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Evaluation of Current Operating Standards for Chlorine Dioxide in Disinfection of Dump Tank and Flume for Fresh Tomatoes†

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ABSTRACT

Standard postharvest unit operations that rely on copious water contact, such as fruit unloading and washing, approach the criteria for a true critical control point in fresh tomato production. Performance data for approved sanitizers that reflect commercial systems are needed to set standards for audit compliance. This study was conducted to evaluate the efficacy of chlorine dioxide (ClO_2) for water disinfection as an objective assessment of recent industry-adopted standards for dump tank and flume management in fresh tomato packing operations. On-site assessments were conducted during eight temporally distinct shifts in two Florida packinghouses and one California packinghouse. Microbiological analyses of incoming and washed fruit and dump and flume system water were evaluated. Water temperature, pH, turbidity, conductivity, and oxidation-reduction potential (ORP) were monitored. Reduction in populations of mesophilic and coliform bacteria on fruit was not significant, and populations were significantly higher ($P < 0.05$) after washing. *Escherichia coli* was near the limit of detection in dump tanks but consistently below the detection limit in flumes. Turbidity and conductivity increased with loads of incoming tomatoes. Water temperature varied during daily operations, but pH and ORP mostly remained constant. The industry standard positive temperature differential of 5.5°C between water and fruit pulp was not maintained in tanks during the full daily operation. ORP values were significantly higher in the flume than in the dump tank. A positive correlation was found between ORP and temperature, and negative correlations were found between ORP and turbidity, total mesophilic bacteria, and coliforms. This study provides in-plant data indicating that ClO_2 can be an effective sanitizer in flume and spray-wash systems, but current operational limitations restrict its performance in dump tanks. Under current conditions, ClO_2 alone is unlikely to allow the fresh tomato industry to meet its microbiological quality goals under typical commercial conditions.

In 2010, an international group led by the United Fresh Produce Association with representation from all sectors of the fresh tomato supply chain released the *Food Safety Programs and Auditing Protocol for the Fresh Tomato Supply Chain* (Tomato Audit Protocol) *(34)*. This more prescriptive commodity-specific standard and audit protocol was preceded by the U.S. Food and Drug Administration (FDA) ''Guide to Minimize Microbial Food Safety Hazards of Fresh-cut Fruits and Vegetables'' *(37)* and the United Fresh *Commodity Specific Food Safety Guidelines for the Fresh Tomato Supply Chain (33)*. These programs provided recommendations for food safety practices intended to minimize the microbiological hazards associated with fresh and fresh-cut tomato products.

Tomatoes are frequently handled in packinghouses that typically employ systems of recirculated water, such as dump tanks and flume systems for unloading tomatoes from harvest bins or larger gondolas. The water used in these systems can become a point of cross-contamination for spoilage organisms and plant pathogens, which may lead to quality loss and decay, and human pathogens may cause outbreaks of foodborne diseases *(14)*. Process water is disinfected to inactivate or destroy pathogenic and other spoilage microorganisms in the source water and to prevent the transfer of these organisms from process water to produce and from one produce item to another during postharvest handling *(28)*. Effective treatment is an important preventive measure to increase the likelihood that the produce will be microbiologically safe for human consumption. Chlorination (150 mg/liter, pH 6.5 to 7.5, 2 min) is a current approved method for sanitization of dump tank and flume water in packinghouses and is part of the state regulations for tomatoes in Florida *(9)*.

Chlorine in its various available forms, including chlorine gas (Cl_2), sodium hypochlorite (NaClO), and calcium hypochlorite ($CaOCl_2$), are potent disinfectants with strong oxidizing properties. However, disadvantages

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related to their use include the formation of potentially hazardous disinfection by-products *(10),* strong pH dependence for optimal antimicrobial function, and the potential for irritating aerosols or gas emission that may affect worker comfort and health *(23, 25).* Because of these potential problems, chlorine dioxide (ClO_2) has been increasingly adopted as an alternative to chlorine for sanitization in the fresh and fresh-cut produce industry *(2, 11).* ClO_2 used as a disinfectant has several advantages over chlorine, including larger oxidant capacity *(7),* lower reactivity with organic matter *(12),* and high effectiveness at low concentrations *(15).* In contrast with NaClO, the most widely used form of chlorine in the fresh produce industry, ClO_2 is effective as an antimicrobial agent against such bacteria as *Salmonella* and *Escherichia coli* over a broad pH range (3 to 8) *(15).* ClO_2 and its main by-product, chlorite (ClO_2^-), are classified as noncarcinogenic products *(1, 17, 35).*

The ability of ClO_2 to inhibit several foodborne pathogens in various commodities has been reported previously. ClO_2 concentrations of 4 to 5 mg/liter were effective for reducing *Salmonella, E. coli* O157:H7, and *Listeria monocytogenes* inoculated onto cabbage, carrot, lettuce, strawberry, and melon *(21, 31, 32).*

Although several studies related to the antimicrobial effect of ClO_2 on foodborne pathogens of concern have been conducted on a laboratory scale, insufficient data are available to assess performance on an industrial scale. Few studies have been performed to determine the effect of pH, temperature, and suspended matter on the disinfection efficacy of ClO_2 for fresh produce on both commercial and laboratory scales, and the small amount of information available is not clear or consistent.

In addition to the use of chemical sanitizers for postharvest water management at the dump and flume handling stage, the Tomato Audit Protocol is very specific that wash water temperature is a critical point of preventive control management during tomato postharvest handling. Tomatoes are a warm-season crop, and high pulp temperatures can be reached during harvesting and transport to the packinghouse. Internalization of soft-rot bacteria into the fruit stem scar and subtending tissue can occur when tomatoes are submerged in water that is cooler than the pulp temperature *(4, 6).* As the tomato fruit cools and tissues contract, a vacuum is created, causing water and any potentially pathogenic organisms suspended in the water to be drawn into microwounds, pores, or other natural openings in the fruit. The standards and audit criteria for fresh tomatoes stipulate that in general cold water immersion as a cooling technique should not be used, and when a dump tank is used, the water temperature must be maintained at 5.5°C above the temperature of the incoming fruit pulp to minimize the risk of internalization *(5, 16, 34).* Therefore, water temperature relative to pulp temperature, disinfectant treatments, and other water quality constituents are jointly critical considerations for maintaining the safety and quality of tomatoes.

The overall goal of this study was to evaluate the current operating standards for use of ClO_2 in postharvest washing of fresh tomatoes and to generate data to support scientific audit criteria. The specific objectives were (i) to conduct on-site evaluation of the wash water management system using ClO_2 as the sole water disinfectant and to quantitatively evaluate the microbiological quality of the water in commercial dump tank and flume systems and (ii) to determine the comparative efficacy of using the oxidation-reduction potential (ORP) and other physicochemical parameters such as pH, temperature, and water turbidity to monitor, control, and document water disinfection status within various commercial tomato packing operations.

MATERIALS AND METHODS

Tomato packing facilities. Cooperating tomato packinghouses in Florida (facilities A and B) and California (facility C) running with fully matched ClO_2 generation and injection systems for all water contact units were visited during their respective tomato production seasons in 2010. Four site visits in each state were conducted randomly during the respective seasons. In general, the washing system of the three packinghouses consisted of a dump tank and a flume system and their associated recirculation system tanks.

Water analysis. Water samples were taken from the dump tank and flume system and their respective recirculation system tanks every 30 min during each selected processing day (up to 6 h/day). Water samples were taken to assess physicochemical parameters and microbiological quality. All measurements were repeated in triplicate.

Water samples of 500 ml were collected at each sample point to determine the ORP (millivolts), ClO_2 concentration (milligrams per liter), and turbidity (FAU). The pH, water temperature (degrees Celsius), and conductivity (Siemens per centimeter) were directly measured at a consistent location of each tank and the respective recirculation systems. The instruments used were a portable pH and temperature meter (Russell RL060P, Thermo Fisher Scientific, Inc., Waltham, MA) for temperature and pH, an ORP meter (Thermo Fisher Scientific) for ORP, and portable colorimeters for turbidity (DR/850, Hach Company, Loveland, CO) and ClO_2 residuals (Pocket Colorimeter II, Hach Company). Data were collected three times for each physicochemical parameter, sampling area, and time.

Standard enumeration methods were used to determine viable culturable mesophilic bacteria suspended in water. Two random water samples of 100 ml were collected from each sample area and immediately neutralized with 0.6% sodium thiosulfate (1 N $Na_2S_2O_3$) or 100 μl of 1 N $Na_2S_2O_3$ in the Florida and California facilities, respectively. The amount of $Na_2S_2O_3$ used was previously tested in the laboratory and was added in excess to ensure complete ClO_2 neutralization. Samples were immediately placed in refrigerated coolers and transported to the relevant Florida (Citrus Research and Education Center, University of Florida, Lake Alfred) and California (University of California, Davis) laboratories, where they were analyzed within 14 h of collection. Tenfold dilution series were prepared in 9 ml of sterile 0.1% buffered peptone water (BD, Franklin Lakes, NJ). Total aerobic mesophilic bacteria (TAM) and total coliforms were enumerated from water samples. Plate count agar (PCA; BD) incubated at 29°C for 48 h was used to enumerate TAM, and Chrom-ECC agar (Chrom Agar, Paris, France) incubated at 37°C for 24 h was used to enumerate total coliforms and presumptive *E. coli.* For trials 3 and 4 performed in California, the populations of total coliforms and *E. coli* were determined with the QuantiTray

Colilert System (Idexx Laboratories Inc., Westbrook, ME). All microbial counts were reported as log CFU per 100 ml of sample.

Tomato analysis. Tomato samples were collected every 30 min during each selected processing day (approximately 5 to 6 h) at each tomato facility. The sampling areas were the top and bottom of the field transport gondola (before washing) or field transport MACX48 bulk harvest bin (before washing) and the end of the water contact area before grading or worker contact (after spray brush bed washing).

Five tomatoes were collected from each sampling area to determine on-site pulp temperature of the fruit using a portable calibrated temperature probe. The probe was calibrated in an ice water slurry before each sampling day. For consistency, the pulp temperature was measured for each tomato at the stem scar region (approximately 1 cm deep).

For tomato microbiological analyses, two randomly collected samples of five tomatoes each were collected in sterile bags at each sampling area and immediately placed in refrigerated coolers, transported to the respective California and Florida laboratories, stored at 2.5°C, and processed within 14 h. Tomatoes were removed from temporary storage, and 100 ml of sterile potassium phosphate buffer (3.9 mM KH_2PO_4 and 6.1 mM K_2HPO_4) supplemented with 0.05% Tween 20 (Fisher, Fair Lawn, NJ) was added. Tomatoes were vigorously rubbed by hand for 1 min to remove the attached bacteria from the tomato surface. Tenfold dilution series were prepared in 9 ml of sterile 0.1% buffered peptone water and TAM and total coliforms were recovered from the tomato samples with PCA incubated at 29°C for 48 h for TAM and Chrom-ECC agar incubated at 37°C for 24 h for total coliforms and presumptive *E. coli*. All microbial counts were reported as log CFU per fruit.

Efficacy of the washing system for inoculated tomatoes. *Pseudomonas fluorescens* TVS074 is a biological control formulation isolate registered with the U.S. Environmental Protection Agency (EPA) by T. Suslow in 1994 after required EPA toxicological and nontarget organism studies and approved for commercial use on all fresh produce categories with no residue tolerance restrictions. This strain, which is resistant to rifampin (80 mg/liter), was isolated via spontaneous mutation and used in this study to facilitate detection and recovery of the bacteria of interest by minimizing interference with indigenous bacteria. TVS074 was grown overnight on King's medium B plates (0.15% $MgSO_4$ [Fisher], 1% glycerol [Fisher], 2% K_2HPO_4 [EMD Chemicals, Tokyo, Japan], 2% proteose peptone [BD], and 15% agar [BD]) supplemented with 80 mg/liter rifampin at 29°C. After incubation, cells were detached from the plates and resuspended in Butterfield's buffer (Whatman Inc., Piscataway, NJ). The cell suspension was washed twice by centrifugation at $1,500 \times g$ for 10 min and resuspended in Butterfield's phosphate buffer. The final cell pellet was suspended in Butterfield's phosphate buffer, and the culture was adjusted to achieve approximately 9 log CFU/ml. The final level was confirmed by plating on King's B plates supplemented with 80 mg/liter rifampin.

Four sets of 10 mature green tomatoes (*Lycopersicon esculentum* cv. Shadylady) were inoculated at room temperature by spot inoculation around the peduncle hemisphere but not within the stem scar itself. The inoculated area was marked with indelible ink on the tomato surface. Twenty drops (5 μl) of inoculum (10^9 CFU/ml) were placed onto the surface. The inoculum was allowed to dry overnight at room temperature before processing. Inoculated tomatoes had an average inoculum level of 8 log CFU per fruit after inoculation.

For processing, each set of tomatoes was marked with a different color tape to differentiate them from the noninoculated tomatoes and to facilitate sample collection. Inoculated tomatoes were transported to facility C and introduced to the system during standard washing operations and mixed with tomatoes from the field in the dump tank. Each set of inoculated fruit was introduced at different processing times with different water quality conditions, which were recorded at each time. The tomatoes were collected after the final water contact and before grading and worker contact. Tomatoes were briefly air dried, placed in sterile bags, stored in chilled coolers, transported to the laboratory, and stored at 2.5°C. All tomatoes were processed within 14 h after collection for microbiological analyses. To determine the potential nonwashed level of *P. fluorescens* TVS 074 on tomato surfaces, an inoculated fruit not subjected to the commercial dump and wash system ($n = 10$) was used as an internal control for survival. This set of control tomatoes was stored and transported under the same conditions as the sample tomatoes that were processed at the facility.

Bacteria were enumerated by placing tomatoes individually in sterile bags (Whirl-Pak, Nasco, Modesto, CA) containing 50 ml of sterile potassium phosphate buffer (3.9 mM KH_2PO_4 and 6.1 mM K_2HPO_4) supplemented with 0.05% of Tween 20 (Fisher) and vigorously rubbed by hand. For *P. fluorescens* recovery, cultures on King's B plates supplemented with 80 μg/liter rifampin were enumerated after incubation at 29°C for 24 h. All microbial counts were reported as log CFU per fruit.

Contribution of process wash water to the final tomato microbial load. Four sets of 10 tomatoes each were submerged in a 0.1% silver nitrate solution for 1 min at room temperature and rubbed and dried with sterile paper towels and ethanol (70%, vol/vol) by hand for surface sterilization. Tomatoes were immediately introduced into the dump tank and then collected, transported, and analyzed as previously described. This assessment of process water was performed only in facility C. Populations of TAM and total coliforms were recovered from the tomato samples using the same methods previously described. Surface sterilized fruit not subjected to the dump and flume wash system were used as internal controls.

Statistical analysis. To evaluate temporal, water quality, and treatment differences between dump and flume tanks for all physicochemical parameters and bacterial populations, statistical analyses were performed using the MIX procedure function of the Statistical Analysis System (version 9.2, SAS Institute, Cary, NC), and means were separated with Tukey's pair comparison. To determine differences in bacterial populations between tomatoes collected from top and bottom of the harvest gondola, a similar analysis was performed. When no significant changes were found during the sampling time, data for all time points were averaged, and a comparison between dump and flume tanks was carried out with a *t* test paired comparison or one-way analysis of variance to determine differences among facilities using JMP (version 8, SAS Institute). Statistical significance was established when P values were smaller than 0.05. A correlation matrix for all physicochemical parameters and bacterial populations was constructed using the CORR procedure of SAS, which provided a value for the Pearson correlation (R) and the associated P value.

RESULTS

Water physicochemical conditions. The physicochemical parameters pH, ORP, turbidity, conductivity, and temperature were assessed in water utilized to wash

TABLE 1. *ORP, turbidity, and conductivity values in the tomato dump tank and flume system for each facility and each day of operation*[a]

Facility	Trial	ORP (mV)[b]		Conductivity (S/cm)[b]		Turbidity (FAU)[c]	
		Dump tank	Flume system	Dump tank	Flume system	Dump tank	Flume system
Florida							
A	1	427 ± 106 C b	530 ± 110 B a	1,025 ± 322 D a	1,240 ± 160 D b	2.33–172 D a	1.66–40.0 D b
	2	465 ± 94.6 BC b	466 ± 123 C a	764 ± 129 E a	1,090 ± 107 D b	4.50–131 E a	3.00–45.0 D b
B	1	537 ± 147 AB b	637 ± 67.0 A a	1,180 ± 94.1 CD a	1,216 ± 177 BC a	2.00–57.0 CD a	2.33–53.3 B,C a
	2	594 ± 89.3 A b	630 ± 59.9 A a	1,476 ± 289 AB a	1,234 ± 303 A a	11.0–201 AB a	4.17–197 A a
California							
C	1	437 ± 33.7 C b	488 ± 83.0 B a	1,512 ± 400 A a	806 ± 219 B b	15.8–169 A a	9.00–32.0 B b
	2	430 ± 90.2 C b	518 ± 117 BC a	1,311 ± 206 C a	880 ± 137 C b	3.50–383 C a	2.00–45.2 C b
	3	431 ± 146 C b	669 ± 12.5 A a	1,346 ± 200 BC b	1,187 ± 99.3 B b	0.16–305 BC a	0.66–42.3 B b
	4	302 ± 99.7 D b	650 ± 54.3 A a	1,147 ± 166 D a	1,476 ± 292 B a	0.50–316 D a	0.0–110 B b

[a] Within each column, values with different uppercase letters denote a significant difference among trials for all tomato facilities ($P < 0.05$). Within each row, values with different lowercase letters denote a significant difference between the dump tank and flume system ($P < 0.05$).

[b] Values represent the mean ± standard deviation ($n = 3$) for each trial during each operation day.

[c] Values represent the range of turbidity from the beginning to the end of the operation time.

tomatoes at three different facilities. For all trials and facilities no significant difference ($P > 0.05$) was found between fruit contact tanks and their recirculation tanks, so values were averaged for a total of six observations for both dump and flume tanks (Supplementary Figs. 1 through 3).

During each trial, physicochemical parameters were monitored during a regular processing day (up to 330 min). On-site measurements of ClO_2 were highly variable depending on the point within the handling system. The accuracy of the colorimetric measurements was severely impaired within the range of daily operational increasing water turbidity; thus, the data collected on site were not included in this report. Based on data from the devices utilized by the cooperating facilities to measure ClO_2, which matched the data from the devices used in this study, the flume appeared to be in compliance with the commodity-specific tomato standards *(33),* but the dump tank did not. Overall for all trials, ORP values were significantly higher in the flume than in the dump tanks ($P < 0.05$), with the exception of facility A during trial 2 (Table 1). During the period of sampling on a given date, ORP values tended to remain constant for facilities B and C; however, for facility A the ORP values of the flume tended to increase during the time course of daily operations (Supplementary Table 1). For facilities A and C, the dump tank ORP values were consistently below 600 mV; however, in flume tanks ORP values of >600 mV were noted over time. In contrast, facility B maintained ORP values of >600 mV in both tanks (Table 1). For facility A during trial 2 and facility C during trials 1 and 2, ORP values remained below the target values in both tanks (Table 1).

Conductivity and turbidity were the only parameters that had a significant bivariate correlation ($P < 0.05$) during the time frame of each site visit. Values for both parameters increased during the sampling period (Table 1 and Supplementary Tables 2 and 3) and were significantly higher ($P < 0.05$) in dump tanks than in the flume, with the exception of facility B where both parameters were not significantly different between dump and flume tanks (Table 1). When comparing the trials, different conditions for each daily operation were recorded, and conductivity and turbidity were significantly different between location and among trials (Table 1).

Turbidity values in flume tanks rarely exceeded 45 FAU. After daily cleaning and a complete change with fresh water at start up, water turbidity was directly related to the condition of the incoming fruit load, which carries varying amounts of soil and nonproduct organic matter. In several trials, cumulative turbidity increases exceeding 300 FAU were noted in the dump tank water (trials 2 and 4, facility C), far greater levels that noted in the other trials (Supplementary Table 3). In general, turbidity increased during the course of daily operations with occasional large spikes due to incoming loads (Table 1). For example, a change in turbidity from 264 to 320 FAU after 180 min (trial 2, facility C) was observed in a tank that held more than 500 kl. In the same way, turbidity values increased from 316 to 594 FAU after 210 min in facility C during trial 4. Reductions in FAU values were periodically observed because of the periodic partial exchanges with clean water (approximately 30% turnover) to the system by the facility operational staff. Although both conductivity and turbidity values increased during sampling, ORP remained constant (Table 1 and Supplementary Table 1).

Water temperature and pH were largely invariant during trials, and values were not significantly correlated with the time course of a daily event. Consequently, values for the entire trial at each location were averaged. In Florida facilities, no significant differences in pH and temperature were noted between the dump tank and flume system (Table 2). In contrast, facility C had larger pH variation and lower temperature in the dump tank than in the flume system (Table 2).

For all trials and locations, the Pearson correlation among the parameters revealed that ORP was negatively correlated with turbidity and positively correlated with

TABLE 2. *Water temperature and pH in the tomato dump tank and flume system for each facility and day of operation*[a]

Facility	Trial	pH		Temp (°C)	
		Dump tank	Flume system	Dump tank	Flume system
Florida					
A	1	7.65 ± 0.14 B a	7.77 ± 0.13 BC a	34.4 ± 0.94 C b	36.1 ± 0.75 CD a
	2	7.66 ± 0.17 B a	7.65 ± 0.19 C a	34.9 ± 1.42 BC a	35.7 ± 0.61 D a
B	1	7.36 ± 0.11 C a	7.41 ± 0.15 D a	36.8 ± 0.27 AB a	36.9 ± 0.32 CD a
	2	7.11 ± 0.27 D a	7.14 ± 0.29 E a	37.2 ± 2.64 A a	37.3 ± 2.59 C a
California					
C	1	7.32 ± 0.19 C b	7.83 ± 0.16 B a	34.2 ± 2.45 C b	42.3 ± 1.76 B a
	2	7.24 ± 0.40 CD b	7.90 ± 0.35 B a	34.6 ± 3.44 BC b	43.8 ± 3.06 A a
	3	7.94 ± 0.41 A b	8.21 ± 0.45 A a	33.9 ± 4.89 C b	42.1 ± 4.13 B a
	4	8.09 ± 0.25 A a	8.12 ± 0.39 A a	35.1 ± 3.33 BC b	44.3 ± 1.96 A a

[a] Values represent the mean ± standard deviation ($n = 3$) for each trial during each operation day. Within each column, values with different uppercase letters denote a significant difference among trials for all tomato facilities ($P < 0.05$). Within each row, values with different lowercase letters denote a significant difference between the dump tank and flume system ($P < 0.05$).

temperature (Table 3). In contrast, pH was not correlated ($P > 0.05$) with ORP but was negatively correlated with conductivity (Table 3).

To minimize water infiltration of fruit during packing, incoming tomatoes and processing water temperature should have a negative differential of at least 5.5°C, i.e., fruit pulp must be cooler than the water *(5, 19, 35)*. The temperature of the water and the incoming fruit and the temperature differential were monitored throughout the site survey intervals for all trials (Figs. 1 and 2). For facilities A and B, temperature differentials of less than 5.5°C were found in trial A2 and B2. In facility A during the trial 2, the temperature differential was less than the target point for most of the daily operations. For facility C, at times during trials 1 and 2 this industry standard was not met.

Water microbiological quality. The TAM and total coliforms in water were determined at each location during the operational monitoring period. For Florida facilities A and B, populations of TAM were not significantly different between dump and flume tanks ($P > 0.05$) (Table 4A). In contrast, the population of TAM was always significantly lower in the flume than in the dump tank system in the California facility. Comparison among trials within each facility revealed that the populations of TAM were similar within each location, but a significant difference was evident between the three facilities (Table 4A). The general trend indicated that populations of TAM remained constant during the sampling day (Table 4A). Pearson correlation analysis revealed that populations of TAM were negatively correlated with ORP, pH, and temperature but positively correlated with turbidity (Table 3).

Populations of total coliforms were significantly higher in the dump tank than in the flume system ($P < 0.05$) for all trials (Table 4B). Comparison among trials revealed that the population of total coliforms in the dump tank was similar for all tomato facilities. In contrast, differences in coliform populations were found in the flume system among trials (Table 4B). As observed with TAM, the population of total coliforms remained mostly constant during the each survey period (Table 4B). Total coliforms were significantly positively correlated with turbidity, but significantly negatively correlated with the remaining physicochemical parameters monitored, with the exception of pH (Table 3). Evaluation for *E. coli* in the dump tank was mostly negative for all trials in Florida and the first two trials in California, although in dump tank samples colonies characteristic of *E. coli* were sporadically detected on Chrom-ECC. For facility C, when the detection and enumeration method was changed to the QuantiTray Colilert System for trials 3 and 4 to improve sensitivity, *E. coli* was detected in the dump tank 60 min after the introduction of the tomato load. However, this bacterium was not detected in the flume tank at any sampling point (data not shown).

TABLE 3. *Matrix of Pearson correlation values among water physicochemical parameters and microbial populations*[a]

Parameter	pH	ORP	Turbidity	Conductivity	Temp	TAM	Coliforms
pH	1	0.011	−0.305*	−0.483*	0.431*	−0.169*	−0.075
ORP		1	−0.409*	0.037	0.372*	−0.308*	−0.335*
Turbidity			1	0.416*	−0.439*	0.270*	0.166*
Conductivity				1	−0.054	0.032	−0.215*
Temp					1	−0.531*	−0.446*
TAM						1	0.624*
Coliforms							1

[a] Asterisk denotes significance of the correlation value ($P < 0.05$).

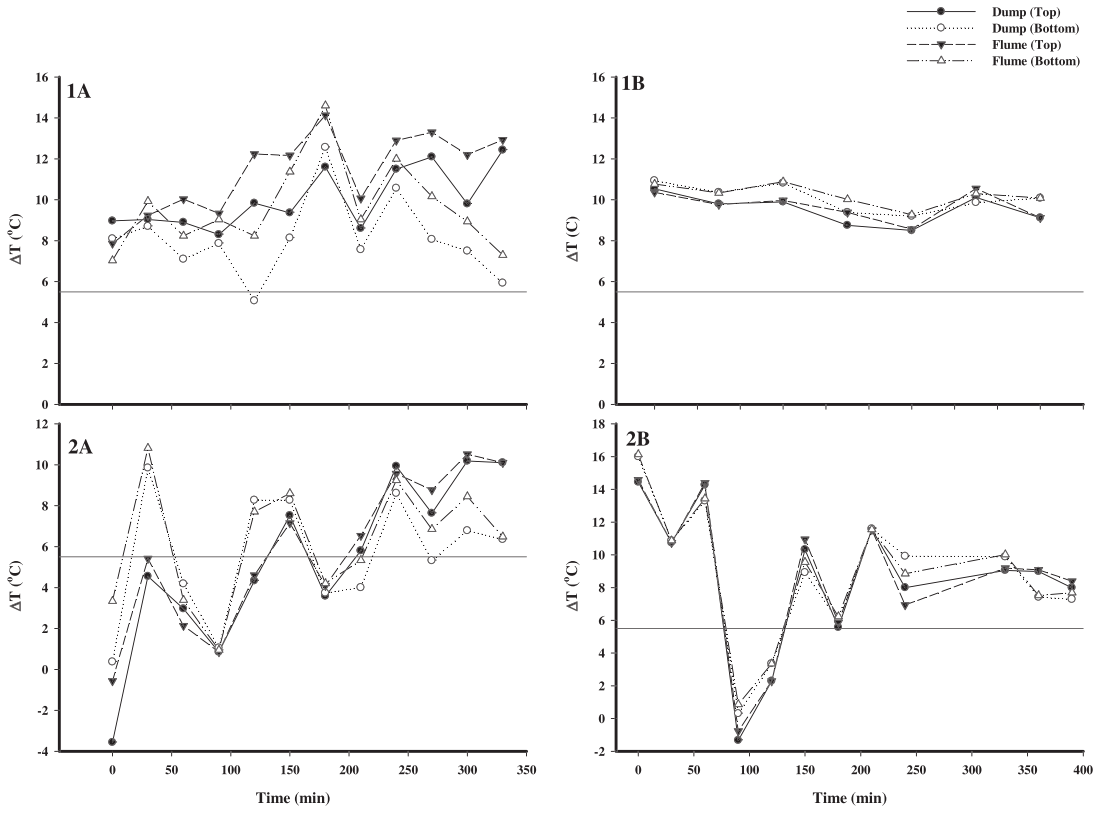

FIGURE 1. *Tomato and dump water temperature differential at tomato facility A (trials 1 and 2) and facility B (trials 1 and 2) in Florida. ''Top'' and ''bottom'' refer to the sampling areas in the gondola carrying incoming fruit. The temperature differential was calculated between incoming tomatoes and the wash water. The horizontal solid line represents the minimum safe temperature differential (5.5°C) according to the Tomato Audit Protocol.*

Washing effectiveness of dump tank and flume systems on tomato surface microbial populations. Tomato samples were collected from the top and bottom of the gondolas or bulk harvest bins for each trial during each operational day. No significant differences in the microbial populations from the top and bottom of the tomato lot or between the tomato lots arriving at the dump tank were found. Thus, these values were averaged by trial. TAM populations and total coliforms did not change during the sampling intervals, and values were averaged by trial.

In general, populations of TAM and total coliforms on tomatoes were not significantly different before and after washing ($P > 0.05$). TAM populations were greater after washing in three of the eight trials, and total coliforms were greater after washing in six of the seven trials (Table 5A and 5B). Both microbial populations tended to be greater in tomatoes from California (bush production) than in those from Florida (pole production).

To study the efficiency of the washing process for reducing the microbial load of challenge-inoculated tomatoes, *P. fluorescens* TVS 074 was applied to clean fruit at approximately 8 log CFU per fruit. During a regular operational shift, marked inoculated tomatoes were introduced into the washing system with arriving bulk fruit and were collected at the end of the washing line. Populations of *P. fluorescens* TVS 074 were reduced by approximately 4 log units (Table 6). A set of tests were conducted to determine whether microbial loads would increase during washing. Three trials of surface-sterilized tomatoes were processed during a regular packing shift. Populations of TAM increased from undetectable levels to approximately 3 log CFU per fruit after passing through the washing system. No significant difference was found among the three trials despite substantial variation in dump tank ORP values and more uniform and ORP levels in flume tanks, which seemed to meet the industry standards (Table 7). Populations of TAM were below the limit of detection for surface sterilized tomatoes that were not washed and used as wash system contact controls. Coliforms were not detected (detection limit = 2 log CFU per fruit).

DISCUSSION

The implementation of practices that can minimize the potential risk of cross-contamination with foodborne pathogens, especially whenever water contact is involved, is essential for a successful food safety program. Disinfection of water is a vital management step for minimizing transmission of pathogens within and between produce lots. Contamination with plant pathogens has long been a concern for the fresh produce handling industry, including tomatoes, but in recent years *Salmonella* infection outbreaks associated with consumption of raw tomatoes has emphasized the importance of postharvest practices to minimize risk to consumers *(13)*. In tomato packinghouses, large volumes of water are used during postharvest washing and handling. Water recirculation is a common practice in the

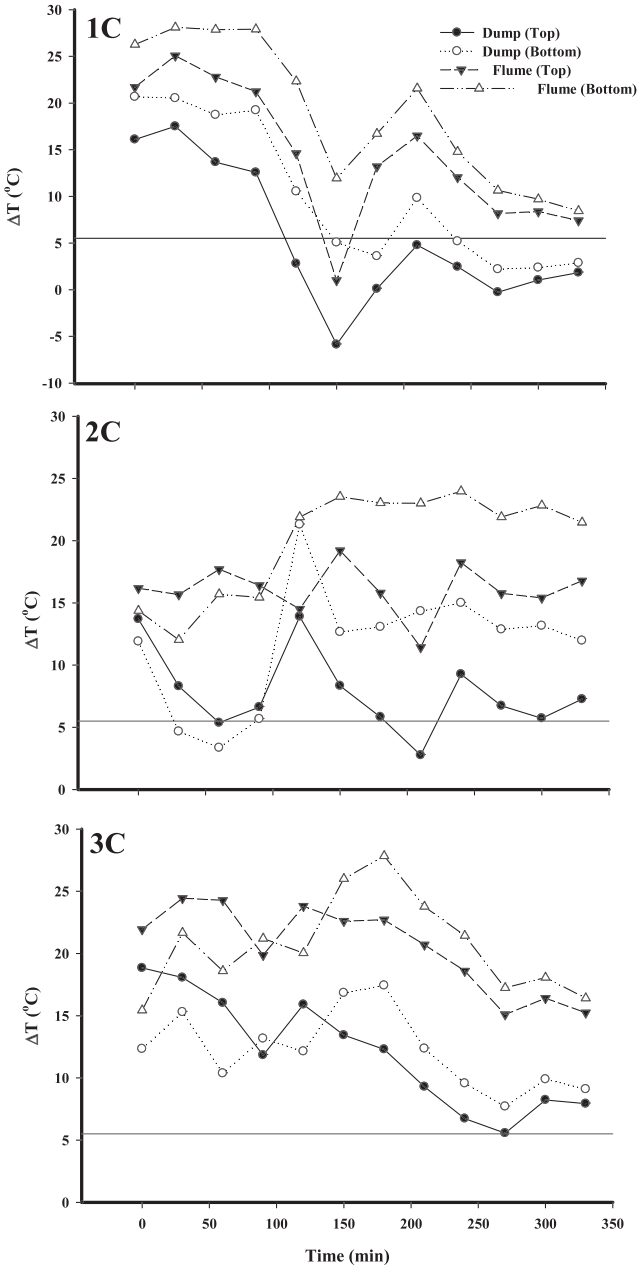

FIGURE 2. *Tomato and dump water temperature differential for trials 1, 2, and 3 at the California tomato facility C. ''Top'' and ''bottom'' refer to the sampling areas on the gondola carrying incoming fruit. The temperature differential was calculated between incoming tomato and wash water. The horizontal solid line represents the minimum safe temperature differential (5.5°C) according to the Tomato Audit Protocol.*

industry because of economic considerations associated with treatment costs, especially heating and wastewater discharge regulations; thus, effective reconditioning and disinfection strategies are essential.

The use of ClO_2 is currently accepted within the guidelines for tomato good agricultural practices for postharvest water disinfection *(9)*. The FDA allows the use of ClO_2 as an antimicrobial agent in water used to wash fresh fruit and vegetables. The residual amount must be less than 3 mg/liter, and this treatment must be followed by a rinse in potable water *(36)*.

TABLE 4. *Comparison of populations of total aerobic mesophilic bacteria (A) and total coliforms (B) between tomato dump and flume tank water*[a]

Facility location	Trial	Dump	Flume	P[b]
A. Total aerobic mesophilic bacteria (log CFU/ml)				
Florida				
A	1	4.01 ± 0.36 BC	3.78 ± 0.64 A	0.0318
	2	3.44 ± 0.98 C	3.67 ± 0.91 A	0.8519
B	1	3.82 ± 0.58 ABC	3.02 ± 1.21 AB	0.0052
	2	3.39 ± 0.79 C	3.08 ± 0.70 AB	0.0579
California				
C	1	4.74 ± 1.12 A	2.22 ± 0.74 B	<0.0001
	2	4.68 ± 1.35 A	0.78 ± 1.12 C	<0.0001
	3	4.53 ± 1.30 AB	2.44 ± 2.86 B	<0.0001
	4	ND[c]	ND	
B. Total coliforms (log CFU/100 ml)				
Florida				
A	1	4.76 ± 1.24 AB	3.50 ± 2.28 A	0.0009
	2	3.63 ± 2.18 BC	3.43 ± 1.97 A	0.3418
B	1	5.00 ± 0.69 AB	3.34 ± 2.53 A	0.0079
	2	4.24 ± 2.80 AB	1.64 ± 2.29 BC	0.0004
California				
C	1	2.85 ± 2.07 C	1.11 ± 1.84 CD	<0.0001
	2	4.98 ± 1.63 A	0.17 ± 0.71 D	<0.0001
	3	2.69 ± 1.31 C	1.35 ± 1.51 C	<0.0001
	4	5.12 ± 0.69 A	2.78 ± 1.03 AB	<0.0001

[a] Values are means ± standard deviations. Within each column, values with different letters denote a significant difference among trials for all tomato facilities.

[b] P values for the t test comparison of bacterial populations between dump and flume tanks for each trial.

[c] ND, not determined for that trial.

In the present study, water treated with ClO_2 increased ORP, which has been adopted by the Tomato Audit Protocol *(34)* and the commodity-specific guidelines for tomatoes *(33)* as a primary parameter for standardizing water disinfection monitoring, dose management, and documentation. Although the relationship of ORP to dose is generally nonlinear, ORP is a potentially useful measurement of the ability of treated water to inactivate microorganisms and is an important component of a sound postharvest quality and safety program *(30)*. In this study, ORP values were significantly higher ($P < 0.05$) and more consistently maintained in the flume system than in the dump tank for all tomato facilities (Table 1). In water systems of high quality, ORP values of 650 to 700 mV are effective for inactivating spoilage bacteria and foodborne pathogens (*E. coli* and *Salmonella*) within a few seconds and for inactivating spoilage yeasts and fungi within a few minutes *(20, 24, 29, 30)*. In general, these values were not achieved in the dump tank for all the facilities evaluated. However, adequate ORP values were found in flume tanks except in facility A (Table 1). ORP values were near 600 mV at the beginning of the operation in facility C and throughout trial 2 in facility B, but after the first load of tomatoes in the dump tank, values dropped to approximately

TABLE 5. *Comparison of populations of total aerobic mesophilic bacteria (A) and total coliforms (B) on tomato surfaces between incoming tomatoes in the dump tank and tomatoes exiting the flume system*[a]

Facility location	Trial	Before washing	After washing	P[b]
A. Total aerobic mesophilic bacteria (log CFU/fruit)				
Florida				
A	1	4.98 ± 1.03 BC	4.67 ± 0.70 B	0.922
	2	4.06 ± 1.05 CD	4.38 ± 0.32 B	0.032
B	1	4.62 ± 0.65 D	4.39 ± 0.68 B	0.836
	2	4.85 ± 0.55 BC	4.60 ± 0.85 B	0.902
California				
C	1	5.18 ± 0.74 BC	6.16 ± 0.45 A	<0.0001
	2	5.25 ± 0.83 B	6.13 ± 0.55 A	<0.0001
	3	6.53 ± 0.97 A	5.50 ± 1.21 A	0.999
	4	ND[c]	ND	
B. Total coliforms (log CFU/fruit)				
Florida				
A	1	3.77 ± 1.11 AB	3.35 ± 0.79 C	0.936
	2	3.18 ± 1.12 BC	3.41 ± 0.62 C	0.148
B	1	3.12 ± 1.22 BC	3.17 ± 0.54 C	0.427
	2	3.02 ± 1.34 BC	3.39 ± 1.01 C	0.131
California				
C	1	2.70 ± 1.39 C	4.85 ± 0.75 AB	<0.0001
	2	3.80 ± 1.38 AB	5.31 ± 0.65 A	<0.0001
	3	4.30 ± 1.59 A	4.44 ± 1.43 B	0.358
	4	ND	ND	

[a] Values are means ± standard deviations. Within each column, values with different letters denote a significant difference among trials for all tomato facilities.
[b] P values for the t test comparison of bacterial populations between incoming unwashed tomatoes and tomatoes after washing for each trial.
[c] ND, not determined for that trial.

TABLE 6. *Reduction of* P. fluorescens *TVS 074 populations on tomatoes after processing through dump and flume tanks at the California tomato facility*[a]

Trial	ORP (mV)		Population after washing (log CFU/fruit)[b]	Log reduction[c]
	Dump	Flume		
1	354	725	3.63 ± 1.36	4.77
2	410	729	4.81 ± 0.64	3.59
3	410	725	4.07 ± 0.94	4.33
4	711	709	4.02 ± 1.17	4.38

[a] Average temperatures for three trials were 32.0 ± 1.28°C and 45.2 ± 0.53°C for dump and flume tanks, respectively. Average turbidity values for three trials were 166 ± 0.6 FAU and 21.3 ± 7.4 FAU for dump and flume tanks, respectively.
[b] Values are means ± standard deviations (n = 10 tomatoes) after washing.
[c] Log reduction was calculated based on the mean *P. fluorescens* population on surfaces of tomatoes that were not subjected to any washing treatment (approximately 8 log CFU per fruit).

TABLE 7. *Contribution of water to the population of total aerobic mesophilic bacteria on surface-sterilized tomatoes after introduction to a commercial dump and flume tank*[a]

Trial	ORP (mV)		Population (log CFU/fruit)[b]
	Dump	Flume	
1	470	802	3.84 ± 0.36
2	825	787	3.61 ± 0.52
3	779	790	2.84 ± 0.21

[a] Average temperatures for three trials were 28.2 ± 12.5°C and 40.6 ± 0.37°C for dump and flume tanks, respectively. Average turbidity values for three trials were 325 ± 39.7 FAU and 55.0 ± 9.64 FAU for dump and flume tanks, respectively. Coliforms were not detected on tomatoes for any of the three trials (detection limit = 2 log CFU per fruit).
[b] Values are means ± standard deviations (n = 10 tomatoes).

400 mV and remained constant during the sampling period (Supplementary Table 1). Cross-contamination of produce can occur when adequate water disinfection cannot be achieved in the dump tank.

Turbidity and conductivity increased over time. Turbidity levels especially increased (Table 1) because of incoming soil and organic matter associated with tomato harvests. Contrary to ORP findings, turbidity was significantly higher in the dump tank than in the flume water except in facility B, trial 2 (Table 1). This finding was supported by significant Pearson correlation coefficients ($P < 0.05$) for ORP and turbidity. Similarly, populations of TAM and total coliforms were significantly lower in the flume than in the dump water (Table 4), and their populations were negatively correlated ($P < 0.05$) with ORP but positively correlated with turbidity (Table 3). Conductivity was significantly higher in the dump tank than in the flume water in facility C and in facility B, trial 2.

The efficacy of ClO_2 for disinfection has been demonstrated in studies using regular tap water in which the presence of organic matter has not been considered. In studies of the disinfection efficiency of ClO_2 in wastewater, suspended solids and organic load had unfavorable effects on disinfection efficiency, mostly related to the ability of bacteria to attach to or derive protection from suspended organic matter *(3, 22)*. In the present study, *E. coli* was detected in dump tanks but not in the flume. High turbidity values in the dump tank presumably resulted in the inability of the dump tank system to effectively inactivate *E. coli* cells within the short retention times. Although ORP is considered a primary indicator of oxidative disinfection for the inactivation of food-related pathogens on a laboratory scale *(19)*, our results suggest that reliance on ORP values alone is insufficient for predicting microbial water quality within commercial tomato operations using ClO_2; thus, standard methods for accurate monitoring of the ClO_2 dose should be incorporated into future guidance documents and audit performance standards. Other physicochemical parameters should be taken into account when optimizing microbial controls, particularly in dump tank management.

Parameters such as pH and temperature remained mostly constant throughout the tomato washing operation, particularly in facility C, but significant differences between the dump tank and the flume water for these conditions were found (Table 2). However, pH and temperature were not significantly different ($P > 0.05$) in facilities A and B. Overall, pH was not correlated with ORP but was negatively correlated with turbidity and TAM population (Table 3). The oxidizing power of ClO_2 may not be pH dependent within typical operating ranges, primarily because ClO_2 does not hydrolyze in water to form hypochlorous acid *(8)*. Additional studies have revealed that bacteria such as *E. coli* may be killed effectively with liquid ClO_2 in a pH range of 3 to 8 in water *(15)*.

In addition to the water disinfection treatment, water temperature also can impact the microbial safety and quality of tomatoes. Standard recommendations based on previous studies are for tomato dump tanks to be maintained at least 5.5°C above the temperature of the incoming fruit to prevent water infiltration and potential contamination with pathogens *(4–6, 16, 33)*. In the present survey study, water remained at a consistent, slightly elevated temperature, relative to ambient conditions, during the washing operation in all trials. However, there was some question of whether the measured differential between water and tomato pulp temperature met specifications and followed current best practice recommendations to limit water uptake by the fruit. The temperature differential differed among trials but was lower than the audit criterion of 5.5°C at some points during the daily shift, which may pose a risk to tomato safety and quality. Variations among trials could be attributed to changes in environmental temperatures during the day and harvesting conditions for each location. This temperature differential is considered by the industry as a difficult point to control, particularly for different environmental and product conditions that can affect incoming product temperature. Thus, special attention to maintaining the temperature differential between water and tomato above 5.5°C must be taken to ensure tomato safety without altering the sensory attributes of the product. Overall results among trials differed for all parameters measured in water, presumably because of environmental and incoming fruit conditions and variability in daily operational management.

The produce washing system is designed to reduce the microbial load that the product has acquired in the field or at minimum to prevent acquisition of an unacceptable microbial load during postharvest washing. In the present study, populations of TAM and coliforms were not significantly different ($P > 0.05$) or were significantly higher ($P < 0.05$) in unwashed compared with washed tomatoes (Table 5). In a previous study in a tomato facility at Charleston, SC, total plate counts and *Enterobacteriaceae* counts were higher on tomatoes washed with chlorine (90 to 140 mg/liter) than on unwashed tomatoes *(27)*. ClO_2 at 5 to 20 mg/liter was effective against *Salmonella enterica* and *Erwinia carotovora* in water, although the sanitizing effects were reduced for inoculated tomatoes, particularly when the inoculum suspension was allowed to dry on the tomato surface *(26)*. ClO_2 probably is less effective against strongly attached microorganisms, which may form protective aggregates over time *(18)*. In addition to washing by immersion in water plus disinfectant, physical removal of contaminant by methods such as a spray brush-bed could improve reduction of the microbial load on tomato surfaces. The combination of physical removal of material with disinfectant roller brushers and ClO_2 disinfection by water immersion resulted in significantly greater reduction of *Salmonella* on tomato surfaces than did water immersion alone *(26)*. In the present study, at facility C passage through the commercial washing operation achieved up to a 4-log reduction of inoculated *P. fluorescens* on tomatoes. In contrast, surface-sterilized tomatoes acquired TAM at approximately 3 log CFU per fruit but did not acquire coliforms during passage with incoming fruit loads through these washing operations.

The antimicrobial efficiency of ClO_2 has been successfully demonstrated with several commodities through laboratory scale studies *(11)*. However, the findings of the present study indicate that although ClO_2 can be an effective sanitizer for flume and spray-wash systems, current operational limitations greatly restrict ClO_2 efficacy in dump tanks. Under current conditions, the application of ClO_2 alone is unlikely to allow the fresh tomato industry to meet microbiological quality goals for dump tank management under typical commercial conditions. These findings suggest that water disinfection with ClO_2 should be considered only part of an integrated strategy for preventing contamination of tomatoes by human or plant pathogens.

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