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ARTICLE

Chlorine dioxide: an evaluation based on a microbial decay approach during mango packing process

María Belia Contreras-Soto[a], José Andrés Medrano-Félix[b], Benigno Valdez-Torres[a], Cristóbal Chaidez[a] and Nohelia Castro-del Campo[a]

[a]Laboratorio Nacional para la Investigación en Inocuidad Alimentaria, Centro de Investigación en Alimentación y Desarrollo, Sinaloa, Mexico; [b]CONACYT-Centro de Investigación en Alimentación y Desarrollo, Sinaloa, Mexico

ABSTRACT

Mango is highly consumed worldwide; nonetheless, its consumption has been related to foodborne outbreaks. This study was performed to evaluate bacterial transference during mango postharvest management and the feasibility of adopting chlorine dioxide as first choice disinfectant in mango packinghouse. Chlorine dioxide (3 and 5 ppm) and sodium hypochlorite (100 and 200 ppm) were evaluated at different turbidity and times against *Salmonella* Choleraesuis and *Listeria monocytogenes*. Bacterial transference was higher from water to fruit than vice-versa (49.17%). Chlorine dioxide (5 ppm) achieved the highest *Salmonella* reductions at low turbidity reaching 2.13 Log_{10} at 10 min; meanwhile, *Listeria* was totally reduced in all conditions. Bacterial decay kinetic showed that chlorine dioxide 5 ppm was 34-fold faster than sodium hypochlorite at 200 ppm in reducing 1 Log_{10} of *Salmonella*. Chlorine dioxide reached faster bacterial inactivation decay over sodium hypochlorite; its usage is safe and meets the regulatory standards set for mango processing.

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Mango; *Salmonella* Choleraesuis; *Listeria monocytogenes*; chlorine dioxide; microbial decay

Introduction

Mango is one of the most consumed fruits worldwide, dealing in important profits for producer countries (Sivakumar et al. 2011; Evans 2017). Nonetheless, in recent years, mango has been related to *Salmonella* outbreaks involving Newport, Saintpaul and Braenderup serotypes (Sivapalasingam et al. 2003; Beatty et al. 2004; CDC 2012), while the presence of *Listeria monocytogenes* has been detected on its surface (FDA 2014). Bacterial mango contamination may occur right at the hydrotreatment processes, which consists of three processes: (1) a short-term (minor to 2 min) first wash in a reception tank in order to remove foreign particles from the field; (2) hydrothermal treatment where mangoes are immersed in hot water at 46.1°C, following the USDA-APHIS protocols to ensure fruit fly control; (3) hydro-cooling process at 21°C for 30 min allows a rapid decrease in the temperature of the mango pulp, decelerating warming-respiratory activity (Sivapalasingam et al. 2003; Bordini et al. 2007; Soto et al. 2007; USDA-APHIS 2019). The use of hot and cool water is meant to avoid the presence of anthracnose and the fruit larvae, two non-permitted infestation in mango importers countries (USA and Japan) (Penteado et al. 2004). However, differentials in water/fruit temperature might facilitate bacterial internalization into the mango pulp, including *Salmonella*. After a mango-related multistate Salmonellosis outbreak, Sivapalasingam et al. (2003) reported hot water treatment as a possible cause of contamination; several factors were taken into account, such as

CONTACT Nohelia Castro-del Campo ncastro@ciad.mx Food Safety, Centro de Investigación en Alimentación y Desarrollo, Carr. a Eldorado km. 5.5, Campo El diez, Culiacan, 80110 Mexico

the reuse of water, which tend to the accumulation of organic matter, affecting negatively the adequate chemical disinfection.

As product of the high volumes of water used and the scarcity of this liquid, it is difficult to consider as viable the total substitution of water used for mango processing, which favours organic matter accumulation. For that reason, it is necessary to evaluate alternatives for achieving the best disinfection and diminishing bacterial transference possibilities required to avoid surface or internal mango contamination (USDA-APHIS 2019). In this regard, water disinfection using sodium/calcium hypochlorite has been the most commonly used practice worldwide to reduce microbial load, given its low cost, ease to use and wide-spectrum action (USDA-APHIS 2015).

Nonetheless, the efficacy of chlorine depends on some factors such as pH, time, temperature and concentration as well as organic matter demand, which in turn may result in the formation of trihalomethanes (THMs), as product of this interaction. THMs can be absorbed and represent a potential risk to consumers' health (WHO 2005; Ongeng et al. 2006; López-Velasco et al. 2012; Coroneo et al. 2017).

In order to solve this problematic, the search for disinfection alternatives has directed into considering new products. Chlorine dioxide is a powerful oxidizing agent approved by the United States Food and Drug Administration for the elimination of microbial pathogens on fresh produce (FDA, 2018) and unlike sodium hypochlorite, it is less affected by abiotic factors like pH, water temperature and turbidity (Dychala 1991; Han et al. 2001; Mahmoud et al. 2007, 2008; López-Cuevas et al. 2017).

This study was performed to evaluate: (1) two-via bacterial transference rate: from mango to water and from water to mango; (2) disinfectant challenge: chlorine dioxide (3 and 5 ppm) and sodium hypochlorite (100 and 200 ppm) at two different water turbidity (2 and 50 NTU); (3) decay kinetics of *Salmonella* Choleraesuis and *Listeria monocytogenes* on the surface of artificially inoculated mangoes under simulated mango hydro-cooling process.

Materials and methods

Fruit selection and disinfection

This study was performed at the Laboratorio Nacional para la Investigación en Inocuidad Alimentaria (LANIIA) from the Centro de Investigación en Alimentación y Desarrollo A.C Culiacán station. Whole Tommy Atkins mangoes in physiological maturity without splits or surface cracks, previously disinfected with sodium hypochlorite (Cloralex® at 5.25%) at 200 ppm free chlorine, were used in this study. After disinfection, mangoes were rinsed with 1% sodium thiosulfate (Faga-Lab, México), followed by sterile water in order to ensure the absence of chlorine traces. Across every step, fruits were gently rubbed during 1 min. Finally, fruits were dried out during 60 min by placing them in a laminar flow A2 class biosafety cabinet (Labconco, USA). Disinfection process was performed to eliminate interference caused by natural occurring microbiological load on mango surface.

Disinfectant preparation

Sodium hypochlorite (Cloralex® at 5.25%) solutions were adjusted at 100 and 200 ppm, while chlorine dioxide solutions were prepared at 3 and 5 ppm as per the manufacturer´s instructions (TwinOxide™, Holland). Sodium hypochlorite (NaClO) and chlorine dioxide (ClO_2) concentrations were measured by spectrophotometry (HANNA DR 3900, USA), using DPD (N, N-diethyl-p-phenylenediamine) methods 10126 and 8021 for total and free chlorine, respectively (APHA 2012).

Inoculum preparation

Salmonella enterica subsp. *enterica* serovar Choleraesuis (ATCC 10708) and *Listeria monocytogenes* (ATCC 7644) were used in this study. The inoculum was prepared reactivating the strains contained in cryo-preservation vials stored at −80°C following manufacturer´s instructions (Microbank^{TM}, Pro Labs Diagnostics Inc. USA). Briefly, each strain was spread by plate into Xylose-Lysine-Deoxycholate agar (XLD) (BD Bioxon, México) and Palcam agar (Fluka, Switzerland) and incubated at 37°C for 24 and 48 h ± 2 h for *Salmonella* and *Listeria*, respectively. After incubation, one typical colony-forming unit (CFU) was cultured in Trypticase Soy Broth (TSB, BD Difco, USA) for *Salmonella*, and in TSB-YE (Trypticase Soy Broth and yeast extract at 0.6%) (BD Difco, USA) for *Listeria* and incubated at 37°C for 18 ± 2 h. Next, bacterial cultures were centrifuged twice at 13,800 *g* for 10 min at 4°C; supernatants were discarded and pellets were washed using phosphate buffer solution (pH 7.2 ± 0.2) (APHA, 2015). Bacterial pellets were re-suspended in phosphate buffer; bacterial concentration was determined by spread plate using XLD and Palcam agar and expressed as $CFUmL^{-1}$.

Mango inoculation

Mango inoculation was performed according to Ukuku and Sapers (2001) and Chaidez et al. (2007) with some modifications. Briefly, mangoes were placed in a 5-L plastic beaker containing the bacterial suspension (7 Log_{10} of *Salmonella* Choleraesuis or *Listeria monocytogenes*) with constant agitation during 60 min in orbital agitator (Thermo Scientific™, MA, USA). After inoculation, fruits were placed in a biosafety level II cabinet (Labconco, USA) for drying (1 h) and allowing bacterial adherence on mango. Bacterial adherence was measured as follow: inoculated fruits were randomly selected and placed in independent plastic bags containing phosphate buffer solution pH 7.2 ± 0.2 (relation 1:1 weight-volume), and gently rubbed to detach bacteria from mango surface; serial dilutions were performed and plated on selective medium. Plates were incubated at 37°C for 24 and 48 h ± 2 h for *Salmonella* and *Listeria*, respectively.

Controls (2) were used in all trials: a non-inoculated and non-treated mango, to ensure the absence of naturally occurring microbiota and *Salmonella*; an inoculated, non-disinfectant treated control was used for comparing non-treated against treated samples.

Bacterial transference rate from mango to water

Eight mangoes weighed per separate (average weight: 300–400 g) were inoculated with either *Salmonella* Choleraesuis or *Listeria monocytogenes* and five fruits were placed in a stainless steel container with 12 L of sterile distilled water at 25°C and constant agitation during 30 min (time that hydro-cooling treatment lasts). After this time, three fruits and three water samples were collected to measure bacterial concentration (by duplicate), as previously described and calculated using Equation (1).

$$TR = \frac{Log_{10}\,(acceptor)}{Log_{10}\,(donator)} \times 100 \quad (1)$$

Where:

-Log_{10} (acceptor): Bacterial Log_{10} CFU mL^{-1} of water

-Log_{10} (donator): Bacterial Log_{10} CFU mL^{-1} of mango rinsing water

Bacterial transference rate from water to mango

Transfer rate from water to fruit was determined by immersing five disinfected mangoes (weighed per separate) into a stainless steel water-bath at 25°C with 12 L of sterile distilled water and constant agitation during 30 min previously inoculated with an inoculum (8 Log_{10} mL^{-1}) of either *Salmonella* Choleraesuis or *Listeria monocytogenes*. After contact time, three fruits and three water samples were collected to measure bacterial concentration transferred to their surfaces and calculated according to Equation (2).

$$TR = \frac{Log_{10}\,(acceptor)}{Log_{10}\,(donator)} \times 100 \tag{2}$$

Where:

-Log_{10} (acceptor): Bacterial Log_{10} CFU mL^{-1} of mango rinsing water
-Log_{10} (donator): Bacterial Log_{10} CFU on mL^{-1} of water

Disinfectant challenge

For the United States, Environmental Protection Agency (US EPA 1997) the criterion to declare a disinfectant agent as effective is when it achieves at least 2 Log_{10} of the microorganism adhered to the surface of fruits and vegetables. To know disinfectants efficacy, sterile water at 25°C was placed in a water bath and turbidity was adjusted with previously sterilized mango crop soil in order to simulate 2 and 50 NTU water turbidity conditions. Turbidity was measured by spectrometry (HACH 2100p, USA). Following, disinfectants were added in order to achieve 3 or 5 ppm and 100 or 200 ppm for ClO_2 and NaClO, respectively. Immediately after this, inoculated mangoes with *Salmonella* or *Listeria* were submerged in a water bath in order to evaluate the microbicide effect of both NaClO and ClO_2 at 0, 1, 5 and 10 min. After each contact time, mangoes were collected and washed in a bag containing sterile water and 0.5 mL of neutralizing solution (BD Difco Laboratories, USA) to stop the residual activity of disinfectants after contact time. After treatment, bacterial count was measured as described before, and results were expressed as $CFUmL^{-1}$. Additionally, wash water temperatures were monitored throughout the course of the experiments. The detection limits in fruits and water were <10 $CFUmL^{-1}$ for a 1:10 dilution on the selected agar for *Salmonella* or *Listeria*.

Modelling bacterial survival curves through decay kinetics

The effectiveness of a food preservation process is assessed through kinetics of microbial death (Mafart et al. 2002; Buzrul et al. 2005). Among several models proposed for microbial survival curve, the Weibull distribution model stands out over the rest. This distribution considers biological variation of a microorganism´s population based on the resistance spectrum of that population to a lethal agent at different concentrations (Mafart et al. 2002; Couvert et al. 2005; Alzamora et al. 2008).

The decay kinetics of *Salmonella* Choleraesuis and *Listeria monocytogenes* inoculated in mango mediated by chlorine dioxide and sodium hypochlorite at different concentrations were analysed through a non-linear regression. The survival curves were fitted by the following decimal logarithm form of the Weibull model, which has two parameters α and β, shown in Equation (3).

$$Log_{10}\left(\frac{N_t}{N_0}\right) = -\frac{1}{2.303}\left(\frac{t}{\propto}\right)^{\beta} \tag{3}$$

Where:

Nt number of microorganisms at treatment time t (s or min)
N_0 number of microorganisms at treatment time 0
t time (min) at which a decrease in bacterial survival of 1 Log_{10} is achieved

α parameter representing a time unit
β shape parameter (without units)

This equation is a non-linear model used to study bacterial inactivation. If the parameter $\beta < 1$ the curve shows concave upwards, if $\beta > 1$ the curve shows concave downwards and if $\beta = 1$ is a straight line (Peleg and Penchina 2000; Van Boekel 2002; Alzamora et al. 2008).

Statistical analysis

A completely randomized three-factor design (Table 1) was established for disinfectant challenge analysis, while a non-linear regression was used to determine the Weibull model parameters. The analysis of variance (ANOVA) was performed using the MINITAB Software version 17.0 (Minitab, Inc., Minneapolis) using as a dependent variable the rate of bacterial decay. Mean comparison was performed by Tukey test, with a confidence interval of p value <0.05. All trials were developed per triplicate.

Results

Bacterial transference rate

Previous studies have established the correlation between water microbial load and fruit hydrocooling process. In the present study, the transference rate from water to fruit and from fruit to water was carried out, results for bacterial transference from water to fruit were 49.17% and 11.2% for *Salmonella* Choleraesuis and *Listeria monocytogenes*, respectively, whereas from fruit to water was 37.45% for *Salmonella* and non-detected for *Listeria* (Table 2).

Disinfectant challenge against Salmonella Choleraesuis

Results for chlorine dioxide at 5 ppm achieved the highest reductions of *Salmonella* Choleraesuis reaching 1.13, 1.87, 2.13 Log_{10} at 1, 5 and 10 min, respectively. Sodium hypochlorite at 100 ppm resulted in the lowest reductions, with values of 0.10, 0.60, 0.80 Log_{10} at 1, 5 and 10 min, respectively (Figure 1). The positive control recovery rate (time 0) ranged between 2.7 and 3 $CFUmL^{-1}$ for both disinfectants (data not shown).

Logarithmic reduction of *Salmonella* Choleraesuis showed a highly significant difference with p values of 0.000, 0.000, 0.002 and 0.013 for the effects of contact time, type of disinfectant and

Table 1. Disinfection challenge treatments.

	Contact Time (min)							
	0		1		5		10	
	Turbidity (NTU)							
Disinfectant (ppm)	2	50	2	50	2	50	2	50
NaClO 100	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
NaClO 200	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
ClO_2 3	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
ClO_2 5	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx

x: replicates of each treatment.

Table 2. Transference rates from water to fruit and fruit to water for *Salmonella* Choleraesuis and *Listeria monocytogenes*.

Microorganism	Transference rate from water to fruit (%)	Transference rate from fruit to water (%)
Salmonella Choleraesuis	49.17 ± 0.01	37.45 ± 0.13
Listeria monocytogenes	11.20 ± 0.22	ND

Values (means ±SD) of three replicates; ND Non-detected

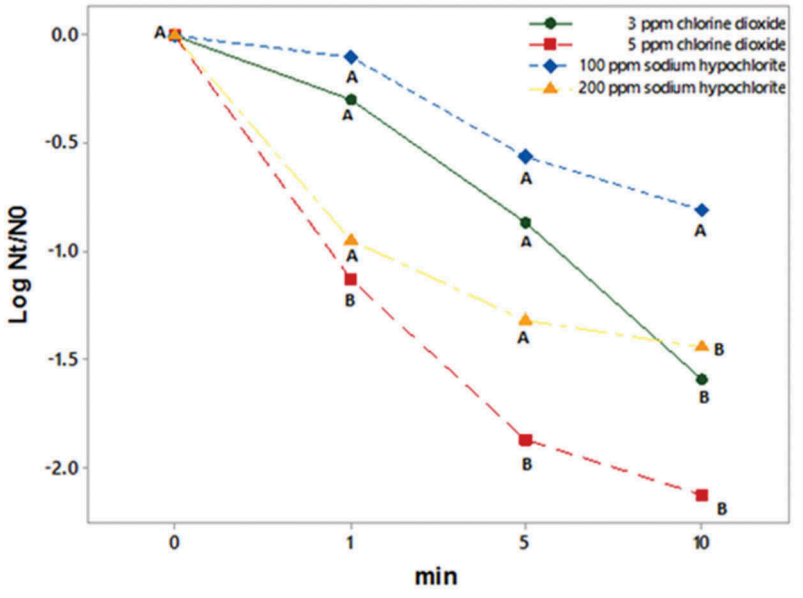

Figure 1. Reduction of *Salmonella* Choleraesuis survival. Different letters indicate significant differences ($p < 0.05$) by the Tukey test. The comparison of means is done for each contact time (columns). Mean of 3 replicates.

interaction effects, turbidity*disinfectant and time*disinfectant, respectively. No interaction effects were observed between variables turbidity and turbidity*time (p= 0.746 and 0.844, respectively) indicating the same microbial behavior in response to each test variable (Table 3). The interaction between turbidity*disinfectant showed that sodium hypochlorite at 100 ppm and 50 NTU were significantly lower ($p < 0.05$) in reducing the survival of *Salmonella* Choleraesuis (Figure 2).

Disinfectant challenge against Listeria monocytogenes

The concentration of *Listeria monocytogenes* adhered to mango surface was almost null (0.7 Log_{10}) and eliminated from the first contact time by all the disinfectants, which did not allow obtaining data at the different contact times defined to carry out a statistical analysis of the experiment nor the decay kinetic analysis.

Decay kinetic of Salmonella Choleraesuis

The best disinfectant challenge treatments were used for disinfection kinetics.

A non-linear regression analysis based on the Weibull model for *Salmonella* Choleraesuis survival using chlorine dioxide yielded the following estimated parameters: α 0.00015 and β 0.15081 (Table 4).

Table 3. Analysis of variance (ANOVA) of reduction of *Salmonella* Choleraesuis by chlorine dioxide at 3 and 5 ppm and sodium hypochlorite at 100 and 200 ppm.

Source	Degree of freedom	Sum of square	F	p-level[a]
Turbidity	1	0.2240	0.11	0.746
Time	3	30.6136	48.01	0.000
Disinfectant	3	10.7068	16.79	0.000
Turbidity*Time	3	0.1743	0.27	0.844
Turbity*Disinfectant	3	3.5071	5.50	0.002
Time*Disinfectant	9	4.8687	2.54	0.013

[a]Alpha≤0.05 significance level

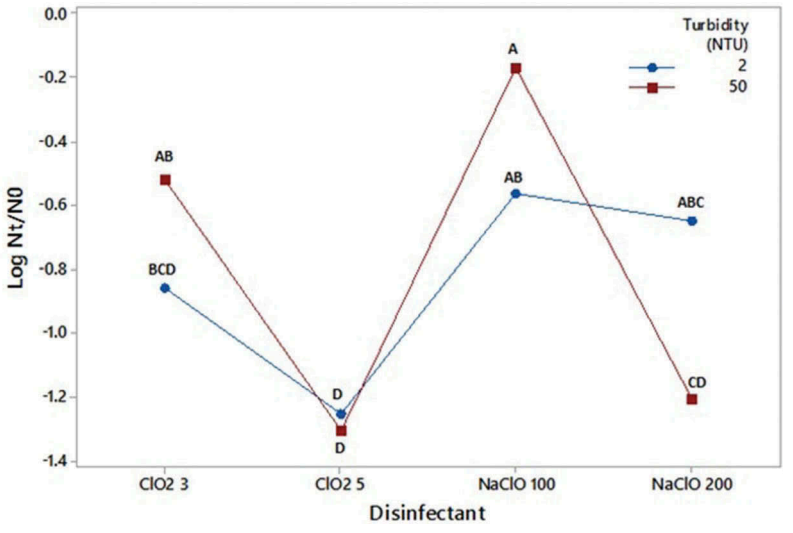

Figure 2. Interaction effects turbidity * Disinfectant for *Salmonella* Choleraesuis. Different letters indicate significant differences ($P < 0.05$) for the Tukey test. Mean of 3 replicates.

Table 4. Non-linear regression analysis based on the Weibull model for the survival of *Salmonella* Choleraesuis using chlorine dioxide at 5 ppm and sodium hypochlorite at 200 ppm.

	Parameter	Estimated value	Estimation error
Chlorine dioxide 5 ppm	α	0.00015	0.00116
	β	0.15081	0.11240
Sodium hypochlorite 200 ppm	α	0.02807	0.06028
	β	0.13521	0.05598

The lack of adjustment of the model was not significant (P = 0.844) (Table 5); hence, it can be stated that the estimated Weibull model is adequate. From this, the estimated prediction model is shown in Equation (4)

$$Log\left(\frac{Nt}{No}\right) = -\frac{1}{2.303} \times \left[\left(\frac{Time}{0.00015}\right)\right]^{0.15081} \quad (4)$$

Solving this equation, the time estimation at which a 1 Log_{10} decrease occurs in *Salmonella* survival by chlorine dioxide at 5 ppm is reached at 0.39 min (Figure 3).

On the other hand, the non-linear regression analysis, based on the Weibull model for the survival of *Salmonella* Choleraesuis using sodium hypochlorite, yielded the following estimated parameters: α 0.02807 and β 0.13521 (Table 4). The lack of adjustment of the model was not significant (P = 0.608) (Table 5), so it can be stated that the estimated Weibull model is adequate. The estimated prediction model is shown in Equation (5)

Table 5. ANOVA test to adjust the model using chlorine dioxide at 5 ppm and sodium hypochlorite at 200 ppm.

Disinfectant	Source	DF	SS	SM	F value	P value
Chlorine dioxide 5 ppm	Error	6	2.16071	0.36011		
	Lack of adjustment	1	0.01844	0.01844	0.04	0.844
	Pure error	5	2.14227	0.42845		
Sodium hypochlorite 200 ppm	Error	4	0.04302	0.01076		
	Lack of adjustment	1	0.00421	0.00421	0.33	0.608
	Pure error	3	2.14227	0.42845		

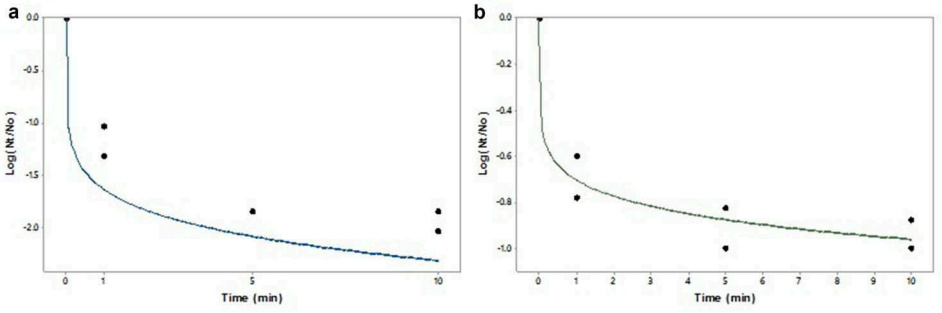

Figure 3. Chlorine dioxide at 5 ppm (a) and sodium hypochlorite at 200 ppm (b) effects on survival of *Salmonella* Choleraesuis inoculated in mango surface at 2 NTU. Curves are fitted using the Weibull model (Mafart et al. 2002).

$$Log\left(\frac{Nt}{No}\right) = -\frac{1}{2.303} \times \left[\left(\frac{Time}{0.02807}\right)\right]^{0.13521} \quad (5)$$

Solving this equation, the time estimation at which a 1 Log_{10} decrease occurs in *Salmonella* survival by sodium hypochlorite at 200 ppm is reached at 13.42 min (Figure 3).

Discussion

In this study, transference rate from water to fruit was considerable higher than from fruit to water, which concur with previous studies such as Holvoet et al. (2014), Allende et al. (2008) and Rana et al. (2010) suggesting that microbial transference occurs in a greater proportion from water to product; those studies indicate that although water is a useful tool to reduce contamination, the inadequate microbiological quality of this resource represents a vehicle for fresh product contamination. The bacterial transference may be related to bacterial characteristics, *Salmonella* as a gram-negative bacterium contains a bi-layer lipid membrane divided by a thin cell wall of peptidoglycan, additional to the presence of lipopolysaccharides that are complex polymers with fatty acid residues as lipophilic part and characteristic chains of oligosaccharides and polysaccharides that form part of the outer membrane of these bacteria (Maier et al. 2009). It is possible that *Salmonella* cell wall characteristics may influence adhesion ability, which is controlled by proteins, lipopolysaccharides and lipoteichoic acids (Maier et al. 2009) and the characteristic of fruit surface has a great importance for the adhesion process. Fernandes et al. (2014) showed that the surface of *Salmonella* is hydrophilic and the surface of the mango is also hydrophilic, suggesting that *Salmonella* should have a lower level of adhesion to mango fruit; however, the high roughness of mango surface allows bacterial adherence to this surface.

On the other hand, *Listeria* as gram-positive bacterium with one lipid membrane and a thick peptidoglycan wall, to which the negatively charged teichoic acids bind, contribute to the negative charge of the bacterial cell wall (Maier et al. 2009). Low levels of the cell membrane lipopolysaccharides in some microorganisms exhibit a reduction in surface hydrophobicity, affecting the adhesion to hydrophilic surfaces, as mango surface (Davey and O'Toole 2000). Based on the cellular structure, the low bacterial adherence to the mango surface and the low transference rate of *Listeria monocytogenes* demonstrated here may be one of the reasons why there have been no reports of *Listeria* in the mango packing plant and cases related to this product. This suggests that the chances of this bacterium being involved in a foodborne outbreak due to mango consumption are limited. Based on our results, it is necessary to perform more studies with different gram-positive bacteria to determine if the low adherence of *Listeria monocytogenes* is due to the fact that it is a gram-positive or is a characteristic of this bacterium, which leads the research to another type of studies in order to know the causes of its low adhesion capacity on this surface. According

to our results, it is also necessary to emphasize the vulnerability of hydro-cooling process as a crucial step for microbial control or bacterial transference, specifically when the knowledge about the proper use of disinfectants is limited or tools for the adequate water monitoring are limited or inexistent within packinghouses facilities.

For the United States Environmental Protection Agency (US EPA 1997) an effective disinfectant agent is the one that reduces at least 2 Log_{10} of the microorganism adhered to the surface of fruits and vegetables; therefore, chlorine dioxide at 5 ppm represents an alternative for fresh produce disinfection, based on *Salmonella* Choleraesuis reduction of 2.12 Log_{10}. Our results show that chlorine dioxide was 2.6 fold more effective than sodium hypochlorite for the reduction of *Salmonella* Choleraesuis, which makes it more effective for inactivating bacteria on mango surface.

Despite of disinfectant type and concentration are the main factors influencing the inactivation of a microorganism, it is important to consider the simultaneous effect of both factors and not by each one, since those not necessary occur individually in real scenarios. Under an ideal scenario, microbial reduction rate would be affected by complex interactions among factors present at that specific exposition time; nonetheless, the optimal range of one factor is prone to change when another factor is not optimal. Because of this, interaction results give a wide and meaningful insight on how disinfectants perform at a given condition.

Results for chlorine dioxide at 3 ppm and sodium hypochlorite at 100 ppm were more affected by turbidity than chlorine dioxide at 5 ppm and sodium hypochlorite at 200 ppm in the logarithmic reduction of *Salmonella* Choleraesuis, since there are higher free chlorinated compounds to react with the bacterial load because of the higher disinfectant concentration. In this regard, high levels of turbidity may relate to the lowering microbicide activity of chlorine dioxide at 3 ppm and sodium hypochlorite at 100 ppm. The turbidity in water is composed of inorganic (e.g. silt, clay, iron oxides) and organic (e.g. algae and carbon fines) matter as well as microbial cells (Silverman et al. 1983; LeChevallier et al. 1988). To date, the presence of organic matter around the target organisms, type of cell wall, membrane composition, bacterial growth phase, biofilm production and clump formation are likely to influence the cell adhesion characteristics and reduce the lethal effect of disinfectants (Solomon et al. 2002; Chaidez et al. 2003; Steenackers et al. 2012).

In this study, we evaluated the activity of chlorine dioxide as a disinfectant alternative to sodium hypochlorite to be used during the hydro-cooling process of mango packaging, where temperature is set at 25°C. Even though temperature during this process may not have a negative effect on disinfectants performance, it has been proved that elevated levels of this factor inflict a detrimental effect on the vast majority of chemical used to this purpose. However, López-Velasco et al. (2012) reported a faster achievement of 6 Log reduction of *S. enterica* inoculated in fresh tomato processing water through the use of ClO_2 (5 mgL^{-1}) as the water temperature increased (10, 25, 40°C), while the turbidity had little effect on bacterial reductions; an increase in temperature can promote better dissolution of ClO_2 in water, hence increasing its oxidative action favoring inactivation of *Salmonella* cells.

Several studies have shown chlorine dioxide to exceed sodium hypochlorite performance as demonstrated by several authors like Korich et al. (1990) inactivating *Cryptosporidium parvum* oocyst; Hinenoya et al. (2015) reducing various multidrug-resistant bacterial strains; and López-Cuevas et al. (2017) eliminating *Escherichia coli* O157:H7 on bell pepper surface.

Opposing results are reported by Mathew et al. (2018) whom concluded sodium hypochlorite (200 ppm) was more effective than chlorine dioxide (5 ppm) against *Salmonella enterica* in simulated hydrotreatment mango processes stating chlorine dioxide was more affected by organic matter (15–320 ppm) and water temperature (46°C). Ours and another authors' results may differ to this study due to details such as fruit inoculation as well as the ClO_2 formulation components, dealing in differences on stability of the chlorine dioxide used.

For inactivation kinetics, chlorine dioxide at 5 ppm for 1 Log_{10} reduction in *Salmonella* survival was reached at 0.39 min; meanwhile other studies focused on inactivation kinetics of *Salmonella*, such as Mahmoud et al. (2007, 2008) showing a reduction of 1 Log_{10} CFU in 2.7 min on strawberry and 1.5 min on cantaloupe when using 5 ppm of gaseous chlorine dioxide, respectively. According to our results, the

reduction of 1 Log_{10} to 0.39 min of *Salmonella* Choleraesuis on the mango surface suggests that the cause of this divergence could be the form of chlorine dioxide used, since the application of the disinfectant in aqueous form instead of gaseous, has a greater contact with the whole surface of the fruit.

The reduction in survival of 1 Log_{10} of *Salmonella* Choleraesuis at 13.42 min by sodium hypochlorite at 200 ppm shows that this disinfectant it is effective; however, its microbicide effect is lower than chlorine dioxide in reducing the microbial load adhered to the surface of the mango.

The data obtained through the Weibull model showed that the parameter β was less than 1 for all the evaluated treatments, which explains its concavity upwards (Figure 3); this means that not all bacterial cells die at the same time nor have the capacity to adapt to the applied stressful condition applied showing, in this case, more resistance to inactivation (Van Boekel 2002); for that reason, it is important to emphasize the importance of beta values in order to comprehend bacterial behavior depending on certain stimuli, hence ensuring the total elimination load during the disinfection process.

In addition to these results, it is necessary to consider that chlorine-based disinfectants are affected by parameters such as turbidity, organic matter, pH and temperature, which reduces their effectiveness; also, various parameters are taken into account when selecting a disinfectant, such as safety, ease of use, availability and cost. It is recommended to expand studies on chlorine dioxide efficiency against various pathogens in all simulated mango packaging hydro treatments.

Finally, it is important to note that the results obtained in the present study regarding the performance of a disinfectant alternative were attained based on a microbial decay kinetics through the Weibull model showing is an accurate approach for estimating bacterial inactivation curves by a given compound.

Conclusions

This study proposes aqueous chlorine dioxide as a disinfection method of *Salmonella* Choleraesuis and *Listeria monocytogenes* in the hydro-cooling process in the mango industry. *Salmonella* Choleraesuis is able to be transferred through water and adhere on fruit surface during mango handling, which might represent a high risk for consumers, contrarily to *Listeria monocytogenes* given its low adherence. Based on a microbial decay approach as well as on a simulated hydro-cooling scenario, aqueous chlorine dioxide showed to be a more effective disinfectant compared to sodium hypochlorite; nevertheless, good manufacture practices should never be neglected in order to minimize the presence of pathogens in mango packaging.

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