Food Control 26 (2012) 28–35

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Chlorine dioxide dose, water quality and temperature affect the oxidative status of tomato processing water and its ability to inactivate *Salmonella*

Gabriela López-Velasco [a,1], Alejandro Tomás-Callejas [a,b,1], Adrian Sbodio [a], Francisco Artés-Hernández [b], Trevor V. Suslow [a,*]

[a] Department of Plant Sciences, University of California, One Shields Avenue, Davis, CA 95616, USA
[b] Postharvest and Refrigeration Group, Department of Food Engineering & Institute of Plant Biotechnology, Technical University of Cartagena, Paseo Alfonso XIII 48, Cartagena, Murcia 30203, Spain

ARTICLE INFO

Article history:
Received 16 August 2011
Received in revised form
9 December 2011
Accepted 14 December 2011

Keywords:
Tomato postharvest water
Dump tank
Chlorine dioxide
Turbidity
Salmonella inactivation

ABSTRACT

Water disinfection is a vital control-point to minimize transmission of diverse pathogens from water sources or produce within and among lots at harvest, during initial postharvest handling, and within subsequent postharvest re-packing and processing. The use of ClO_2 as an alternative to NaClO for water disinfection is being adopted by the fresh tomato postharvest handling industry. Lack of data to define performance for this specific water sanitizer under commercial conditions is a barrier to setting meaningful standards and audit criteria for water sanitation and associated food safety goals. The current work aims to establish a correlative capacity of the Oxidation Reduction Potential (ORP) to ClO_2 dose, under different conditions of water turbidity and temperature, and their potential to reflect the efficacy in inactivation of *Salmonella enterica* in tomato process wash water during primary packing. ORP was monitored at delivered doses of 1, 3 and 5 mg/L ClO_2, 10, 25 and 40 °C water temperature, and varying turbidity (0, 40 and 160 NTU). As main results, inverse correlations between water turbidity, temperature and ORP were observed. An increase in turbidity significantly reduced the final ORP and increased the contact time required for a 5-log inactivation of *S. enterica* at any assayed temperature. An increase in temperature and ClO_2 concentration reduced the contact time and achieved a 6-log reduction of *S. enterica* within a 2 min of contact time. Additionally, differences in the required contact time were determined for inactivation of seven different *S. enterica* serovars. ClO_2 was effective in achieving a 6-log reduction, or greater, of *S. enterica* under a range of water quality and temperature. However, water composition strongly affects the dynamic ORP status which can limit total inactivation of *S. enterica*. Data acquired demonstrated the impacts of water quality and temperature to maintain an effective ORP toward inactivation of *S. enterica*. The outcomes of these studies will likely be useful in reassessing the current definition of adequate water quality and safety standards where ClO_2 is used.

1. Introduction

Consumption of raw fresh tomatoes has been frequently associated with outbreaks of human salmonellosis over the past four decades (FDACS, 2006). Diverse *Salmonella enterica* serotypes have been implicated in multiple cases of illness and outbreaks, including several large multi-state episodes with hundreds of clinical cases, including Newport, Typhimurium, Javiana, Anatum, Thompson and Muenchen (CDC, 2005, 2007). In order to minimize the microbiological hazards associated with whole fresh and fresh-cut tomato products the United Fresh Produce Association (United Fresh) in collaboration with the North American Tomato Trade Work Group published the 'Commodity Specific Food Safety Guidelines for the Fresh Tomato Supply Chain' (FDACS, 2006; United Fresh, 2010). Most recently, in 2010, United Fresh posted the collaborative effort to develop *Food Safety Programs and Auditing Protocol for the Fresh Tomato Supply Chain* (Tomato Audit Protocol) which includes standards for postharvest water quality management and minimum process control criteria for antimicrobial dose (United Fresh, 2010).

Washing fresh produce with clean water can reduce potential contamination associated with the crop and non-food materials (e.g. soil, leaf trash, and other foreign materials) often co-accumulated in harvest containers. However wash and product handling water, such as in dump tanks and flumes, may also be

* Corresponding author. Tel.: +1 530 754 8313; fax: +1 530 752 4554.
E-mail address: tvsuslow@ucdavis.edu (T.V. Suslow).
[1] These authors contributed equally to this work.

0956-7135/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodcont.2011.12.016

a source of contamination or a major point of cross-contamination. Due to regional water shortage, increasing cost of assuring potable water quality for food contact, and regulated wastewater treatments and handling, reconditioning and reuse of postharvest water is a common practice and recommended by the United States Department of Agriculture (FSIS-USDA, 1999). Large volumes of re-circulated water are commonly used in packinghouses for tomato postharvest handling operations. The re-circulated water systems, including dump tanks, flumes, and re-packing systems often lead to accumulation of organic matter and can potentially become a vehicle of cross-contamination for incoming fresh product (Ongeng, Devlieghere, Debevere, Coosemans, & Ryckeboer, 2006). In particular, the wash water can easily become laden with pathogens from contaminated raw tomatoes and spread throughout the supply chain. Dump tank and flume water have received particular attention as a potential source of enteric pathogen contamination or cross-contamination during processing operations (FDACS, 2006). Left untreated, water intended to clean produce functions for a brief period early in a shift but contaminates produce later in the daily operation (Brackett, 1999). Moreover, for the specific case of the tomato postharvest handling, an inadequate washing management program may result in the infiltration of water to the interior of fruit and cause microorganism internalization (Bartz, 1982).

The addition of antimicrobial agents to recycled water can inactivate bacterial cells and fungal conidia or spores in water, helping minimize cross-contamination. As fresh market tomatoes are commonly consumed raw, disinfection in the washing and packing phase constitute an available and practical means of risk reduction. Application of sodium hypochlorite is the most common sanitizing technique used to minimize the microbiological hazards associated in the fresh plant produce industry (Artes, Gomez, Aguayo, Escalona, & Artes-Hernandez, 2009). However, some problems have long been recognized related to its use, such as potentially hazardous disinfection-by-products formation, its strong pH dependence for rapid antimicrobial action, and the potential for gas emission that may affect workers comfort and safety (Olmez & Kretzchmar, 2009). Other approved postharvest water sanitizers include peroxiacetic acid, aqueous ClO_2 and ozone. Due to the above mentioned issues, efforts to identify and evaluate alternative sanitizing agents to chlorine has become of increasing concern and priority for various industry sectors. The U.S. Food and Drug Administration (FDA) approved formulations of ClO_2 as an antimicrobial agent in water used to wash fresh fruit and vegetables in a residual amount less than 3 mg/L. Also, this treatment must be followed by a potable water rinse (CFR, 2007). Based on its molecular weight and its ability to accept electrons, ClO_2 has approximately 263% available chlorine, which is more than 2.5 times the oxidizing capacity of hypochlorous acid (Hass, 2009; Suslow, 2004).

The use of ClO_2 as a water treatment aide for minimally processed produce has been recently reviewed (Gomez-Lopez, Rajkovic, Ragaert, Smigic, & Devlieghere, 2009). However, there is limited research available about its antimicrobial effect on plant processing water and the level of restriction of cross-contamination potential. A limitation to chlorine dioxide adoption within commercial systems revolves around the fact that most research related to the evaluation of the antimicrobial effectiveness in reduction of pathogens during washing are conducted solely under laboratory scale studies, that used clean tap water or other optimal water constituent conditions, that do not anticipate the full range of industrial scenarios. In particular, the bactericidal effect of ClO_2 toward human pathogens, such as *Salmonella* has been studied in model systems that partially mimic typical commercial system conditions with variations of temperature and levels of organic matter that could affect efficiency of a given disinfectant (Junli, Li, Nanqui, & Frank, 1997). When evaluating a sanitizer, water quality parameters such as pH, temperature, turbidity, conductivity, organic matter content should be considered, in order to avoid the generation of data under unrealistic conditions with limited capacity to translate into practical applications (Gil, Selma, Lopez-Galvez, & Allende, 2009). Presence of organic loads in water can provide protection to bacteria through stabilization of the cell membranes and restricting access of a sanitizing agent to key cellular components for inactivation (Virto, Manas, Alvarez, Condon, & Raso, 2005). Microorganisms attached or embedded in particles have been shown to demonstrate an apparent increased resistance to inactivation by chlorine compared to non-attached microorganisms (Bohrerova & Linden, 2006; Dietrich, Basagaoglu, Loge, & Ginn, 2003; LeChevallier, Evans, & Seidler, 1981). Cross-contamination with *Escherichia coli* between inoculated and non inoculated fresh-cut escarole washed with different water quality was impacted by microbial and organic load present in re-circulation water (Allende, Selma, Lopez-Galvez, Villaescusa, & Gil, 2008). During produce washing, it has been demonstrated that once cross-contamination occurs, further washing with disinfectant solutions, including ClO_2, were ineffective in complete control of the attached contamination (Lopez-Galvez et al., 2010; Nou et al., 2011).

Previous assessment at different tomato packinghouses in California, by the research lead, documented fluctuations in physico-chemical water conditions including temperature, turbidity, conductivity and pH during multiple daily operational surveys. Turbidity can vary from 0 to 300 NTU, or greater, and from 0 to 50 NTU for dump tank and flume systems, respectively, during a regular processing day. Moreover, processing water temperature in dump tank and flume can reach 40 °C, or higher, to avoid water infiltration into tomatoes (Tomás-Callejas et al., 2011).

The objectives of the current work were to (1) assay the effect of ClO_2 on the survival of several *S. enterica* serotypes in fresh tomato processing water and (2) to evaluate the effect of ClO_2 concentration, water temperature and turbidity on the system control-point ORP levels under different temperatures and water quality conditions.

2. Materials and methods

2.1. Synthetic water preparation

Synthetic tomato processing water was designed to reproduce a consensus processed water composition based on previous water assessment at tomato packinghouses (Tomás-Callejas et al., 2011). To simulate the background oxidative demand of processing water, tomato plants with adhering soil were collected from University of California (Davis, CA) research farm. Plants were submerged in water and agitated to increase the amount of solids suspended in water. Water was diluted with tap water to adjust the turbidity up to 0, 22, 43 and 160 NTU. Synthetic water was autoclaved to minimize interference with other bacteria and to facilitate enumeration of inoculated *Salmonella*.

2.2. Bacterial strains and growth conditions

Seven *S. enterica* serotypes including Poona (PTVS026), Garminara (PTVS041), Michigan (PTVS042), Enteriditis (PTVS044), Agona (PTVS043), Montevideo (PTVS045) and Newport (PTVS077) were used. An antibiotic-resistant derivative strain to rifampicin (80 mg/L) was isolated for each isolate via spontaneous mutation and used to minimize interference with other bacteria and to facilitate the detection and recovery for each serotype. *S. enterica* was grown overnight in 9 mL of tryptic soy broth (BD Diagnostics, Sparks, MD, USA) supplemented with 80 mg/L of rifampicin at 37 °C. After incubation, cultures were centrifuged at 4000 rpm for 10 min. The

pellet was re-suspended and washed twice with Butterfield's phosphate buffer (Whatman Inc., Piscataway, NJ, USA). The final cell pellet was suspended in Butterfield's phosphate buffer to make an initial cell density of approximately 10^9 CFU/mL. The final concentration was confirmed by plating on Tryptic Soy Agar (TSA) (BD Diagnostics, Sparks, MD, USA) supplemented with 80 mg/L of rifampicin (TSA-rif).

2.3. Experiment design, detection and recovery of S. enterica

A factorial combination of the following conditions was evaluated: water turbidity (0, 22, 43 and 160 NTU), water temperature (10, 25 and 40 °C), and ClO_2 concentration (1, 3 and 5 mg/L). For each condition, *S. enterica* sv. Newport cells were added to 100 mL of synthetic water to achieve a final concentration of 10^7 CFU/mL. After homogenous distribution of the inoculum, ClO_2 was added to reach 1, 3 or 5 mg/L with continuous stirring. Immediately, an aliquot of 1 mL of sample was taken and dispensed in a tube containing 9 mL of Dey/Engley (DE) neutralizing broth (BD Diagnostics, Sparks, MD, USA) supplemented with 80 mg/L of rifampicin to inactivate residual ClO_2 at the end of contact times of 5, 10, 15, 30, 45, 60, 75, 90 and 120 s. Samples were plated on TSA-rif and incubated at 37 °C for 24 h to determine the log reduction of *S. enterica* sv. Newport for each treatment interval. TSA-rif plates were also supplemented with 1 g/L of sodium pyruvate {$C_3H_3NaO_3$;(TSARP)} during preparation to facilitate resuscitation of sub-lethally injured cells (Knudsen, Yamamoto, & Harris, 2001). Additionally, tubes were incubated at 37 °C for 24 h to establish an enrichment-based presence/absence test for samples anticipated to be below the limit of detection for direct enumeration. Enrichment allowed a qualitative determination of the contact time needed for complete inactivation of *S. enterica* sv. Newport. These experiments were compared to a standard disinfection using NaClO at 3 concentration levels (5, 25 and 50 mg/L; pH = 7) at 25 °C. For the remaining serotypes, an enrichment-based qualitative test, as described above, was used. All the turbidity and ClO_2 conditions were tested at 25 °C. All analyses were made in triplicate.

2.4. Physicochemical analysis

Changes in the ORP (mV), pH, and residual ClO_2 concentration (mg/L) were monitored in a separate test without pathogen inoculation, for biosafety considerations, and using the same factorial experimental design (water temperature = 10, 25 and 40 °C; Turbidity: 0, 22, 43 and 160 NTU). The ORP, pH and residual ClO_2 concentration were measured in a system containing 100 mL of synthetic water and after the addition of ClO_2 for 2 min as described above. All physicochemical parameters were determined using standard protocols. Specifications of the instruments used are listed below: portable pH/temperature meter (Russell RL060P, Thermo Fisher Scientific Inc., Waltham, MA, USA) for temperature and pH; ORP-meter (Thermo Fisher Scientific Inc., Waltham, MA, USA) for ORP; portable colorimeters for turbidity (DR/850, Hach Company, Loveland, CO, USA) and ClO_2 residual (Pocket Colorimeter™ II, Hach Company, Loveland, CO, USA). The samples regarding 160 NTU water were pre-filtered by using a 0.45 μm filter before measuring the ClO_2 residual to avoid interference from suspended solids and the ClO_2 colorimeter. All analyses were made in triplicate.

2.5. Statistical design

The experiment was based on a 3 × 3 × 3 factorial design: turbidity with three levels: 0, 22, 43 and 160 NTU × temperature with three levels10, 25 and 40 °C and ClO_2 concentration with three levels 1, 3 and 5 mg/L. The resulting data was analyzed using Statistical Analyses System (SAS) 9.2 (SAS Institute, Cary, NC, USA), with analysis of variance (ANOVA). A correlation matrix among all physicochemical parameters and bacterial population was constructed using CORRELATION procedure function of SAS which provided a value of Pearson correlation (R) and the p-value of the correlation. For all comparisons, a significant difference was established when $p < 0.05$.

3. Results

3.1. Effect of temperature and turbidity on S. enterica survival in water treated with different ClO_2 concentrations

Water temperature had a pronounced effect on the efficiency of ClO_2 to inactivate *S. enterica* sv. Newport. An increase in water temperature led to greater log reduction of the pathogen at all ClO_2 concentrations tested (Fig. 1). Under water conditions of 25 and 40 °C, an approximately 7-log reduction was achieved within 30 s when ClO_2 concentration was 3 or 5 mg/L (Fig. 1A and B). However at when water temperature was adjusted to 10 °C, at a concentration of ClO_2 of 1 mg/L, only a 4-log reduction of *Salmonella* population was achieved (Fig. 1C). In contrast to the temperature effect, and increment in water turbidity limits the inactivation of *Salmonella* by chlorine dioxide (Fig. 2). This tendency was more evident for 1 and 3 mg/L ClO_2 (Fig. 2 B and C), however when the ClO_2 concentration was 5 mg/L, the log reduction was not significantly different regardless of turbidity conditions within the test system. Analysis of variance evidenced not only a significant effect of the temperature, turbidity and ClO_2 concentration on the population of *Salmonella*, but also a significant interaction between turbidity and ClO2 dose as well as the water turbidity and temperature (Table 1).

3.2. Effect of temperature and turbidity on the contact time needed for inactivation of S. enterica in water treated with different ClO_2 concentrations

During each disinfection performance assessment, temporal sample aliquots were further enriched to determine total inactivation of *S. enterica* within a 2 min period (Table 2). As in the enumerative survival experiments, contact times for inactivation were of shorter periodicity at higher temperature and ClO_2 concentration but were extended as larger values of water turbidity were introduced (Table 2). For water turbidity of 160 NTU, 1 and 3 mg/L ClO_2 were not effective in inactivating *S. enterica* sv. Newport regardless of the water temperature. A 5 mg ClO_2/L concentration was sufficient to inactivate *S. enterica* in water (160 NTU) at 25 and 40 °C after 75 and 120 s, respectively. For water temperature of 10 °C, ClO_2 levels of 1 and 3 mg/L were unable to completely inactivate freely suspended *Salmonella* cells (Table 2).

Comparisons between ClO_2 and NaClO, for their ability to inactivate *S. enterica* cells were determined. For NaClO, shorter contact times were needed to inactivate approximately 6-log CFU/mL of *Salmonella* in a range of 5–50 mg/L at 25 °C. When NaClO was utilized as disinfectant, *S. enterica* populations dropped within the first 5 s to a recoverable population below the limit of detection, except when water turbidity increased to 160 NTU (Table 3).

3.3. Comparison of the susceptibility of different S. enterica strains to ClO_2 under different conditions of water temperature and turbidity

In general, heterogeneous inactivation times for the different serotypes regarding turbidity and ClO_2 concentration were observed (Table 4). A ClO_2 dose of 1 mg/L was not able to inactivate

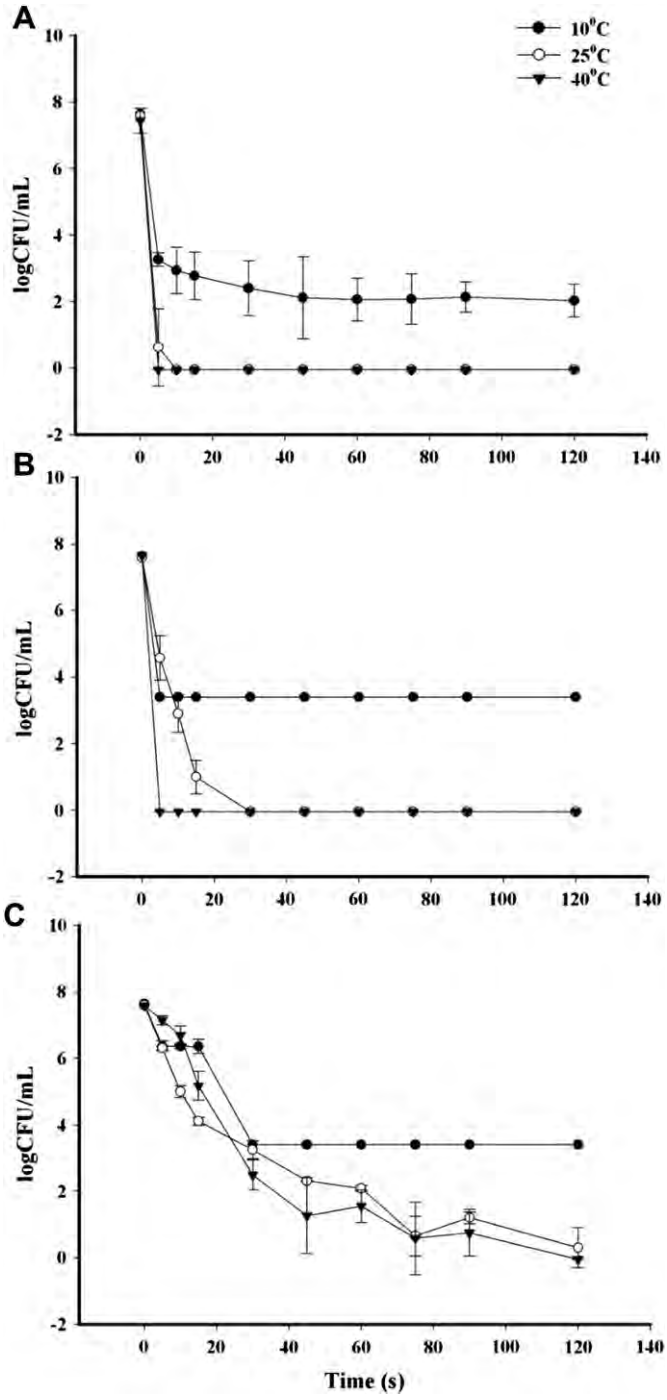

Fig. 1. Effect of temperature on the survival of *S. enterica* by sanitizing with (A) 5 mg/L, (B) 3 mg/L, (C) 1 mg/L ClO_2 within 2 min of contact time at 43 NTU water turbidity. Error bars represent standard deviation of the mean of three independent replicates.

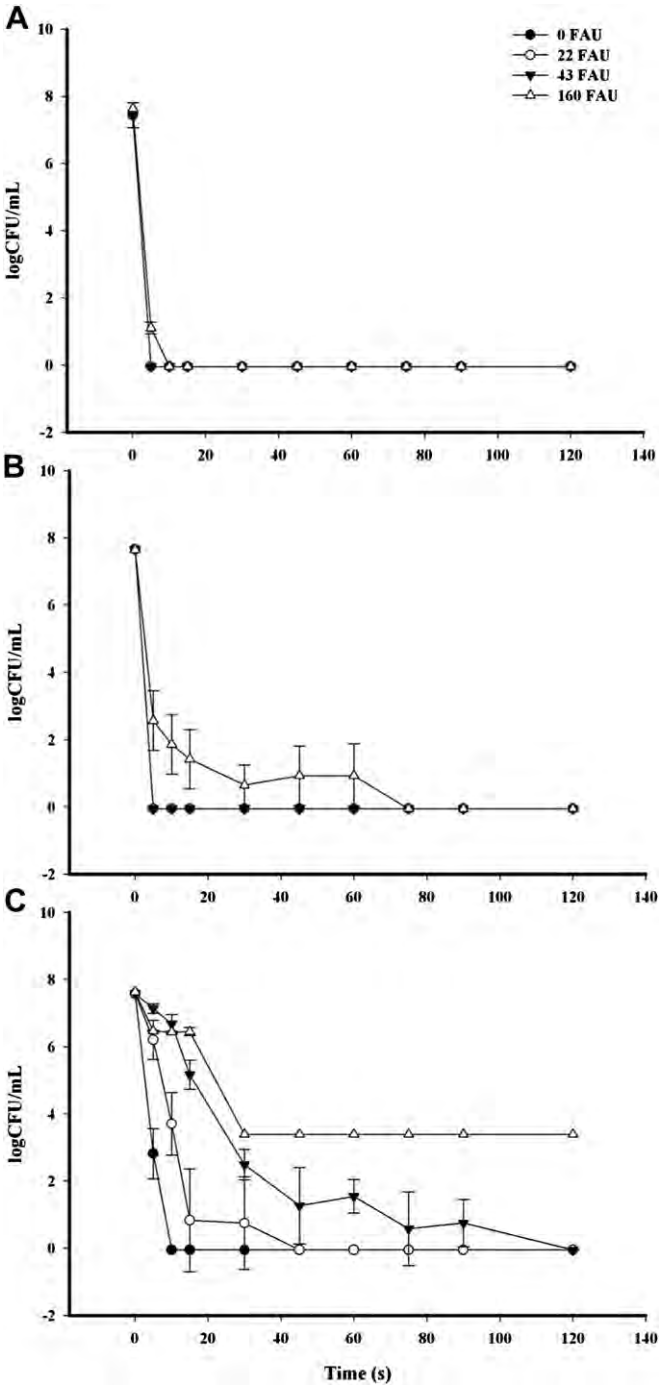

Fig. 2. Effect of turbidity on the survival of *S. enterica* by sanitizing with (A) 5 mg/L, (B) 3 mg/L, (C) 1 mg/L ClO_2 within 2 min of contact time at 40 °C water temperature. Error bars represent standard deviation of the mean of three independent replicates.

any strain within 120 s regardless of the water turbidity. A ClO_2 concentration of 3 mg/L effectively inactivated the serotypes Newport, Gaminara, Poona and Enteriditis after 30, 60, 75 and 120 s, respectively, for a turbidity value of 43 NTU, but it was unable to inactivate *Salmonella* Montevideo and Michigan serotypes. However, 3 mg/L ClO_2 was not able to inactivate any *Salmonella* serotype at 160 NTU. Differences among *Salmonella* serotypes, turbidity and ClO_2 concentrations on the inactivation time were found. All serotypes were inactivated in timeframes less than 120 s by using 5 mg/L ClO_2 at the 43 NTU condition. However, at 160 NTU, only the Newport serotype was inactivated within 120 s. Consequently *Salmonella* serotypes could be ranked according to their resistance to the ClO_2 doses tested as follows: Newport $<$ $<$ Poona $<$ Enteriditis $<$ Agona $<$ Montevideo $=$ Michigan.

3.4. Effect of water turbidity, temperature and ClO_2 concentration on the oxidation reduction potential

Water temperature and turbidity in the model system affected ORP values. ClO_2 concentrations of 3 and 5 mg/L were sufficient to maintain ORP values greater than 650 mV when water turbidity was adjusted at 22 and 43 NTU (Fig. 3A and B). However, when water

Table 1
Analysis of variance of population of *S. enterica*, ORP, pH and ClO_2 residual as influenced by water temperature (*T*), turbidity (*F*) and ClO_2 dose ([ClO_2]).

Source of variation[a]	DF[b]	Population of *Salmonella*		ORP		pH		ClO_2 residual	
		F value	*p*-value	*F* value	*p*-value	*F* value	*p*-value	*F* value	*p*-value
Main effects									
T	2	14.8	<0.0001	6.1	0.0024	0.8	0.4241	0.7	0.5216
F	2	5.8	0.0006	212.1	<0.0001	1.5	0.2281	1.2	0.3118
[ClO_2]	2	45.4	<0.001	78.9	<0.0001	0.8	0.4591	2.1	0.1299
Two-way interactions									
$T \times F$	4	6.9	<0.0001	2.5	0.0392	0.9	0.4734	0.7	0.5987
$T \times$ [ClO_2]	4	1.7	0.1488	0.2	0.9364	0.9	0.4866	0.7	0.5797
$F \times$ [ClO_2]	4	2.9	0.008	16.5	<0.0001	0.9	0.4430	0.8	0.5560
Three way interactions									
$T \times F \times$ [ClO_2]	8	1.1	0.3392	0.7	<0.6930	0.9	0.4883	0.8	0.6211

[a] Variable levels: (*T*) Temperature (10, 25 and 40 °C), (*F*) Turbidity (22, 43 and 160 NTU), [ClO_2] chlorine dioxide dose (1, 3 and 5 mg/L).
[b] (DF) Degrees of freedom.

turbidity was 160 NTU, ClO_2 failed to maintain ORP values larger than 500 mV. When 1 mg/L ClO_2 was used at 22 NTU water turbidity, ORP values greater than 650 mV were achieved. Nevertheless, for water adjusted to 43 or 160 NTU, ORP tended to decrease or failed to achieve an oxidative status greater than 300 mV (Fig. 3C).

Under conditions held at 5 mg/L ClO_2 and 160 NTU turbidity, ORP was observed to achieve values greater than 650 mV within 10 s. However, ORP tended to decrease when the water temperature was 25 or 40 °C, but remained relatively constant when temperature was held at 10 °C (data not shown). For 1 and 3 mg/L ClO_2 ORP values were not significantly different whichever water temperature was tested, reaching values lower than 600 mV (data not shown). For water turbidity of 22 and 43 NTU, ORP values in excess of 650 mV were reached regardless temperature conditions (data not shown).

Residual ClO_2 concentrations were monitored after its addition during 2 min. For the three ClO_2 concentrations assayed, after 5 s of their addition a lowering in the residual ClO_2 concentration was documented. Lower loses of ClO_2 were observed at 10 °C than at 40 °C as well as at 22 NTU than at 160 NTU (Table 5).

From the statistical analysis on the effect of the main factors studied in the model system, it was shown that ORP was significantly affected ($p < 0.05$) by water temperature, turbidity and ClO_2 concentration (Table 1). Interactions among factors were significant between turbidity and both, temperature and ClO_2 concentration, but not between temperature and ClO_2 concentration (Table 1). Changes in pH and residual ClO_2 throughout time were monitored, but no significant effect associated to different conditions of temperature, turbidity or ClO_2 concentration was found (Table 1).

3.5. Relationship between water physicochemical parameters and inactivation of S. enterica

Pearson correlation (*R*) among all physicochemical parameters and the inactivation of *S. enterica* was determined (Table 6). Population of *S. enterica* in disinfected water was negatively correlated ($p < 0.05$) to contact time, temperature, ORP and ClO_2 concentration but positively correlated to turbidity (Table 6). ORP was positively correlated to contact time, ClO_2 concentration and pH, but negatively correlated to water temperature and turbidity.

4. Discussion

Water turbidity and temperature and ClO_2 concentration showed a significant interacting effect and were well correlated with the survival potential of *S. enterica* and in the associated ORP of the simulated process water (Tables 1 and 6). Contact times of 30 s were sufficient to provide a ~7-log reduction of the pathogen in a range of 25–40 °C, 3–5 mg/L ClO_2 and water turbidity of 0–40 NTU (Figs. 1 and 2) These water quality conditions are often present in commercial flume tanks handling fresh market tomatoes. However when *S. enterica* cells were exposed to lower water temperature (10 °C), high values of water turbidity (160 NTU) or lower ClO_2 concentration (1 mg/L), the survival and the contact time needed for inactivation significantly increased and conditions were often insufficient to eliminate the pathogen (Tables 2 and 4). These findings were correlated with the ORP, where higher values of turbidity limited the ability of the water system to maintain values of ORP higher than 650 (Fig. 3), which are often specified for an adequate bactericidal effect in industry guidance standards (United Fresh, 2010).

Although the interaction between ClO_2 and organic matter does not produce toxic by-products, as occurs with NaClO, ClO_2 is able to oxidize a large fraction of natural organic matter (Swietlik & Sikorska, 2004). Thus, it is likely that oxidation of organic matter diminish its ability to increase and maintain the oxidation status of the processing water (Fig. 3). The unfavorable influences of organic matter load in terms of chemical oxygen demand (COD) for the disinfection efficacy of ClO_2 in wastewater have been reported. The inactivation of *E. coli* in artificial wastewater decreased as the COD

Table 2
Effect of ClO_2 concentration, water temperature (*T*) and turbidity on the contact time (s) needed for inactivation of 6-log of *S. enterica* sv. Newport.

Turbidity (NTU)	*T* (°C)								
	10 °C	25 °C	40 °C	10 °C	25 °C	40 °C	10 °C	25 °C	40 °C
	1 mg ClO_2/L			3 mg ClO_2/L			5 mg ClO_2/L		
0	ND	60	5	ND	10	<5	ND	10	<5
22	>120	90	45	>120	15	5	45	5	<5
43	>120	>120	90	>120	30	5	>120	5	<5
160	>120	>120	>120	>120	>120	>120	>120	120	75

Results represent the average contact time in seconds needed for the inactivation of 6-log of *S. enterica* sv. Newport.
ND: Not determined.

Table 3
Effect of NaClO concentration (mg/L) and water turbidity (FAU) on the contact time needed (s) for inactivation of *S. enterica* sv. Newport.

Water turbidity (NTU)	Concentration of NaClO (mg/L)		
	5	25	50
0	5	<5	<5
43	120	15	<5
60	>120	75	15

Results represent the average contact time in seconds needed to inactivate 6-log of *Salmonella enterica* at 25 °C ($n = 3$ independent replicates).
For all conditions plate counts were recorded. Except for 160 NTU and 5 mg/L NaClO, all treatments had counts lower than the limit of detection (0.4 log CFU/mL) after 5 s of NaClO addition.

Table 4
Comparison of the contact time needed for inactivation of 6-log among different *S. enterica* serovars under different conditions water turbidity and ClO_2 concentration at 25 °C of water temperature.

Turbidity (NTU)	*Salmonella* strain						
	Newport	Gaminara	Poona	Enteritidis	Agona	Montevideo	Michigan
1 mg ClO_2/L							
42	>120[a]	>120	>120	>120	>120	>120	>120
160	>120	>120	>120	>120	>120	>120	>120
3 mg ClO_2/L							
42	30	60	75	120	>120	>120	>120
160	>120	>120	>120	>120	>120	>120	>120
5 mg ClO_2/L							
42	5	30	60	90	90	120	120
160	120	>120	>120	>120	>120	>120	>120

[a] Results represent the average contact time in seconds needed to inactivate 6-log of *Salmonella enterica* at 25 °C ($n = 3$ independent replicates).

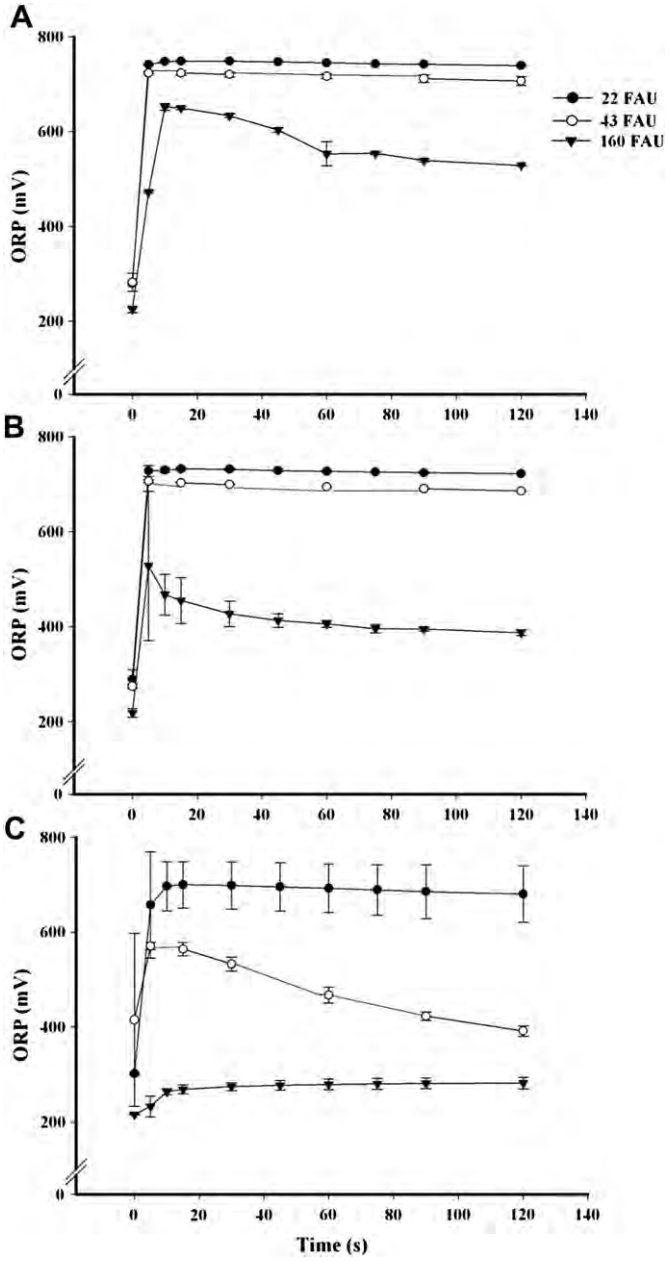

Fig. 3. Effect of turbidity on the ORP with (A) 5 mg/L, (B) 3 mg/L, (C) 1 mg/L ClO_2 within 2 min of contact time at 40 °C water temperature. Error bars represent standard deviation of the mean of three independent replicates.

increased at the same ClO_2 concentration (Ayyildiz, Ileri, & Sanik, 2009). In addition, suspended solids in solution have a protective role in limiting microorganism inactivation by ClO_2 due to entrapment of the bacteria within organic matrix or through bacterial aggregation in water, which enhances protection against diverse disinfectant reagents (Berbeau, Desjardins, Mysore, & Prevost, 2005; Narkis, Armon, Offer, & Friedland, 1994). Similar effects associated with organic load in the processing water have been reported for other water disinfection strategies such as acidic electrolyzed water, where its bactericidal effect toward *E. coli* O157:H7, *S. enterica* sv. Typhimurium and *Listeria monocytogenes* and spoilage microorganisms was reduced with an increase in water organic demand (Ongeng et al., 2006; Park, Alexander, Taylor, Costa, & Kang, 2009). It is recommended that chlorinated water should not reach turbidity values greater than 300 NTU, after which, it should be replenished with clean water (Suslow, 2004). However this study indicates that ineffectiveness of the disinfectant solution could also occur with lower levels of turbidity, and often dump tanks in fresh tomato handling industry can surpass these values. Consequently the control of presence of large amounts of organic matter and total suspended solids appears to be a key step in an adequate washing management program with ClO_2.

Temperature is an important variable impacting the activity of oxidant-based disinfectants (Berbeau et al., 2005). Elevated water temperatures and disinfectant doses and contact times generally

Table 5
Effect of water turbidity and water temperature on the residual ClO_2 concentration in modeled tomato processing water during time.

Time (s)	Initial concentration of ClO_2 (mg/L)[a]								
	1			3			5		
	T (°C)								
	10	25	40	10	25	40	10	25	40
Water turbidity 22 NTU									
5	0.67[b]	0.66	0.55	3.3	2.57	2.24	4.61	3.99	3.54
15	0.69	0.64	0.5	3.14	2.45	2.12	4.39	3.77	3.40
60	0.73	0.60	0.51	2.91	2.25	1.91	4.32	3.62	3.31
120	0.67	0.57	0.48	3.04	2.21	1.97	4.20	3.61	3.08
Water turbidity 43 NTU									
5	0.37	1.01	1.06	2.87	3.19	3.06	4.05	3.94	4.43
15	0.28	1.05	1.03	2.11	3.24	3.04	3.87	3.9	4.65
60	0.20	1.00	0.95	1.96	3.00	2.79	3.62	3.77	3.97
120	0.19	0.94	0.88	2.01	2.85	2.28	3.66	3.40	3.33
Water turbidity 160 NTU									
5	0.49	0.43	0.43	1.92	1.13	1.35	1.58	1.93	0.26
60	0.50	0.46	0.36	1.16	0.99	0.97	1.76	1.50	0.21
120	0.50	0.32	0.39	1.17	0.96	0.84	1.59	1.42	0.23

Results are the mean of the chlorine dioxide residual of 3 independent replicates.
[a] Initial ClO_2 concentration at time $t = 0$ s.
[b] Residual ClO_2 concentration (mg/L).

Table 6
Pearson correlation matrix among physicochemical parameters and inactivation of *S. enterica* with ClO_2.

	Contact time	Water temperature	Turbidity	ClO_2	*Salmonella* population	ORP	pH	ClO_2 residual
Contact time	1[a]	0	0	0	−0.529***	0.195***	−0.0612	−0.055
Water temperature		1	0	0	−0.147***	−0.111*	−0.0513	−0.07
Turbidity			1	0	0.064*	−0.609***	0.076	−0.078
ClO_2				1	−0.311***	0.370***	−0.021	0.095
Salmonella population					1	−0.603***	0.063	−0.058
ORP						1	−0.112*	0.069
pH							1	−0.008
ClO_2 residual								1

(*, **, ***) Denotes significance of the correlation at $p < 0.05$, 0.001, 0.0001 respectively.
[a] Values represent Pearson correlation (*R*).

favor the inactivation of microorganisms by ClO_2 (Berbeau et al., 2005; Son et al., 2005). The effect of temperature in this study showed that an increase in temperature reduced the contact time needed for *Salmonella* inactivation (Fig. 1). This effect was previously demonstrated for the inactivation of *E. coli* in tap water, in a range of 5–35 °C, where reduction to levels below the limit of detection varied from 35 to 7 s respectively (Benarde, Snow, Olivieri, & Davidson, 1967). In this study, at 10 °C water temperature, the ORP reached and maintained higher values than when water temperature was 25 or 40 °C, for conditions when ClO_2 dose was set at 5 mg/L (data not shown). Additionally, lower ClO_2 residual was achieved at 10 °C compared to 40 °C (Table 5). The ORP often correlates well with the antimicrobial potential of the water and, with certain dose range limitations, is directly correlated with the concentration of the oxidant (Robbs, Bartz, & Sargent, 1995). Previous studies showed that ClO_2 residual in hot water was lower than in cold water, presumptively related with faster solubilization of ClO_2 (Zhang, Stout, Yu, & Vidic, 2008). Moreover, other studies suggest that in solution, ClO_2 gas quickly equilibrate in the vessel headspace. In the case of dump tanks and open flumes, the airspace is infinite and will presumably reduce the amount of ClO_2 to zero (Mahovic & Bartz, 2002) which can be exacerbated with an increase in temperature and exposure to sunlight. It is likely that although low temperature could maintain higher ORP values by preventing ClO_2 losses, an increase in temperature can promote better dissolution of ClO_2 in water, thus increasing its oxidative action favoring inactivation of *Salmonella* cells. It is important to recognize that it is imperative to monitor ClO_2 and ORP levels, to ensure adequate disinfection, particularly in open systems as dump tanks and open flumes for tomato washing.

Simulating commercial conditions for fresh tomato processing water were considered in this study to establish the variability of the parameters evaluated. Flume tanks, based on our experience in monitoring several operations, will rarely exceed turbidity values higher than 40–50 NTU, and although temperature can affect residual levels of ClO_2 in water, results indicate that the ClO_2 was effective in reducing pathogens levels exceeding a 6-log parameter. In contrast, on-site surveillance at a tomato packinghouse established that dump tanks can reach high turbidity levels during daily operations and thus current permissible regulatory levels of ClO_2 addition might not be sufficient to successfully prevent cross-contamination of plant and human pathogens.

Efficiency of ClO_2 to disinfect dump tanks and flume systems for tomato processing water should be evaluated in a context that considers factors that can impair its effectiveness. Therefore, implementation of any water disinfection strategy should consider parameters of disinfectant concentration, water temperature, suspended solids and organic load to ensure that produce washing can act as a point of control to minimize risk of contamination with human and plant pathogens during production of fresh horticultural produce.

Acknowledgments

This research was funded by Center for Produce Safety (University of California, Davis, USA). The concession of a predoctoral grant by the Fundación Séneca de la Región de Murcia (Spain) to A. Tomás-Callejas is also appreciated. We acknowledge the technical assistance of L.A. Richmond, P. Wei, A. Camacho, T. Vo, and H. Abbott. Thanks are also due to Prof. Dr. F. Artés for his critical review.

Appendix. Supplementary material

Supplementary material related to this article can be found online at doi:10.1016/j.foodcont.2011.12.016.

References

Allende, A., Selma, M. V., Lopez-Galvez, F., Villaescusa, R., & Gil, M. I. (2008). Impact of wash water quality on sensory and microbial quality, including *Escherichia coli* cross-contamination, of fresh-cut escarole. *Journal of Food Protection, 71*, 2514–2518.

Artes, F., Gomez, P., Aguayo, A., Escalona, V., & Artes-Hernandez, F. (2009). Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities. *Postharvest Biology and Technology, 51*(287–296), 287–296.

Ayyildiz, O., Ileri, B., & Sanik, S. (2009). Impacts of organic load on chlorine dioxide disinfection efficacy. *Journal of Hazardous Materials, 168*, 1092–1097.

Bartz, J. A. (1982). Infiltration of tomatoes immersed at different temperatures to different depth in suspensions of *Erwinia carotovora* subsp. *carotovora*. *Plant Disease, 66*, 302–306.

Benarde, M. A., Snow, W. B., Olivieri, V. P., & Davidson, B. (1967). Kinetics and mechanism of bacterial disinfection by chlorine dioxide. *Applied Microbiology, 15*, 257–265.

Berbeau, B., Desjardins, R., Mysore, C., & Prevost, M. (2005). Impacts of water quality on chlorine and chlorine dioxide efficacy in natural waters. *Water Research, 39*, 2024–2033.

Bohrerova, Z., & Linden, K. G. (2006). Ultraviolet and chlorine disinfection of *Mycobacterium* in waste water: effect of aggregation. *Water Environment Research, 78*, 808–814.

Brackett, R. E. (1999). Shelf stability and safety of fresh produce as influenced by sanitation and disinfection. *Journal of Food Protection, 55*, 808–814.

Center for Disease Control and Prevention (CDC). (2005). Outbreaks of *Salmonella* infections associated with eating Roma tomatoes – United states and Canada, 2004. *Morbidity and Mortality Weekly Report, 54*, 325–328.

Center for Disease Control and Prevention CDC. (2007). Multistate outbreaks of *Salmonella* infections associated with raw tomatoes eaten in restaurants – United States, 2005–2006. *Morbidity and Mortality Weekly Report, 56*, 909–911.

Code of Federeal Regulations (CFR). (2007). *Title 21. Part 173.300. Secondary direct food additives permitted in food for human consumption. Chlorine dioxide*. Washington, D.C.: US Government Printing Office.

Dietrich, J. P., Basagaoglu, H., Loge, F. J., & Ginn, T. R. (2003). Preliminary assessment of transport processes influencing the penetration of chlorine into wastewater particles and the subsequent inactivation of particle-associated organisms. *Water Research, 37*, 139–149.

Florida Department of Agriculture and Consumer Services (FDACS). (2006). *Tomato good agricultural practices (T-GAP) and tomato best management practices (T-BMP)*. Available at http://www.freshfromflorida.com/fs/TomatoBestPractices.pdf Accessed February 2010.

FSIS-USDA. (1999). *Sanitation performance standards compliance guide*.

Gil, M. I., Selma, M. V., Lopez-Galvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: problems and solutions. *International Journal of Food Microbiology, 134*, 37–45.

Gomez-Lopez, V. M., Rajkovic, A., Ragaert, P., Smigic, N., & Devlieghere, F. (2009). Chlorine dioxide for minimally processed produce preservation: a review. *Trends in Food Science and Technology, 20*, 17–26.

Hass, C. N. (2009). Disinfection. In F. W. Pontius (Ed.), *Water quality and treatment* (pp. 877–932). New York: McGraw Hill Inc.

Junli, H., Li, W., Nanqui, R., & Frank, M. (1997). Disinfection effect of chlorine dioxide on bacteria in water. *Water Research, 31*, 607–613.

Knudsen, D. M., Yamamoto, S. A., & Harris, L. J. (2001). Survival of *Salmonella* spp. and *E. coli* O157:H7 on fresh and frozen strawberries. *Journal of Food Protection, 64*, 1483–1488.

LeChevallier, M. W., Evans, T. M., & Seidler, R. J. (1981). Effect of turbidity on chlorination efficiency and bacterial persistence in drinking water. *Applied and Environmental Microbiology, 42*, 159–167.

Lopez-Galvez, F., Allende, A., Truchado, P., Martinez-Sanchez, A., Tudela, J. A., Selma, M. V., et al. (2010). Suitability of aqueous chlorine dioxide versus sodium hypochlorite for preserving quality of fresh-cut lettuce while avoiding by-product formation. *Postharvest Biology and Technology, 55*, 53–60.

Mahovic, M. J., & Bartz, J. A. (2002). Improvements in sanitation of flumes or dump tanks in packinghouses. *Proceedings of the Florida State Horticultural Society, 115*, 35–37.

Narkis, N., Armon, R., Offer, R. F. O., & Friedland, E. (1994). Effect of suspended solids on wastewater disinfection efficiency by chlorine dioxide. *Water Research, 29*, 227–236.

Nou, X., Luo, Y., Hollar, L., Yang, Y., Feng, H., Millner, P., et al. (2011). Chlorine stabilizer T-128 enhances efficacy of chlorine against cross-contamination by *E. coli* O157:H7 and *Salmonella* in fresh-cut lettuce processing. *Journal of Food Science, 76*, M218–M224.

Olmez, H., & Kretzchmar, U. (2009). Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *LWT-Food Science and Technology, 42*, 686–693.

Ongeng, D., Devlieghere, F., Debevere, J., Coosemans, J., & Ryckeboer, J. (2006). The efficacy of electrolysed oxidising water for inactivating spoilage microorganisms in process water and on minimally processed vegetables. *International Journal of Food Microbiology, 109*, 187–197.

Park, E. J., Alexander, E., Taylor, G. A., Costa, R., & Kang, D. H. (2009). The decontaminative effects of acidic electrolyzed water for *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* on green onions and tomatoes with differing organic demands. *Food Microbiology, 26*, 386–390.

Robbs, P. G., Bartz, J. A., & Sargent, S. A. (1995). Oxidation–reduction potential of chlorine solutions and their toxicity to *Erwinia carotovora* subsp. *carotovora* and *Geotrichum candidum*. *Plant Disease, 79*, 158–162.

Son, H., Cho, M., Kim, J., Oh, B., Chung, H., & Yoon, J. (2005). Enhanced disinfection efficiency of mechanically mixes oxidants with free chlorine. *Water Research, 39*, 721–727.

Suslow, T. V. (2004). *Oxidation–reduction potential (ORP) for water disinfection monitoring, control, and documentation*. Division of Agriculture and National Resources. Davis, CA: University of California.

Swietlik, J., & Sikorska, E. (2004). Application of fluorescence spectroscopy in the studies of natural organic matter fractions reactivity with chlorine dioxide and ozone. *Water Research, 38*, 3791–3799.

Tomás-Callejas, A., López-Velasco, G., Valadez, A., Sbodio, A., Artés-Hernández, F., Danyluk, M., et al. (2011). Evaluation of current operating standards for chlorine dioxide in disinfection of dump tank and flume for fresh tomatoes. *Journal of Food Protection*, . doi:10.4315/0362-028X.JFP-11-347.

United Fresh. (2010). *Food safety programs and auditing protocol for the fresh tomato supply chain*. Available at http://www.unitedfresh.org/newsviews/food_safety_resource_center/fresh_tomato_supply_chain Accessed February 2010.

Virto, R., Manas, P., Alvarez, I., Condon, S., & Raso, J. (2005). Membrane damage and microbial inactivation by chlorine in the absence and presence of a chlorine-demanding substrate. *Applied and Environmental Microbiology, 71*, 5022–5028.

Zhang, Z., Stout, J. E., Yu, V. L., & Vidic, R. (2008). Effect of pipe corrosion scales on chlorine dioxide consumption in drinking water distribution systems. *Water Research, 42*, 129–136.