subsequent microbial enumeration.

Spin-dried samples were aseptically divided into four 25-g portions. Two portions were packaged in polypropylene bags with an oxygen transmission rate of $29.3 \text{ pmol/s/m}^2/\text{Pa}$ for storage at 5°C over a 14-day period. An enrichment step was carried out at day 0 by adding 225 ml of sterile tryptic soy broth (TSB; Difco) supplemented with nalidixic acid (50 mg/liter, Sigma-Aldrich, St. Louis, Mo.) to each stomacher bag, followed by incubation at 37°C for 24 h and subsequent homogenization in the same medium. Nonenriched samples were homogenized in sterile phosphate-buffered saline (pH 7.4) in a stomacher 400 Biomaster (Seward Limited, London, UK), on days 0, 7, and 14.

Microbial enumeration. Populations of *E. coli* O157:H7, total aerobic bacteria, and yeasts and molds were enumerated by serially diluting samples in sterile phosphate-buffered saline and spiral plating (Wasp II Spiral Plater, DW Scientific, West Yorkshire, UK) onto sorbitol MacConkey agar (Difco) supplemented with nalidixic acid (50 mg/liter), tryptic soy agar (Difco), and potato dextrose agar (Difco) supplemented with chloramphenicol (Difco, 300 mg/liter), respectively. Sorbitol MacConkey-Nal plates were incubated at 37°C for 24 h, tryptic soy agar plates at 28°C for 36 h, and potato dextrose agar-chloramphenicol plates at 25°C for 5 days. Colonies were enumerated with an automated plate counter (ProtoCOL, Synoptics, Cambridge, UK).

Data were analyzed as a three-factor linear model with the Proc Mixed procedure of SAS (SAS Institute, Cary, N.C.), with water condition, sanitizer, and storage time as the main factors. Values represent the means of duplicate determinations for each sample from three replicate experiment trials.

RESULTS AND DISCUSSION

When comparing the relative efficacy of sanitizers in reducing microbial population, a water wash under either process water or tap water conditions was used as the reference in order to account for survivors that might have been removed simply by mechanical action.

Effects on *E. coli* O157:H7 population. Initial *E. coli* O157:H7 population of the no-wash control was 5.25 log CFU/g (Fig. 1). A 0.79-log reduction in the numbers of *E. coli* O157:H7 recovered from artificially inoculated shredded carrots was achieved by washing with regular tap water only. Citric acid-based sanitizer showed a similar effect under both water conditions, whereas washing with chlorine resulted in an additional 0.84-log reduction only when tested in regular tap water. A rapid inactivation of chlorine is clearly evident, with no reduction in the *E. coli* O157:H7 population seen when process water was used. The efficacy of peracetic acid was not affected by the COD level and was as effective as chlorine under regular tap water. Acidified sodium chlorite eliminated all detectable *E. coli* O157:H7 (with a 100 CFU/g limit of detection) under both tap water and process water scenarios. In addition, an enrichment step was performed to determine whether any of the sanitizers were able to completely eliminate the *E. coli* O157:H7 Nal^r population. As shown in Figure 2, no viable cells of *E. coli* O157:H7 were recovered from the samples

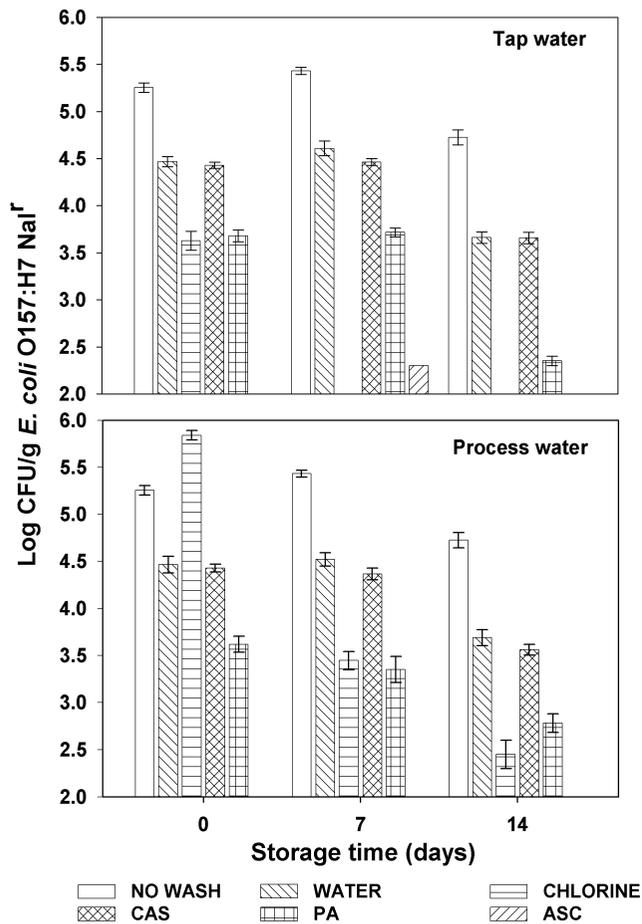


FIGURE 1. The efficacy of sanitizers on the reduction of *E. coli* O157:H7 populations from artificially inoculated shredded carrots. Bars represent the standard errors of the mean from triplicate experiments. The limit of detection was 2.0 log CFU/g of shredded carrots. CAS, 0.66% citric acid-based sanitizer; PA, 80 ppm peroxyacetic acid; ASC, 1,000 ppm acidified sodium chlorite.

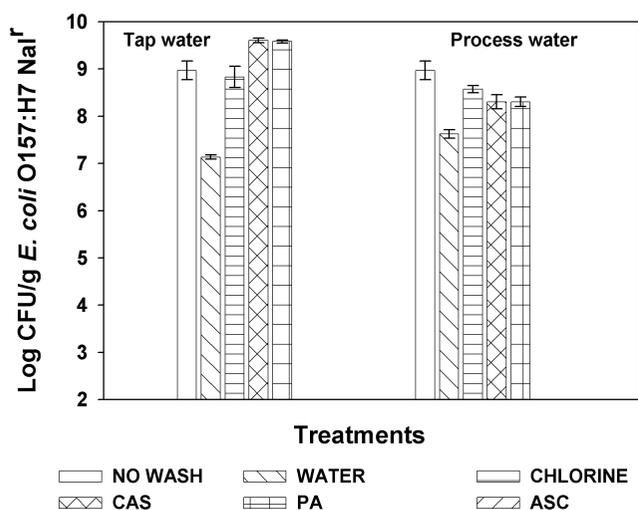


FIGURE 2. Recovery of *E. coli* O157:H7 from artificially inoculated shredded carrots after treatment with sanitizers and 24 h of enrichment. Bars represent the standard errors of the mean from triplicate experiments. The limit of detection was 2.0 log CFU/g of shredded carrots. CAS, 0.66% citric acid-based sanitizer; PA, 80 ppm peroxyacetic acid; ASC, 1,000 ppm acidified sodium chlorite.

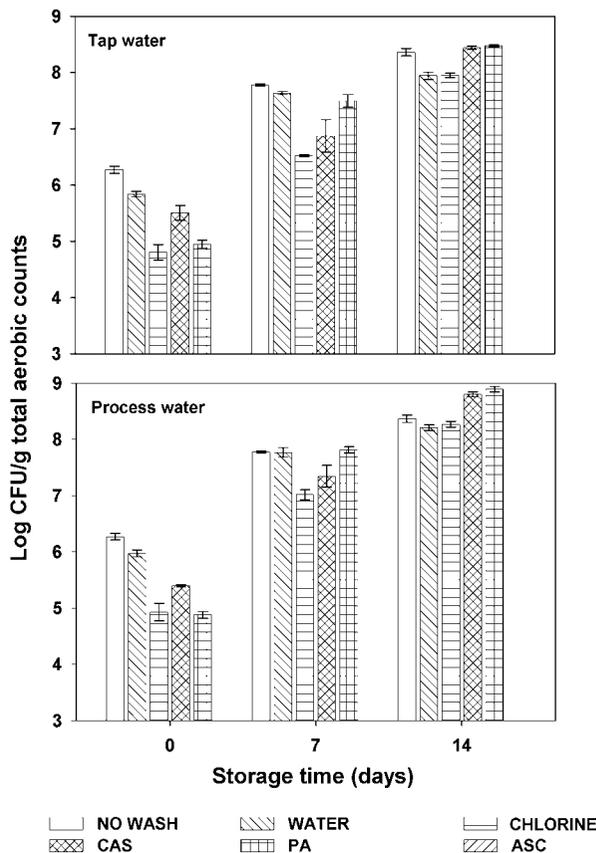


FIGURE 3. The efficacy of sanitizers on the reduction of total aerobic microbial counts from shredded carrots. Bars represent the standard errors of the mean from triplicate experiments. The limit of detection was 3.0 log CFU/g of shredded carrots. CAS, 0.66% citric acid-based sanitizer; PA, 80 ppm peroxyacetic acid; ASC, 1,000 ppm acidified sodium chlorite.

treated with acidified sodium chlorite, whereas all other treatments yielded between 7.13 and 9.60 log CFU/g *E. coli* O157:H7 after enrichment. This result suggests that the acidified sodium chlorite treatment might completely or nearly completely kill the pathogenic population (the equivalent of a 4.46-log reduction compared with the water wash or a 5.25-log reduction compared with the no-wash control). The low pH environment created by organic acids (i.e., citric acid) and the highly oxidative intermediates of acidified sodium chlorite are broad-spectrum germicidal agents (24). *E. coli* O157:H7 cells are tolerant of acidic conditions (5, 10); therefore, the bactericidal action of sanitizers cannot depend on lowering pH only. However, the pH factor cannot be disregarded because bacteria are inactivated more quickly at lower pH conditions (13). The effect of high organic content (COD) in wash water, the type of sanitizer, and a combination of COD level and sanitizer were all highly significant on the reduction of *E. coli* O157:H7 at day 0 (all $P < 0.0001$).

Effect on total aerobic bacterial counts. Washing with water was effective in reducing the total microbial counts found in shredded carrots by only 0.3 to 0.4 log, whereas chlorine and peracetic acid caused an additional 1-log reduction, regardless of the COD level (Fig. 3). Citric acid was effective in the 0.76- to 0.88-log reduction range,

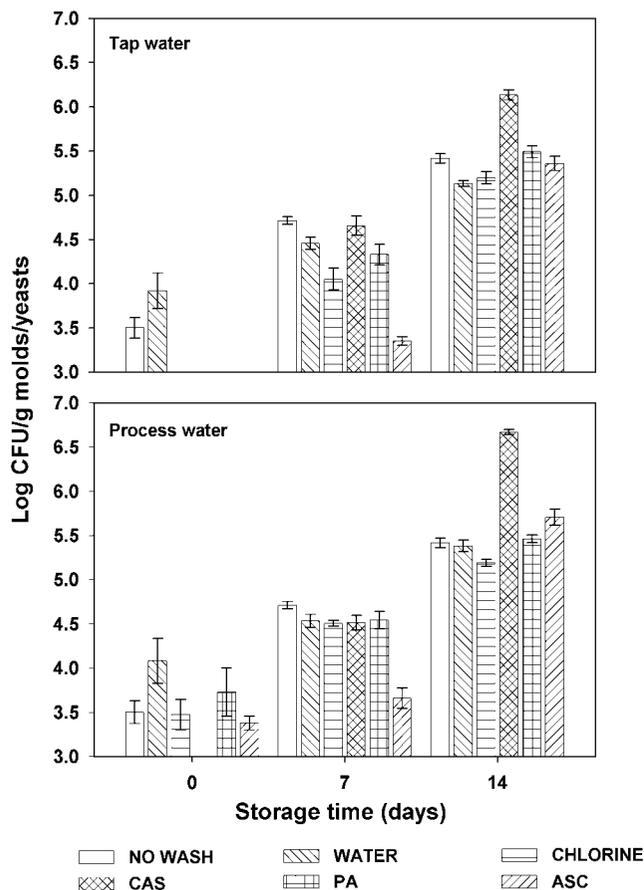


FIGURE 4. The efficacy of sanitizers on the reduction of mold and yeast counts from shredded carrots. Bars represent the standard errors of the mean from triplicate experiments. The limit of detection was 3.0 log CFU/g of shredded carrots. CAS, 0.66% citric acid-based sanitizer; PA, 80 ppm peroxyacetic acid; ASC, 1,000 ppm acidified sodium chlorite.

and acidified sodium chlorite caused at least a 3.27-log reduction under both water conditions. No significant effect from the high level of organic matter in wash water ($P = 0.7099$, comparing all treatments under process and tap water conditions) or a combined effect ($P = 0.3367$) between process wash water and type of sanitizer was observed. Treatment effects were significantly different ($P < 0.0001$).

Effect on yeast and mold counts. Populations of yeasts and molds were not reduced when washing with tap water, regardless of the COD level. Under high levels of organic matter, acidified sodium chlorite caused the highest reduction (0.7 log), followed by chlorine (0.61 log) and peracetic acid (0.35 log), whereas under tap water, the same sanitizers reduced the mold and yeast counts by at least 0.92 log (Fig. 4). Citric acid effectively produced a minimum 0.92-log reduction under both water conditions. Significant differences in recovery of yeasts and molds were from the effects of process water ($P = 0.0281$) and the type of sanitizers ($P = 0.0005$), although not synergistically ($P = 0.3679$).

Residual bacteria in wash water. No recovery of *E. coli* O157:H7 or other bacteria was possible from sanitizer solutions used to disinfect inoculated shredded carrots.

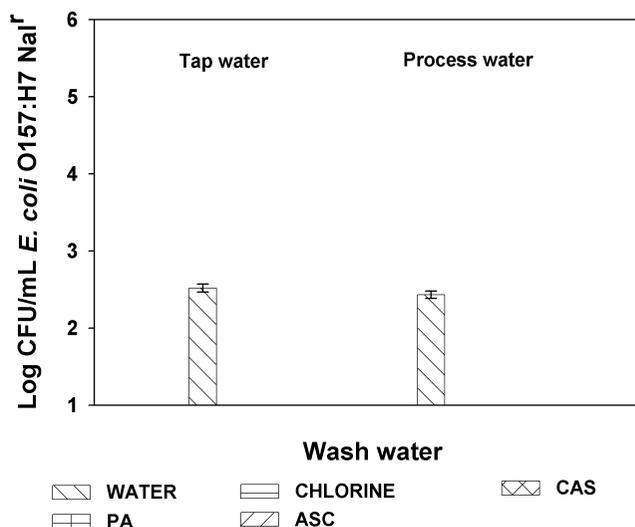


FIGURE 5. The efficacy of sanitizers on the reduction of residual *E. coli* O157:H7 from wash water. Bars represent the standard errors of the mean from triplicate experiments. The limit of detection was 1.0 log CFU/ml of wash water. CAS, 0.66% citric acid-based sanitizer; PA, 80 ppm peroxyacetic acid; ASC, 1,000 ppm acidified sodium chlorite.

When only water was used to wash the carrots, residuals of 2.5 log per ml of *E. coli* O157:H7 and up to 5.3 log per ml of total aerobic bacteria were recovered (Figs. 5 and 6). No significant differences between the effects of sanitizers under different levels of organic load in wash water were observed ($P = 0.0675$ for total aerobic bacteria, $P = 0.2189$ for *E. coli* O157:H7 with water treatment; no growth for all others in both assays) but were observed between plain wash water and wash water from sanitizers ($P < 0.0001$ and $P = 0.0039$ for total aerobic bacteria under tap and process water, respectively; $P < 0.0001$ for

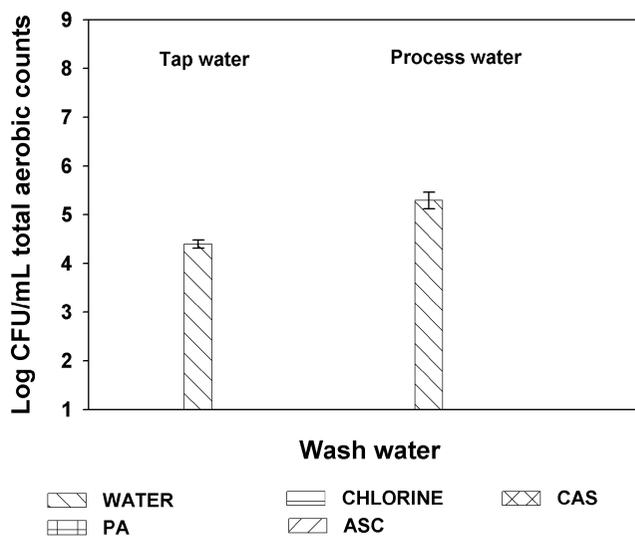


FIGURE 6. The efficacy of sanitizers on the reduction of total aerobic microbial counts from wash water. Bars represent the standard errors of the mean from triplicate experiments. The limit of detection was 1.0 log CFU/ml of wash water. CAS, 0.66% citric acid-based sanitizer; PA, 80 ppm peroxyacetic acid; ASC, 1,000 ppm acidified sodium chlorite.

E. coli O157:H7 under any water condition, when comparing all treatments under both water conditions). Results agree with previous findings in which sanitizing agents were more effective against microorganisms present in water than against those attached to produce surfaces (21). Preventing cross-contamination of fresh-cut produce is essential in maintaining the microbial safety and quality of these commodities.

Effects during storage. Significant differences in the efficacy of chemical sanitizers were observed among treatments at all times over the 14-day storage period ($P < 0.0001$ for all assays in any sampling day). *E. coli* O157:H7 showed a general decline in population, whereas total microbial counts increased over time, with the exception of samples treated with acidified sodium chlorite (Figs. 1 and 3). Besides bacterial death as a result of drying over the storage period and possible overgrowth by the microflora of carrots, cell injury also might have prevented recovery of pathogenic survivors, especially on selective medium. Beuchat and Brackett (8), Nguyen-the and Lund (19, 20), and Babic et al. (4) have demonstrated a lethal effect from carrots on *L. monocytogenes*. An inhibitory or killing effect of carrot tissue fluid on the *E. coli* O157:H7 population was also suggested by Abdul-Raouf et al. (1). In the same study, a reduction of the *E. coli* O157:H7 population was seen on shredded carrots under conditions similar to those in this study, with high numbers of survivors recovered at the end of the incubation period, but substantially fewer numbers recovered from lettuce or cucumber subjected to the same study conditions. No *E. coli* O157:H7 colonies or total bacteria counts were detected when acidified sodium chlorite was used as a sanitizer, regardless of the COD level, at any sampling time during the study. The effect is equivalent to at least a 3.49-log and 5.21-log reduction, respectively, when compared with a water wash.

Interestingly, no colonies were recovered after day 0 on chlorine-treated samples when regular tap water was used. In this case, the *E. coli* O157:H7 population might have fallen just below the level of detection (100 CFU/g), although not completely killed, as suggested by high levels of recovery during enrichment and the corresponding trend of decreasing numbers observed when chlorine was applied under process water conditions. A follow-up study is suggested to determine the exact fate of the *E. coli* O157:H7 population between days 0 and 7.

Peracetic acid treatment decreased pathogenic *E. coli* over days 7 and 14 when compared with a water wash, with a sustained effect that ranged from reductions of 0.89 to 1.31 log under tap water conditions and an average 0.90-log reduction in a process water base. Masson (18) suggested that the residual effect of the acetic acid released when peroxyacetic acid is degraded causes reduced growth in microflora (including fecal coliforms), as observed in ready-to-use salads treated with 90 ppm peracetic acid.

Yeast and mold populations also increased over the storage period under all sanitizer treatments, including acidified sodium chlorite (Fig. 4). A high level of organic matter in wash water caused a significant effect on microbial

reduction only on days 7 and 14, in the case of total aerobic bacteria ($P = 0.0007$ and $P < 0.0001$, respectively), but only on day 14 in the case of yeast and mold recovery ($P < 0.0001$). A combined effect of process water and type of sanitizer significantly affected recovery on day 14 for total aerobic bacteria ($P < 0.0001$) and on days 7 and 14 for yeasts and molds ($P = 0.0285$ and $P < 0.0001$, respectively).

This study further demonstrated that organic matter in fresh-cut process water reduces the efficacy of sanitizers on pathogen reduction, especially with chlorine. Acidified sodium chlorite was more tolerant to the organic loads presented in the fresh-cut process water than other sanitizers tested. Because treatment with acidified sodium chlorite under both tap water and process water conditions produced significant pathogen reduction, these results suggest that acidified sodium chlorite holds considerable promise as an alternative to chlorine as a sanitizer for fresh-cut produce. Regarding microbial safety of wash water, the results also revealed that any of the sanitizers tested in this study will prevent cross-contamination of other produce with *E. coli* O157:H7 and bacteria similar to that found in shredded carrots.

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