CENTER *for* PRODUCE SAFETY

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FINAL PROJECT REPORT

Project Title
Impact of wash water disinfectants on *Salmonella enterica* transfer and survival in mango packing facility water tank operations

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Objectives
1. *To determine the efficacy of water disinfectants to eliminate water-to-mango cross contamination with Salmonella in the presence and absence of organic matter.*
   Objective 1 will be accomplished through the following experiments:
   - *Investigating the efficacy of disinfectants to inactivate Salmonella in wash water*
   - *Investigating the efficacy of disinfectants to prevent and eliminate Salmonella transfer to mangoes from contaminated wash water*

2. *To determine the efficacy of water disinfectants on inactivating Salmonella on the surface of artificially inoculated mangoes in the presence and absence of organic matter.*
   Objective 2 will be accomplished through the following experiments:
   - *Investigating the efficacy of disinfectants to inactivate Salmonella on mangoes*
   - *Investigating the efficacy of disinfectants to prevent and eliminate Salmonella transfer from contaminated mangoes to wash water*

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FINAL REPORT

Abstract

Mangoes have been associated with foodborne outbreaks involving different serovars of *Salmonella*, including *S*. Oranienburg (1998), *S*. Newport (1999) and *S*. Saintpaul (2001 and 2003). A common theme in these outbreaks was the source of contamination which was traced back to wash water used in hydrocooling. In order to reduce mango flesh temperature following hot water treatment, mango producers practice hydrocooling. However, it has been shown that cooling of fruit following heat treatment can lead to potential pathogen internalization into mangoes. Since there are no available technologies to inactivate internalized pathogens, wash water disinfection is a critical processing step in mango production, affecting its quality, safety and shelf-life. Although several commercially available chemical disinfectants are approved for use in wash water, chlorine is the most commonly used chemical sanitizer in the fresh produce industry. Besides chlorine, other commercially used wash solutions include peracetic acid and chlorine dioxide. Although investigations on the efficacy of disinfectants to reduce pathogens on other fruits such as cantaloupe, berries, and apples have been performed, no studies have been conducted on mangoes. Hence in this study we determined the antimicrobial efficacy of water disinfectants on pathogen inactivation in presence and absence of organic matter. In order to simulate the mango packing facility water tank operations, the effect of disinfectants on *Salmonella* transfer from water-to-mango and its control on mango surface was performed in three different stages to mimic washing in the dump tank, hot water treatment and hydrocooling. Relevant results from this study have been shared with the stakeholders through the National Mango Board. The outcomes of this research will benefit the stakeholders especially the mango packing house by providing science based data needed to develop best management practices regarding post-harvest washing of mangoes thereby promoting their microbiological safety.

Background

Fresh fruits and vegetables are rich in nutrients and are recommended by the American dietetic association as part of a healthy diet (Luo et al., 2012). Although consumption of raw fresh produce is considered safe, recent produce associated illnesses have highlighted their potential for transmission of foodborne pathogens (Luo et al., 2012). Consumption of raw mangoes has led to multiple *Salmonella* outbreaks in the US. In all these outbreaks, contamination was traced traced back to the use of contaminated processing water (Hanning et al., 2009). These outbreaks have demonstrated that the quality of water used for post-harvest washing of produce is critical (Gil et al., 2009). As a condition for entry into the US, mangoes are required to receive a hot water immersion treatment as specified in the APHIS PPQ treatment manual (USDA, 2010). However, this treatment has been reported to be the cause of *Salmonella* internalization into mangoes resulting in foodborne outbreaks (Sivapalasingam et al., 2003; Penteado et al., 2004). It has been shown that hydrocooling following hot water treatment can lead to potential pathogen internalization into mangoes (Penteado et al., 2004). Currently, no effective treatment exits to eliminate internalized pathogens from mangoes. This further highlights the critical need for effective treatment of wash water to remove any *Salmonella* contaminating the outside of the fruit and to inactivate *Salmonella* that might be introduced into the wash water (Allende et al., 2009; Nieto-Montenegro, 2013).

Upon arrival at the packing house, mangoes are transferred to the dump tank for initial washing and transfer to the sizing line. Potable water is used to wash mangoes in the dump tank for 30 seconds to not more than 2 minutes (NMB, 2010). Following the initial washing, mangoes are sized for hot water treatment. The hot water treatment is done at 46.1°C and the duration of the treatment varies from 65–110 min depending on the fruit's size and shape. After hot water treatment, fruit are moved into the hydrocooling operations. This treatment helps to reduce the mango flesh temperature and reduce hot water injury. This is performed using chlorinated water maintained at 21.1°C for at least 30 minutes so as to reduce the flesh temperature to 27 to 29°C. Throughout these washing steps it is essential to maintain water quality. Water quality of the dump tank can quickly deteriorate due to accumulation of pathogens, dirt, fruit latex and agricultural chemicals from the field. It is critical to include an effective disinfectant in the dump tank to prevent pathogen carryover into the hot water and hydrocooling treatments. Additionally, the water in the hydrocooling treatment needs to be monitored regularly for free chlorine levels and the pH of the water needs to be adjusted to 7. Moreover, best management practices require the replacement of wash water on a regular basis so as to maintain water quality and sanitation (Adapted from the NMB mango post-harvest best management practices manual, 2010). Since, mango undergoes washing at three different stages at the packing facility; this proposal will evaluate the efficacy of different disinfectants on pathogen inactivation in water and on mangoes under simulated washing conditions in the laboratory.

Water is not only an ideal medium for washing produce and reducing potential contamination but can also result in pathogen transfer (Gil et al., 2009). Therefore, in the absence of practical technologies that provide a necessary kill step for pathogens, mango producers rely on wash water disinfectants to enhance product safety. Wash water quality has been a major concern for the mango industry especially with *Salmonella* outbreaks associated with consumption of raw mangoes (Allende et al., 2009; Hanning et al., 2009). Furthermore, the Food and Drug Administration has also specified the importance of maintenance of wash water quality through use of antimicrobial chemicals in processing water to minimize potential microbial contamination of wash water and subsequent cross contamination of the product (FDA, 2008). Although several commercially available chemical disinfectants are approved for use in wash water, chlorine based disinfectants including chlorine and chlorine dioxide are the most commonly used chemical disinfectant in the fresh produce industry (Shen et al., 2013; Li et al., 2001; Beuchat, 1999). This prevalent use of chlorine is due to its established antimicrobial properties, minimal effect on product quality and low cost (Lopez-Galvez et al., 2009). Although an effective antimicrobial agent, the efficacy of chlorine is quickly compromised in the presence of organic matter, as is commonly the case with routine produce washing (Shen et al., 2013; Keskinen et al., 2009; NMB, 2010). In fact, inadequately chlorinated wash water is thought to be a contributing factor to the outbreaks of *Salmonella* in mangoes (Beatty et al., 2004).Therefore, to be an effective disinfectant, chlorine needs to be frequently replenished in the wash system thus creating a technical challenge for the industry.

Peroxyacetic acid (PAA) is a strong oxidant that has been shown to be effective against foodborne pathogens on different produce surfaces (Vandekinderen et al., 2009, Hilgren et al., 2007). Commercially available peracetic acid based disinfectants contain a considerable amount of hydrogen peroxide that also exhibits antimicrobial activity. PAA are not susceptible to peroxidases and retain their activity presence of organic loads or food residues when compared to chlorine (Fatemi and Frank, 1999; Small et al., 2007; Hilgren et al., 2007). Therefore they provide an alternative to the use of chlorine and the need for frequent free chlorine measurement and replenishment. Furthermore, the use of PAA does not produce any harmful chlorinated decomposition products, the only decomposition products being acetic acid and oxygen (Monarca et al., 2002).

Although investigations on the efficacy of these disinfectants to reduce pathogens on other fruits such as cantaloupe, berries, apples have been performed (Fan et al., 2009; Magnone et al., 2013; Liao and Sapers, 2000; Mattson et al., 2011), no studies have been conducted on mangoes. In addition, produce commodities differ in their ability to support pathogen growth and there is evidence for effect of produce differences on the impact of post-harvest processing and pathogen inactivation (Hoelzer et al., 2012). Moreover, there are several unique differences in post-harvest management practices for mangoes when compared to other fruits (NMB, 2010). Therefore, this study was undertaken to determine the antimicrobial efficacy of common disinfectants including chlorine, chlorine dioxide and PAA for pathogen inactivation on mangoes and in mango wash water under simulated packing house conditions. An understanding of the efficacy of various disinfectants and the interplay of organic matter on their effectiveness in the mango post-harvest processing environment will help develop better management practices that would promote public health and benefit the mango industry.

The overall objective of this proposal was to investigate the efficacy of chlorine, (200 ppm), chlorine dioxide (5 ppm) and PAA (80 ppm) to mitigate *Salmonella* populations from wash water and mangoes during mango packing facility water tank unit operations. The specific objectives are

1. To determine the efficacy of water disinfectants to eliminate water-to-mango cross contamination with *Salmonella* in the presence and absence of organic matter.

Objective 1 was accomplished through the following experiments:

- Investigating the efficacy of disinfectants to inactivate *Salmonella* in wash water
- Investigating the efficacy of disinfectants to prevent and eliminate *Salmonella* transfer to mangoes from contaminated wash water

2. To determine the efficacy of water disinfectants on inactivating *Salmonella* on the surface of artificially inoculated mangoes in the presence and absence of organic matter

Objective 2 was accomplished through the following experiments:

- Investigating the efficacy of disinfectants to inactivate *Salmonella* on mangoes
- Investigating the efficacy of disinfectants to prevent and eliminate *Salmonella* transfer from contaminated mangoes to wash water

In order to simulate mango packing facility water tank operations, efficacy of the disinfectants for *Salmonella* control on mangoes and in wash water was tested in three different stages mimicking mango washing in the dump tank, hot water treatment and hydrocooling.

Based on our results and on further discussions with the mango board, three additional objectives were also addressed

3. To determine the efficacy of water disinfectants to reduce *Salmonella* internalization during hydrocooling of mangoes
4. To determine the effect of organic load on disinfectant concentration in mango wash water
5. To determine effect of disinfectant treatment on mango color

Research Methods and Results

Objective 1: To determine the efficacy of water disinfectants for inactivating *Salmonella* in wash water and preventing water-to-mango cross contamination in the presence and absence of organic matter

Bacterial cultures: One isolate each from five different serovars of *Salmonella enterica* (*S*. Montevideo, *S*. Poona, *S*. Newport, *S*. Baildon and *S*. Braenderup - tomato outbreak isolates) was used for the study. All the five strains were induced for resistance to nalidixic acid (NA; 50 µg/ml) to facilitate selective enumeration of the inoculated pathogens. Each strain was cultured separately in 10 ml of sterile Tryptic soy broth (TSB) containing NA (50 µg/ml) at 37°C for 24 h with agitation (100 rpm). Cultures were then transferred for two consecutive 24 h periods onto Tryptic soy agar plates containing NA (TSAN, 50 µg/ml) to produce a bacterial lawn. To prepare the inoculum, growth from the bacterial lawn was transferred to 0.1% buffered peptone water (BPW). The approximate bacterial count in each culture was determined spectrophotometrically. Equal portions from each of the five strains were combined to make a five-strain mixture of the pathogen. The cells were then sedimented by centrifugation (3600 X g for 15 min), washed twice with sterile BPW, and resuspended in 10 ml BPW. The bacterial population in the five-strain mixture was determined by plating 0.1-ml portions of appropriate dilutions on Xylose lysine deoxycholate agar (XLD) plates supplemented with NA (50 µg/ml; XLDN), followed by incubation at 37°C for 24 h. Appropriate dilutions of the five-strain mixture in BPW was used to obtain the desired level of inoculum (7 log CFU/ml of wash water).

Mangoes: Mangoes (var. Tommy Atkins and Ataulfo) were obtained through the National Mango Board from different packing houses and vendors. Upon receipt, fruits were visually inspected for defects (bruises, moldy growth, breaks in peel) and any defective mango was discarded. All fruits were maintained at 10°C with 90% humidity until use. A day before the experiment, the required number of fruits were transferred to room temperature (21°C) for tempering prior to use.

Wash solutions: Chlorine wash solution (200 ppm) was prepared by diluting 6% NaOCl (Clorox, Oakland, CA) in distilled water (22C) and adjusting the pH to 6.5 ± 0.1 with citric acid. Peroxyacetic acid wash solution (80 ppm) was prepared by diluting 35% solution (Acros Organics) in sterile water. Chlorine dioxide wash solution (5 ppm) was prepared by dissolving Potable Aqua® chlorine dioxide water purification tablets (Wisconsin Pharmacal Company) in sterile water. Sterile water (DW) was used as the negative control.

Organic load simulators: Sterilized knives were used to create nicks on whole mangoes. The mangoes were then placed in sterile filtered stomacher bags, massaged by hand for 2 minutes and vortexed for 2 minutes to obtain mango exudates for use as organic contaminants in wash water. Further in order to achieve the required level of organic contamination, clay-loam soil was also added to wash water. Organic load in all water samples was measured and confirmed using a COD meter (Chemical Oxygen Demand, Hach).

Produce wash: Wash solutions prepared as described above was inoculated with the five strain mix of *S. enterica* to yield 7 log CFU/ml.

<u>**Dump tank wash**</u> The first washing simulated the initial wash with chlorinated water in the dump tank. Mangoes var. Tommy Atkins (n=4/treatment/sampling time) were immersed in sterile

containers (22.2 x 21.1 x 22.2 cm) containing the inoculated wash solutions (5 L) held at 24°C for 2 minutes. DW was used as the control wash solution. To investigate the antimicrobial efficacy of the disinfectants, mangoes were removed from the wash solutions at 30 s, 1 and 2 min of exposure. The mangoes were individually transferred to sterile stomacher bags containing 200 ml of Dey-Engley neutralizing broth (DE broth, Difco). Each mango was hand rubbed for 1 min, and the DE broth was analyzed for presence (by enrichment) and /or *Salmonella* population. Wash water samples (10 ml, n=4) were collected immediately prior to addition of mangoes (zero minute sampling time point), to enumerate the starting bacterial concentration in wash water to account for any immediate reduction in pathogen population on exposure to the disinfectants. Additionally, at each sampling point, 10 ml of wash water (n=4/treatment/time point) was transferred to stomacher bags containing 90 ml DE broth for further microbiological analysis. The experiments were repeated with wash solutions containing organic contamination to evaluate the antimicrobial efficacy of the disinfectants in the presence of organic matter. The entire experiment was repeated twice to obtain a total of 12 mangoes/treatment/time point. A similar experimental protocol was followed for testing Ataulfos. All samples were processed for microbiological analyses as described below.

Hot water treatment To simulate the hot water treatment that is routinely performed at mango packing facilities, mangoes var. Tommy Atkins (n=4/treatment/sampling time) were immersed in containers (22.2 x 21.1 x 22.2cm) containing inoculated wash solutions (5 L) that were maintained at 46°C. The mangoes were immersed in the wash solutions containing the different disinfectants or DW and held at 46°C for 110 mins. Following immersion in the hot wash solutions, mangoes were sampled at 65, 75, 90 and 110 minutes. In addition to the mangoes, wash water samples (n=4/treatment/time point) were collected at each sampling point (0, 65, 75 and 90 min). The experiments were repeated with wash solutions containing organic contamination to evaluate the antimicrobial efficacy of the disinfectants in the presence of organic matter. The entire experiment was repeated twice to obtain a total of 12 mangoes/treatment/time point. In case of Ataulfos, mangoes were sampled at 65 and 75 minutes and wash water was sampled at 0, 65 and 75 minutes to determine the disinfectant efficacy.

Hydrocooling To simulate hydrocooling, inoculated wash solutions containing the different disinfectants or DW was maintained at 21°C. Prior to immersion in the wash solutions (5 L), mangoes var. Tommy Atkins (n=4/treatment/sampling time) were exposed to hot water treatment (sterile distilled water) for 110 min and then subjected to hydrocooling. Following hot water treatment, mangoes were held at ambient temperature before being transferred to hydrocooling containers (22.2 x 21.1 x 22.2cm) for a period of 30 minutes. Following hydrocooling, mangoes were sampled at 30 minutes. In addition to the mangoes, wash water samples (n=4/treatment/time point) were collected at each sampling point (0, 30 min). The mangoes and wash water solutions were processed as described below. The experiments were repeated with wash solutions containing organic contamination to evaluate the antimicrobial efficacy of the disinfectants in the presence of organic matter. The entire experiment was repeated twice to obtain a total of 12 mangoes/treatment/time point. In case of Ataulfos, mangoes were hot water treated for 75 minutes prior to hydrocooling for 30 minutes.

Microbiological analyses: DE broth from mangoes and wash solution samples were serially diluted in BPW and duplicate 0.1 ml of the suspensions will be surface plated on XLDN. In addition to plating, DE broth from the different samples was enriched in Rappaport-Vassiliadis broth R10 (RVB, Difco) and incubated at 43°C for 16-24 h. When counts for the respective samples were negative by direct plating, enrichment broths were streaked on XLDN and incubated at 37°C for 48 h before being examined for presumptive *S. enterica* colonies. The presumptive colonies isolated from the enrichment broths were confirmed by agglutination assays (*Salmonella* latex agglutination test, Oxoid).

Disinfectant and pH measurement: Chlorine concentration was monitored using Chlor – Test strips (MQuant™; EMD Millipore) and visual kit (Chemetrics, Inc.). Chlorine dioxide concentration was monitored using a visual kit (Chemetrics, Inc.). Peracetic acid concentration was monitored using test strips (Hydrion®; Microessential Laboratory) and a visual kit (Chemworld).Furthermore, pH of the wash solutions was monitored at each sampling point for all experiments using a portable pH meter (Thermo Fisher Scientific). Additionally, wash water temperatures were monitored throughout the course of the experiments.

Objective 2: To determine the efficacy of wash water disinfectants in inactivating *Salmonella* on the surface of contaminated mangoes and preventing mango-to-water cross contamination.

Mango inoculation: Five strain mix of *S. enterica* was prepared as described in objective 1. Mangoes were tempered at room temperature prior to inoculation. To artificially contaminate the fruits, mangoes were spot inoculated with 7 log CFU/mango by placing 50 µl of the five strain mix around the stem end. After inoculation, mangoes were held at room temperature in a biosafety hood to allow for the inoculum to dry *S. enterica* populations on mangoes from each experiment that did not undergo washing were transferred to separate sterile stomacher bags containing 200 ml of DE broth, rubbed for 1 min, serially diluted and plated to determine the baseline bacterial populations. The remaining mangoes (n=4/treatment/time point) were immersed in wash solutions containing the different disinfectants or DW as in objective 1. The experiments were performed in three different stages each simulating mango wash in the dump tank, hot water treatment and hydrocooling as described under objective 1. Following exposure to the different wash solutions, surviving *Salmonella* population were enumerated in wash water and on mango surface as described previously. Besides, bacterial enumeration, the pH, temperature and disinfectant concentrations were also be measured as described above.

Results (Objective 1 and 2):

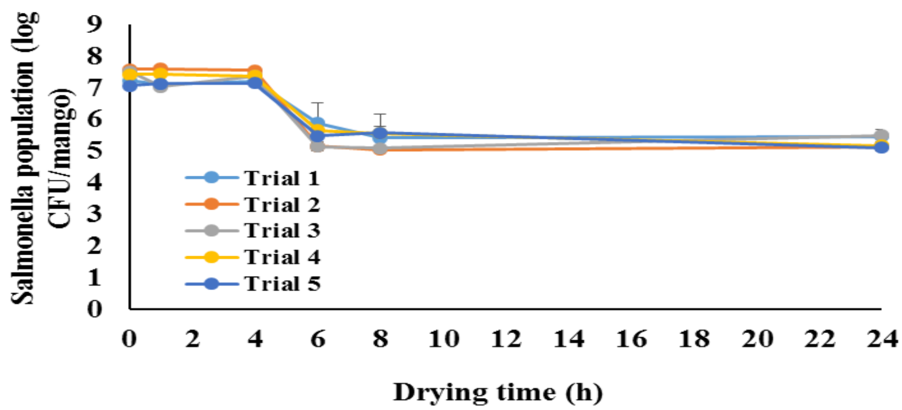
<u>Organic matter estimation in mango wash water samples collected from mango packinghouse:</u>

Water samples from the dump tank, hot water treatment and hydrocooling tank were collected during visit to a commercial mango farm. Samples were processed and chemical oxygen demand (COD) was estimated to assess organic contamination. Based on COD assays, dump tank water had the highest organic content with a COD value of 317. Hot water and hydrocooling water samples had a COD value of 30 and 17, respectively. Using these values as a guideline, experiments were performed to evaluate the effect of organic contamination on disinfectant efficacy. The organic load for the experiments was prepared by mixing sterile clay-loam soil with mango wash water (to simulate latex) in the wash tanks.

<u>Standardization of mango inoculation (spot inoculation) protocol for objective 2:</u>

Mangoes (n=15, 3/trial) were spot inoculated with 7 log CFU/mango by placing 50 µl of a five strain mix around the stem end. After inoculation, mangoes were held at room temperature in a biosafety hood for 1-24 h to allow for the inoculum to dry. Following drying, *Salmonella* populations on inoculated mangoes were enumerated at different times to ascertain initial pathogen load and optimum drying times. As seen from the result below, drying of inoculum on mango surface beyond 4 h resulted in a significantly lower pathogen recovery (from 7 log to 5 log). Hence for objective 2, mangoes will be dried for 4 h following surface inoculation (Fig. 1).

Fig. 1: Effect of inoculum drying time on *Salmonella* recovery from mango surface



Efficacy of wash water disinfectants in inactivating *Salmonella* and preventing cross contamination:

Results of our study demonstrate that fruit variety had no significant effect on disinfectant efficacy. The two varieties used in the study namely Ataulfo and Tommy were selected based on their popularity, difference in size, surface characteristics and duration of hot water treatment. As evidenced from our results, 200 ppm chlorine, 80 ppm PAA and 5 ppm Chlorine dioxide were equally effective against Ataulfo and Tommy both in the presence and absence of organic contaminants.

Dump Tank wash: With the initial washing of mangoes, water samples treated with chlorine and PAA were found to be negative for *Salmonella* (<1 CFU/25 ml of water) almost immediately upon testing. In addition, mangoes sampled 30 s after washing were also found to be negative for *Salmonella*. We hypothesize that the instantaneous kill effect observed with water samples effectively prevented the transfer of *Salmonella* from the water to the mango surface. Additionally, there was no significant effect of organic load on the antimicrobial efficacy of chlorine and PAA. However, in case of chlorine dioxide, following sample enrichment, water and mangoes were positive for *Salmonella* at the initial sampling time particularly in presence of organic contaminants. This reduced efficacy of chlorine dioxide can be attributed to the significant decrease in its residual concentration. While there was no reduction in chlorine and PAA concentrations, chlorine dioxide levels dropped drastically from 5 ppm to 2.0 ppm by the end of the experiment (2 min)

Hot water treatment: In case of the hot water treatment, Ataulfos were washed for up to 75 min and Tommys for 110 min as per the USDA APHIS requirements. Despite the difference in the duration of exposure to the disinfectants we did not observe any significant difference in the antimicrobial efficacy with reference to fruit variety. All three disinfectants were equally effective against Ataulfo and Tommy Atkins. As with the dump tank wash, chlorine and PAA were found to be more effective against *Salmonella* when compared to chlorine dioxide. Furthermore, in the presence of an organic load, water samples and mangoes treated with chlorine dioxide were found to be positive for *Salmonella* throughout the duration of the experiment, 75 min (Ataulfo) and 100 min (Tommy). This reduced antimicrobial efficacy of chlorine dioxide is due to the rapid decrease in its effective concentration from 5 ppm to 0.8 ppm by 75 and 110 min in hot water. Additionally, while chlorine concentrations remained stable, PAA concentrations fell from 80 to 60 ppm in the presence of organic contamination.

Hydrocooling: In order to simulate hydrocooling in the mango industry and to effectively evaluate the role that temperature difference [mangoes going from hot water (46°C) to hydrocooling (21°C)] has on *Salmonella* presence in mangoes, fruits used in the hydrocooling experiments were first hot water treated by washing them in sterile hot water for 75 min (Ataulfo) or 110 min (Tommys). Similar to the dump tank and hot water treatment, fruit variety was found to have no significant effect on disinfectant efficacy. Chlorine (200 ppm) and PAA (80 ppm) were found to be more effective in inactivating *Salmonella* in wash water and preventing water-to-mango cross contamination during hydrocooling. In case of chlorine dioxide, samples (water and mangoes) were found to be positive for *Salmonella* till the end of the experiment both in the presence and absence of organic matter. In addition, while chlorine and PAA remained stable, chlorine dioxide concentrations fell from 5 ppm to 2 ppm by 30 mins of hydrocooling.

Objective 3: Determine the efficacy of water disinfectants to reduce *Salmonella* internalization during hydrocooling of mangoes

Since internalization of *Salmonella* in mangoes is a result of hot water treatment immediately followed by hydrocooling, in order to comprehensively evaluate disinfectant efficacy, we performed internalization experiments. Briefly, mangoes (n=12) were subject to hot water treatment using sterile water for 75 min and then subject to hydrocooling in contaminated water (containing five strain mix of *Salmonella*, 7 log CFU/ml) with different disinfectants in the presence and absence of organic matter. Following hydrocooling, mangoes were air dried in laminar flow hood for 1 h. All mangoes were sanitized by immersion in 1 liter of 2 g/l of sodium hypochlorite solution for 1 minute and air dried. Each mango was then surface sanitized using 70% ethanol. Cuts were made on the surface of mangoes with sterile knives, transferred to stomacher bags containing DE broth and blended in a stomacher. Microbiological analysis was done as described above.

Results:

The internalization experiments revealed that immediate transfer of fruits from hot water tanks into hydrocooling containers led to significant internalization of SE into the mango pulp. With respect to the control treatment where hot water treated mangoes were washed with contaminated water, approximately 2 log CFU of *Salmonella*/mango was recovered from the pulp. This is in corroboration with other studies that have demonstrated similar levels of *Salmonella* internalization during hydrocooling. With reference to the disinfectant treatments, use of chlorine and PAA significantly inhibited internalization when compared to chlorine dioxide particularly in the presence of organic contamination. With chlorine dioxide, *Salmonella* internalization level was similar to that of control samples which were washed in sterile water. This indicates that chlorine dioxide was least effective in preventing *Salmonella* entry into the fruit.

Objective 4: Determine the effect of organic load on disinfectant concentration in mango wash water

In the course of performing objective 1 and 2, disinfectant concentrations were monitored only for the duration of each experiment. However, discussions with the mango board revealed that wash water used in the packing house can go unchanged and re-used for up to 72 h. Since disinfectant efficacy is highly dependent on its effective concentration, we monitored disinfectant concentration over a period of 3 days. Briefly, sterile water in containers (22.2 x 21.1 x 22.2cm) held at either 21-24°C (dump tank/hydrocooling tank) or 46°C (hot water tank) was treated with disinfectants (200 ppm chlorine/80 ppm PAA/5 ppm chlorine dioxide) in the presence and absence of organic matter. Following disinfectant addition, disinfectant concentrations were measured at regular intervals using the test strips and visual kits for chlorine, chlorine dioxide and PAA. Duplicate water containers were set up for each treatment and the entire experiment was repeated three times. Furthermore, at each sampling time duplicate samples were assayed for disinfectant concertation.

Results:

As with previous published research that has been performed with other fruits and vegetables, our study also demonstrated a significant reduction in effective disinfectant concentration over time. Furthermore, the drop in disinfectant concentration was significantly influenced by the organic load and temperature of the wash water. Of the three disinfectants tested, Chlorine and PAA were more stable than chlorine dioxide. With chlorine dioxide, in the presence of organic load, the concentration dropped rapidly to below detection limits (0.8 ppm) by 3 h at room temperature and within 2 min at 46°C in the hot water tank. This further emphasizes the relatively reduce applicability of chlorine dioxide at the mango packing house.

Objective 5: Determine effect of disinfectant treatment on mango color

The attractiveness and appearance of the fruit particularly its color is an important parameter that dictates consumer acceptability. Hence maintenance of an appealing color is critical to promote fruit sale. In this regard, use of strong disinfectants such as chlorine, PAA and chlorine dioxide may have an unwanted effect on fruit color and impact consumer acceptance. Having demonstrated that chlorine and PAA are effective in inactivating *Salmonella* on mangoes and in wash water, based on discussions with the mango board we also evaluated its effect on mango color. For this study, mangoes (Tommy and Ataulfo) were subjected to dumpt tank wash (2 min), hot water treatment (75/110 min) and hydrocooling (30 min) in sterile water containing the disinfectants. The mango color was read before and wash using a HunterLab MiniScan XE Plus spectrophotometer (HunterLab Associates). A total of 12 fruits/variety were sampled under each wash condition. At each time of color determination, reflectance spectra (from 400 to 700 nm, in 10 nm increments) and a* b* values were measured at three random locations on each mango. a* values are representative of the redness of the surface and b* indicate the yellowness of the mango skin.

Results

Since washing mangoes in water containing disinfectants can impact fruit color, we determined mango fruit color before and after washing. Irrespective of the water temperature and duration of washing mangoes (Ataulfo and Tommys), all three disinfectants namely chlorine, PAA and chlorine dioxide did not negatively affect the fruit color and appearance. This is critical since appearance is a critical index that affects consumer acceptability. Hence these disinfectants can be used to effectively reduce *Salmonella* contamination in mangoes without affecting fruit appearance.

Outcomes and Accomplishments

Over the last decade, mangoes have been implicated in three *Salmonella* associated foodborne outbreaks in the US. In all of these outbreaks, *Salmonella* was traced back to the wash water that was used at the packinghouse. This clearly highlights the need to identify effective wash water disinfectants that can be applied during mango washing to help reduce and eventually eliminate pathogens from the mango processing environment. In order to address this requirement, it is important to study the effect of disinfectants under conditions that are routinely encountered at the mango packinghouse. Since each produce is unique and is processed and handled based on their differing characteristics, literature on disinfectant efficacy that has been generated on other produce may not be applicable to mangoes. Hence, to address this critical need we investigated the efficacy of common wash water disinfectants in promoting the microbiological safety of mangoes under simulated packinghouse conditions. One of the major factors that influence disinfectant efficacy is organic load/contamination in the wash water. However organic contamination levels used in literature may not be a true reflection of actual loads at the mango packinghouse. Hence, in order to better simulate the mango processing conditions, we have for the first time analyzed water samples from mango packing house and established realistic levels of organic contamination that may be encountered at these facilities. Further, we have also determined the efficacy of chlorine (200 ppm), PAA (80 ppm) and chlorine dioxide (5 ppm) in inactivating *Salmonella* in the water tanks and preventing cross contamination of mangoes. Our results clearly demonstrate that chlorine and PAA were effective in eliminating *Salmonella* from wash water and also reduced transfer of *Salmonella* from water to mango surface and vice versa. Moreover, our studies also reveal that they are effective in reducing pathogen internalization following hydrocooling. This information is vital since all three mango-associated outbreaks were attributed to *Salmonella* that had internalized into intact whole fruits during hydrocooling. It is expected that results of the study will help the stake holders namely, mango producers and processing facilities to develop best management practices to reduce contamination risks of foodborne pathogens during fruit washing and promote the microbial safety of mangoes.

Summary of Findings and Recommendations

Summary:

- Dump tank has the highest level of organic load that is usually made up of latex, leaves and soil with a COD of 317 ppm.
- Hot water (30 ppm) and hydrocooling (17 ppm) tanks were found to contain lower levels of organic contamination.
- Fruit variety had no influence on the antimicrobial efficacy of the disinfectants tested.
- There are two likely scenarios for contamination of mangoes, namely i) contaminated wash water and ii) contaminated fruit. Hence we investigated both scenarios. Under both these conditions, Chlorine (200 ppm) and PAA (80 ppm) were found to be most effective in inactivating *Salmonella* in contaminated wash water and on contaminated mangoes. Furthermore, washing with chlorine and PAA prevented water-to-mango and mango-to-water cross contamination both in the presence and absence of organic matter.
- Internalization studies revealed that use of chlorine and PAA significantly reduced bacterial uptake into the fruit by greater than 7 log. However, in the presence of organic contamination, treatment with chlorine dioxide did not inhibit *Salmonella* internalization. Approximately 2 log CFU of *Salmonella*/fruit was recovered from the chlorine dioxide treated mangoes.
- Among the three disinfectants used, chlorine dioxide was found to be the least effective due to an observed loss in its effective concentration over time. In fact, specifically in the hot water tank in the presence of organic contamination, chlorine dioxide levels fell below detection limit (0.8 ppm) within 2 minutes of addition to wash water.
- Over time (72 h), there was a significant reduction in the effective concentration of all three disinfectants and this effect was more pronounced in the presence of organic contamination.
- In addition to being effective in controlling *Salmonella* in wash water and on mangoes, all three disinfectants did not adversely affect fruit color.

Recommendations:

Results from this study provide information to producers and mango processing facilities to develop better management practices with particular reference to washing of mangoes. Wash water has been shown to be a major source of *Salmonella* at the packing house. Further, contaminated mangoes either originating at the farm or along the processing line can also contribute to contamination of wash water and other fruits in the lot. We have demonstrated that in either of these scenarios use of 200 ppm chlorine or 80 ppm chlorine dioxide would help reduce the risk for contamination of the fruit. As noticed in the case of the dump tank wash, hot water treatment and hydrocooling, both these disinfectants demonstrated immediate efficacy (> 7 log reduction in *Salmonella* population as early as 30 seconds or less). Further, at these levels, chlorine and PAA also effectively prevented cross contamination and pathogen internalization. Hence for instantaneous treatment, use of sodium hypochlorite (200 ppm) and PAA would be recommended. This particularly relevant to the mango industry since chlorine or sodium hypochlorite is the most commonly employed disinfectant at the packing house. Additionally, to prevent pathogen carry over from one wash tank to the other, we endorse the inclusion of disinfectants in the hot water and hydrocooling tank besides the dump tank. Although these compounds are effective, it is important to note that their antimicrobial effect is highly dependent on their effective concentration in water. Therefore, it is highly recommended that constant monitoring and detection of the effective disinfectant concentrations and replenishment with these compounds is necessary to obtain a sustained and effective antimicrobial activity.

In summary, considering the high inoculum load that we have used in the study and the low population densities that may be routinely encountered in the field, all three tested disinfectants would be effective for wash water disinfection. Albeit the use of simulated packing house conditions, further studies would be necessary to investigate the effect of these disinfectants in large scale commercial fruit washing operations using larger tanks and fruit volume.

NMB collaboration and support: We appreciate the support from Dr. Leonardo Ortega and Wanda Ramos for helping us understand the mango production process and coordinating mango procurements throughout the study. We also thank Mr. Veny Marti, Martex farms, for hosting the PI at his farm and providing us with water samples towards organic load determination.

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APPENDICES

Publications and Presentations (required)

Meeting abstracts

1. Mathew EN and Amalaradjou MAR. 2017. Efficacy of wash water disinfectants in reducing water-to-mango contamination with Salmonella under simulated mango packing house conditions. IAFP annual meeting abstracts (submitted)

Presentations

1. Amalaradjou MAR. June 2017. Investigating the efficacy of disinfectants in inactivating *Salmonella* in wash water and preventing water to mango cross contamination, Center for Produce Safety research symposium, Denver, CO.
2. Amalaradjou MAR. June 2016. Investigating the efficacy of disinfectants in inactivating *Salmonella* in wash water and preventing water to mango cross contamination, Center for Produce Safety research symposium, Seattle, WA.
3. Amalaradjou MAR. June 2015. Impact of wash water disinfectants on Salmonella enterica transfer and survival in mango packing facility water tank operations, Center for Produce Safety research symposium, Atlanta, GA.

Publications

We anticipate submission of a paper on the efficacy of wash water disinfectants for controlling *Salmonella* during mango packing house water tank operations.

Tables and Figures (optional)

Objective 1: To determine the efficacy of wash water disinfectants to eliminate water-to-mango cross contamination with *Salmonella enterica* (SE) in the presence and absence of organic matter.

A. Dump tank wash

Table 1: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in inactivating SE in dump tank wash water (Ataulfo mangoes):

Treatment	***Salmonella* population (log CFU/ml)**							
	0 s		**30 s**		**1 min**		**2 min**	
	OM-	**OM+**	**OM-**	**OM+**	**OM-**	**OM+**	**OM-**	**OM+**
Sterile DW	7.21±0.03	7.16±0.03	7.20±0.03	7.29±0.04	7.19±0.03	7.27±0.04	7.19±0.03	7.25±0.03
Chlorine (200 ppm)	E-	E-	E-	E-	E-	E-	E-	E-
PAA (80 ppm)	E-	E-	E-	E-	E-	E-	E-	E-
Chlorine dioxide (5 ppm)	0.9 ± 0.1	0.9 ± 0.1	E-	E-	E-	E-	E-	E-

OM- : No organic contamination; OM+ : presence of organic contamination (COD 315±5 ppm)
E- : Enrichment negative, < 1 CFU/25 ml of wash water (No *Salmonella* recovered following resuscitation)

Table 2: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in inactivating SE in dump tank wash water (Tommy Atkins mangoes):

Treatment	*Salmonella* population (log CFU/ml)							
	0 s		30 s		1 min		2 min	
	OM-	OM+	OM-	OM+	OM-	OM+	OM-	OM+
Sterile DW	7.29±0.05	7.26±0.04	7.25±0.03	7.24±0.03	7.24±0.03	7.26±0.03	7.28±0.03	7.25±0.03
Chlorine (200 ppm)	E-	E-	E-	E-	E-	E-	E-	E-
PAA (80 ppm)	E-	E-	E-	E-	E-	E-	E-	E-
Chlorine dioxide (5 ppm)	0.9 ± 0.1	0.9 ± 0.1	E-	E-	E-	E-	E-	E-

OM- : no organic contamination; OM+ : presence of organic contamination (COD 315±5 ppm)
E- : Enrichment negative, < 1 CFU/25 ml of wash water (No *Salmonella* recovered following resuscitation)

Table 3: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in preventing transfer of SE from dump tank wash water to mangoes (Ataulfo mangoes):

Treatment	*Salmonella* population (log CFU/mango)					
	30 s		1 min		2 min	
	OM-	OM+	OM-	OM+	OM-	OM+
Sterile DW	7.25±0.027	7.21±0.04	7.24±0.03	7.22±0.03	7.28±0.03	7.19±0.03
Chlorine (200 ppm)	E-	E-	E-	E-	E-	E-
PAA (80 ppm)	E-	E-	E-	E-	E-	E-
Chlorine dioxide (5 ppm)	E-	0.9 ± 0.1	E-	E-	E-	E-

OM- : No organic contamination; OM+ : presence of organic contamination (COD 315±5 ppm)
E- : Enrichment negative, < 1 CFU/mango (No *Salmonella* recovered following resuscitation)

Table 4: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in preventing transfer of SE from dump tank wash water to mangoes (Tommy Atkins mangoes):

Treatment	*Salmonella* population (log CFU/mango)					
	30 s		1 min		2 min	
	OM-	OM+	OM-	OM+	OM-	OM+
Sterile DW	7.2±0.04	7.21±0.02	7.24±0.03	7.24±0.03	7.23±0.04	7.23±0.02
Chlorine (200 ppm)	E-	E-	E-	E-	E-	E-
PAA (80 ppm)	E-	E-	E-	E-	E-	E-
Chlorine dioxide (5 ppm)	E-	0.9 ± 0.1	E-	E-	E-	E-

OM- : No organic contamination; OM+ : presence of organic contamination (COD 315±5 ppm)
E- : Enrichment negative, < 1 CFU/mango (No *Salmonella* recovered following resuscitation)

B. Hot water treatment

Table 5: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in inactivating SE in hot water tank (Ataulfo mangoes):

Treatment	*Salmonella* population (log CFU/ml)					
	0 min		65 min		75 min	
	OM-	OM+	OM-	OM+	OM-	OM+
Sterile DW	7.29±0.03	7.2±0.05	7.29±0.02	7.22±0.05	7.28±0.02	7.19±0.04
Chlorine (200 ppm)	E-	0.9 ± 0.1	E-	E-	E-	E-
PAA (80 ppm)	E-	0.9 ± 0.1	E-	E-	E-	E-
Chlorine dioxide (5 ppm)	0.9 ± 0.1	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 30±5 ppm)
E- : Enrichment negative, < 1 CFU/25 ml of wash water (No *Salmonella* recovered following resuscitation)

Table 6: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in inactivating SE in hot water tank (Tommy Atkins mangoes):

Treatment	*Salmonella* population (log CFU/ml)									
	0 min		65 min		75 min		90 min		110 min	
	OM-	OM+	OM-	OM+	OM-	OM+	OM-	OM+	OM-	OM+
Sterile DW	7.38±0.04	7.3±0.05	7.37±0.05	7.28±0.04	7.26±0.09	7.28±0.04	7.22±0.09	7.28±0.06	7.2±0.09	7.26±0.05
Chlorine (200 ppm)	E-	0.9 ± 0.1	E-	E-	E-	E-	E-	E-	E-	E-
PAA (80 ppm)	E-	0.9 ± 0.1	E-	E-	E-	E-	E-	E-	E-	E-
Chlorine dioxide (5 ppm)	0.9 ± 0.1	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 30±5 ppm)
E- : Enrichment negative, < 1 CFU/25 ml of wash water (No *Salmonella* recovered following resuscitation)

Table 7: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in preventing transfer of SE from hot wash water to mangoes (Ataulfo mangoes):

Treatment	*Salmonella* population (log CFU/mango)			
	65 min		75 min	
	OM-	OM+	OM-	OM+
Sterile DW	7.25±0.025	7.14±0.04	7.23±0.024	7.12±0.04
Chlorine (200 ppm)	E-	E-	E-	E-
PAA (80 ppm)	E-	E-	E-	E-
Chlorine dioxide (5 ppm)	0.9 ± 0.1	0.9 ± 0.1	E-	0.9 ± 0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 30±5 ppm)
E- : Enrichment negative, < 1 CFU/mango (No *Salmonella* recovered following resuscitation)

Table 8: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in preventing transfer of SE from hot wash water to mangoes (Tommy Atkins mangoes):

Treatment	*Salmonella* population (log CFU/mango)							
	65 min		75 min		90 min		110 min	
	OM-	OM+	OM-	OM+	OM-	OM+	OM-	OM+
Sterile DW	7.39 ± 0.05	7.24± 0.05	7.39 ± 0.03	7.24 ± 0.04	7.38 ± 0.03	7.23 ± 0.04	7.39 ± 0.04	7.26 ± 0.04
Chlorine (200 ppm)	E-	0.9 ± 0.1	0.9 ± 0.1	E-	E-	E-	E-	E-
PAA (80 ppm)	E-	0.9 ± 0.1	E-	E-	E-	E-	E-	E-
Chlorine dioxide (5 ppm)	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 30±5 ppm)
E- : Enrichment negative, < 1 CFU/mango (No *Salmonella* recovered following resuscitation)

C. Hydrocooling

Table 9: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in inactivating SE in hydrocooling tank (Ataulfo mangoes):

Treatment	*Salmonella* population (log CFU/ml)			
	0 min		30 min	
	OM-	OM+	OM-	OM+
Sterile DW	7±0.04	7.08±0.03	6.92±0.05	7.06±0.02
Chlorine (200 ppm)	E-	0.9±0.1	E-	E-
PAA (80 ppm)	E-	0.9±0.1	E-	E-
Chlorine dioxide (5 ppm)	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 15±5 ppm)
E- : Enrichment negative, < 1 CFU/25 ml of wash water (No *Salmonella* recovered following resuscitation)

Table 10: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in inactivating SE in hydrocooling tank (Tommy Atkins mangoes):

Treatment	*Salmonella* population (log CFU/ml)			
	0 min		30 min	
	OM-	OM+	OM-	OM+
Sterile DW	7.11±0.01	7.253±0.05	7.05±0.03	7.178±0.04
Chlorine (200 ppm)	E-	0.9±0.1	E-	E-
PAA (80 ppm)	E-	0.9±0.1	E-	E-
Chlorine dioxide (5 ppm)	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 15±5 ppm)
E- : Enrichment negative, < 1 CFU/25 ml of wash water (No *Salmonella* recovered following resuscitation)

Table 11: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in preventing transfer of SE from hydrocooling water to mangoes (Ataulfo mangoes):

Treatment	*Salmonella* population (log CFU/mango)	
	30 min	
	OM-	OM+
Sterile DW	7.18±0.059	7.1±0.014
Chlorine (200 ppm)	E-	E-
PAA (80 ppm)	E-	E-
Chlorine dioxide (5 ppm)	0.9±0.1	0.9±0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 15±5 ppm)
E- : Enrichment negative, < 1 CFU/mango (No *Salmonella* recovered following resuscitation)

Table 12: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in preventing transfer of SE from hydrocooling water to mangoes (Tommy Atkins mangoes):

Treatment	*Salmonella* population (log CFU/mango)	
	30 min	
	OM-	OM+
Sterile DW	7.07±0.033	7.18±0.031
Chlorine (200 ppm)	E-	E-
PAA (80 ppm)	E-	E-
Chlorine dioxide (5 ppm)	0.9±0.1	0.9±0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 15±5 ppm)
E- : Enrichment negative, < 1 CFU/mango (No *Salmonella* recovered following resuscitation)

<u>Objective 2:</u> To determine the efficacy of water disinfectants on inactivating *Salmonella enterica* (SE) on the surface of artificially inoculated mangoes in the presence and absence of organic matter.

A. Dump tank wash

<u>Table 1: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in preventing SE transfer from Ataulfo mango surface to dump tank wash water</u>

Treatment	*Salmonella* population (log CFU/ml)							
	0 s		30 s		1 min		2 min	
	OM-	OM+	OM-	OM+	OM-	OM+	OM-	OM+
Sterile DW	6.81±0.01	7.41±0.03	6.7±0.01	7.43±0.04	6.83±0.01	7.42±0.04	6.73±0.02	7.41±0.03
Chlorine (200 ppm)	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1
PAA (80 ppm)	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1
Chlorine dioxide (5 ppm)	0.9 ± 0.1	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 315±5 ppm)
E- : Enrichment negative, < 1 CFU/25 ml of wash water (No *Salmonella* recovered following resuscitation)

Table 2: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in preventing SE transfer from Tommy Atkins mango surface to dump tank wash water

Treatment	***Salmonella* population (log CFU/ml)**							
	0 s		**30 s**		**1 min**		**2 min**	
	OM-	**OM+**	**OM-**	**OM+**	**OM-**	**OM+**	**OM-**	**OM+**
Sterile DW	6.75±0.09	7.35±0.09	6.74±0.08	7.33±0.09	6.81±0.07	7.4±0.06	6.79±0.07	7.4±0.06
Chlorine (200 ppm)	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1
PAA (80 ppm)	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1
Chlorine dioxide (5 ppm)	0.9 ± 0.1	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 315±5 ppm)
E- : Enrichment negative, < 1 CFU/25 ml of wash water (No *Salmonella* recovered following resuscitation)

Table 3: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in inactivating SE on Ataulfo mango surface during simulated dump tank washing

Treatment	*Salmonella* population (log CFU/mango)					
	30 s		1 min		2 min	
	OM-	OM+	OM-	OM+	OM-	OM+
Sterile DW	7.25±0.03	7.44±0.06	7.37±0.03	7.44±0.05	7.34±0.02	7.43±0.05
Chlorine (200 ppm)	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1
PAA (80 ppm)	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1
Chlorine dioxide (5 ppm)	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 315±5 ppm)
E- : Enrichment negative, < 1 CFU/mango (No *Salmonella* recovered following resuscitation)

Table 4: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in inactivating SE on Tommy Atkins mango surface during simulated dump tank washing

Treatment	*Salmonella* population (log CFU/mango)					
	30 s		1 min		2 min	
	OM-	OM+	OM-	OM+	OM-	OM+
Sterile DW	7.84±0.08	7.33±0.07	7.81±0.08	7.35±0.06	7.75±0.07	7.39±0.06
Chlorine (200 ppm)	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1
PAA (80 ppm)	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1
Chlorine dioxide (5 ppm)	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 315±5 ppm)
E- : Enrichment negative, < 1 CFU/mango (No *Salmonella* recovered following resuscitation)

B. Hot water treatment

Table 5: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in preventing SE transfer from Ataulfo mango surface to wash water during hot water treatment

Treatment	*Salmonella* population (log CFU/ml)					
	0 min		65 min		75 min	
	OM-	OM+	OM-	OM+	OM-	OM+
Sterile DW	7.41±0.02	7.37±0.01	7.39±0.02	7.45±0.07	7.47±0.02	7.4±0.05
Chlorine (200 ppm)	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1
PAA (80 ppm)	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1
Chlorine dioxide (5 ppm)	0.9 ± 0.1	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 30±5 ppm)
E- : Enrichment negative, < 1 CFU/25 ml of wash water (No *Salmonella* recovered following resuscitation)

Table 6: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in preventing SE transfer from Tommy Atkins mango surface to wash water during hot water treatment

Treatment	*Salmonella* population (log CFU/ml)									
	0 min		65 min		75 min		90 min		110 min	
	OM-	OM+	OM-	OM+	OM-	OM+	OM-	OM+	OM-	OM+
Sterile DW	7.36±0.08	7.51±0.07	7.32±0.08	7.56±0.06	7.31±0.07	7.56±0.04	7.27±0.07	7.51±0.04	7.45±0.07	7.42±0.05
Chlorine (200 ppm)	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1
PAA (80 ppm)	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1
Chlorine dioxide (5 ppm)	0.9 ± 0.1	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	E-	0.9 ± 0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 30±5 ppm)
E- : Enrichment negative, < 1 CFU/25 ml of wash water (No *Salmonella* recovered following resuscitation)

Table 7: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in inactivating SE on Ataulfo mango surface during simulated hot water treatment

Treatment	***Salmonella* population (log CFU/mango)**			
	65 min		**75 min**	
	OM-	**OM+**	**OM-**	**OM+**
Sterile DW	7.29±0.02	7.38±0.07	7.44±0.05	7.12±0.04
Chlorine (200 ppm)	E-	E-	E-	E-
PAA (80 ppm)	E-	E-	E-	E-
Chlorine dioxide (5 ppm)	E-	0.9 ± 0.1	E-	0.9 ± 0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 30±5 ppm)
E- : Enrichment negative, < 1 CFU/mango (No *Salmonella* recovered following resuscitation)

Table 8: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in inactivating SE on Tommy Atkins mango surface during simulated hot water treatment

Treatment	***Salmonella* population (log CFU/mango)**							
	65 min		**75 min**		**90 min**		**110 min**	
	OM-	**OM+**	**OM-**	**OM+**	**OM-**	**OM+**	**OM-**	**OM+**
Sterile DW	7.28±0.09	7.28±0.08	7.44±0.06	7.29±0.08	7.35±0.05	7.23±0.08	7.28±0.06	7.16±0.05
Chlorine (200 ppm)	E-	E-	E-	E-	E-	E-	E-	E-
PAA (80 ppm)	E-	E-	E-	E-	E-	E-	E-	E-
Chlorine dioxide (5 ppm)	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1	E-	0.9 ± 0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 30±5 ppm)
E- : Enrichment negative, < 1 CFU/mango (No *Salmonella* recovered following resuscitation)

C. Hydrocooling

Table 9: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in preventing SE transfer from Ataulfo mango surface to wash water during hydrocooling

Treatment	*Salmonella* population (log CFU/ml)			
	0 min		30 min	
	OM-	OM+	OM-	OM+
Sterile DW	7.2±0.01	7.2±0.03	7.21±0.01	7.18±0.04
Chlorine (200 ppm)	E-	E-	E-	E-
PAA (80 ppm)	E-	E-	E-	E-
Chlorine dioxide (5 ppm)	E-	0.9±0.1	E-	0.9±0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 15±5 ppm)
E- : Enrichment negative, < 1 CFU/25 ml of wash water (No *Salmonella* recovered following resuscitation)

Table 10: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in preventing SE transfer from Tommy Atkins mango surface to wash water during hydrocooling:

Treatment	*Salmonella* population (log CFU/ml)			
	0 min		30 min	
	OM-	OM+	OM-	OM+
Sterile DW	7.33±0.04	7.41±0.07	7.34±0.04	7.31±0.02
Chlorine (200 ppm)	E-	E-	E-	E-
PAA (80 ppm)	E-	E-	E-	E-
Chlorine dioxide (5 ppm)	E-	0.9±0.1	E-	0.9±0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 15±5 ppm)
E- : Enrichment negative, < 1 CFU/25 ml of wash water (No *Salmonella* recovered following resuscitation)

Table 11: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in inactivating SE on Ataulfo mango surface during simulated hydrocooling

Treatment	***Salmonella* population (log CFU/mango)**	
	30 min	
	OM-	**OM+**
Sterile DW	7.17±0.01	7.32±0.05
Chlorine (200 ppm)	E-	E-
PAA (80 ppm)	E-	E-
Chlorine dioxide (5 ppm)	E-	0.9±0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 15±5 ppm)
E- : Enrichment negative, < 1 CFU/mango (No *Salmonella* recovered following resuscitation)

Table 12: Efficacy of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in inactivating SE on Tommy Atkins mango surface during simulated hydrocooling

Treatment	***Salmonella* population (log CFU/mango)**	
	30 min	
	OM-	**OM+**
Sterile DW	7.30±0.03	7.27±0.03
Chlorine (200 ppm)	E-	E-
PAA (80 ppm)	E-	E-
Chlorine dioxide (5 ppm)	E-	0.9±0.1

OM- : No organic contamination; OM+ : presence of organic contamination (COD 15±5 ppm)
E- : Enrichment negative, < 1 CFU/mango (No *Salmonella* recovered following resuscitation)

<u>Objective 3:</u> Determine the efficacy of water disinfectants to reduce *Salmonella enterica* (SE) internalization during hydrocooling of mangoes

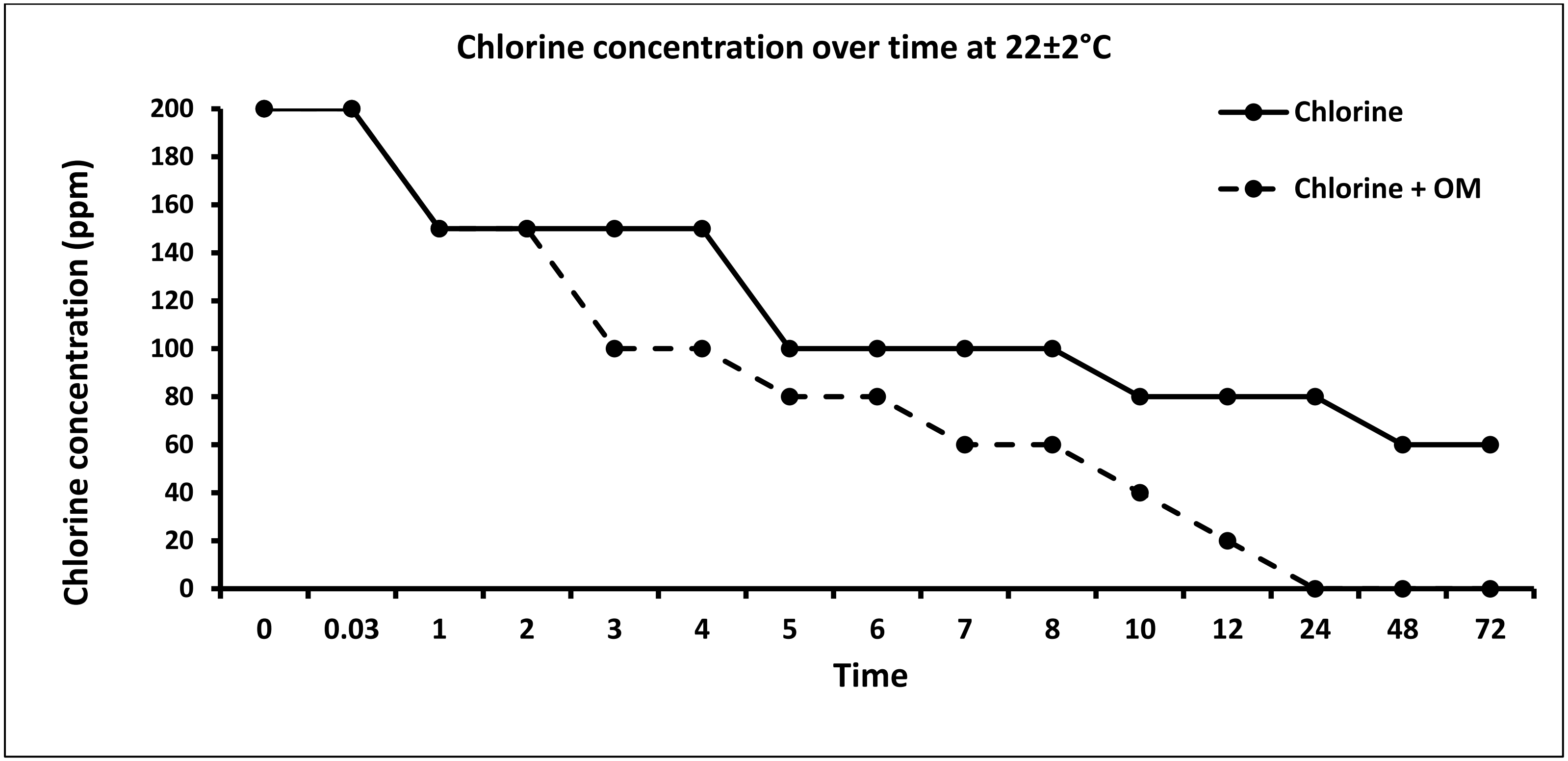
<u>Effect of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide in preventing SE internalization in mangoes following hydrocooling</u>

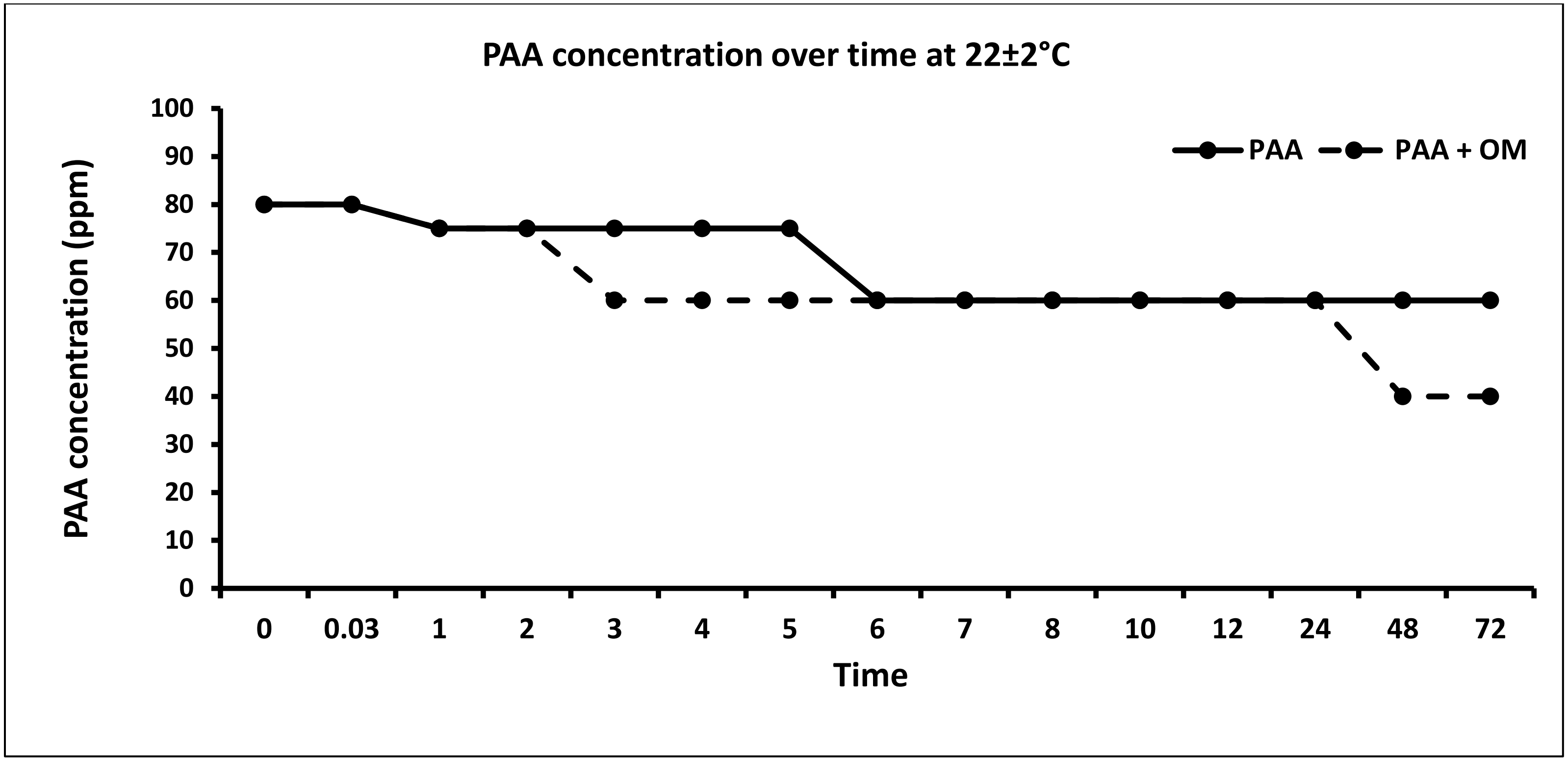
Treatment	***Salmonella* population (log CFU/mango)**	
	OM-	**OM+**
Sterile DW	2.34±0.06	2.39±0.03
Chlorine (200 ppm)	0.9±0.1	0.9±0.1
PAA (80 ppm)	0.9±0.1	0.9±0.1
Chlorine dioxide (5 ppm)	0.9±0.1	2.27±0.05

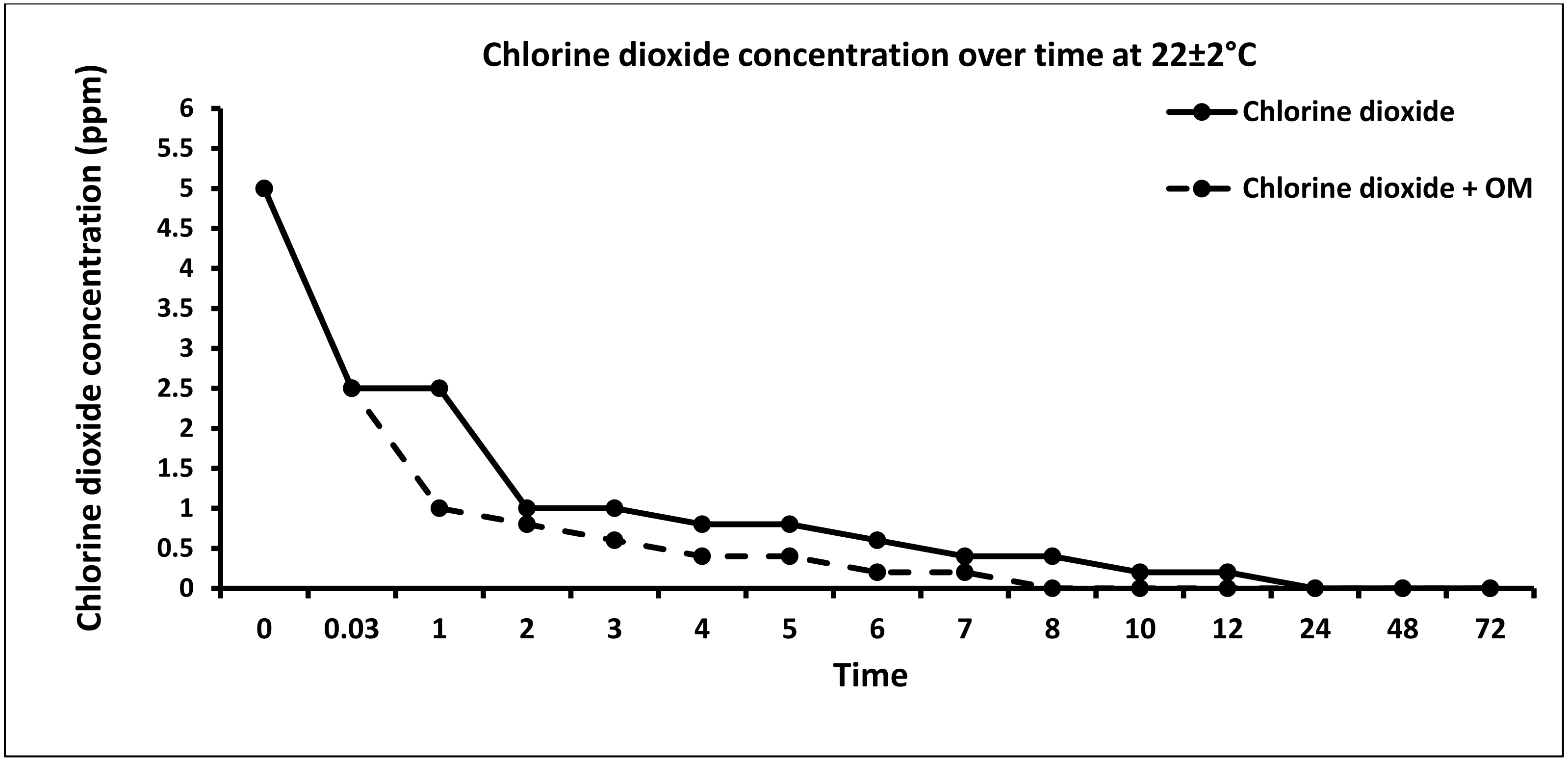
OM- : No organic contamination; OM+ : presence of organic contamination (COD 15±5 ppm)

<u>Objective 4:</u> To determine the effect of organic load on disinfectant concentration in mango wash water

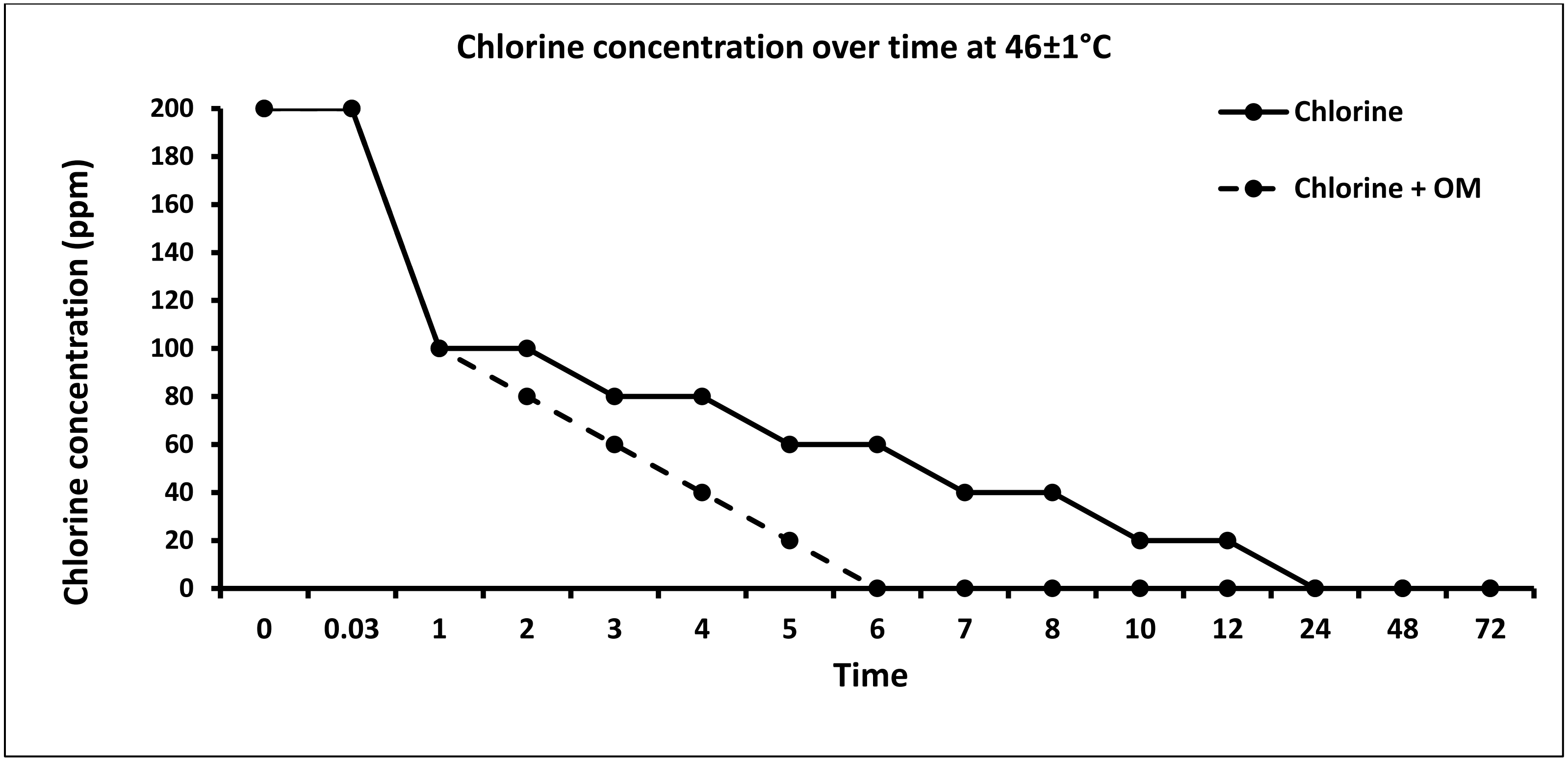
A. Room Temperature: Dump tank/Hydrocooling

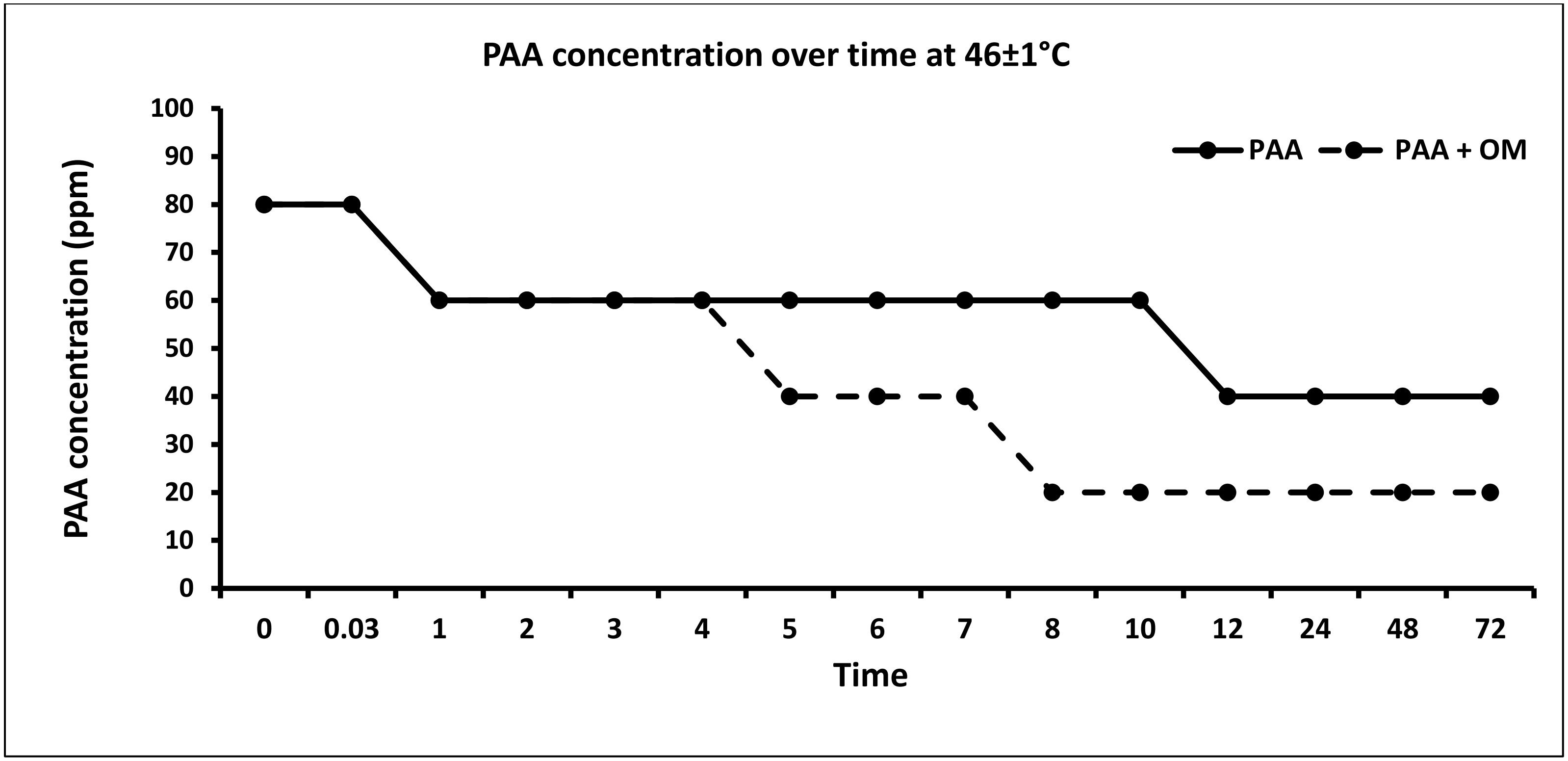


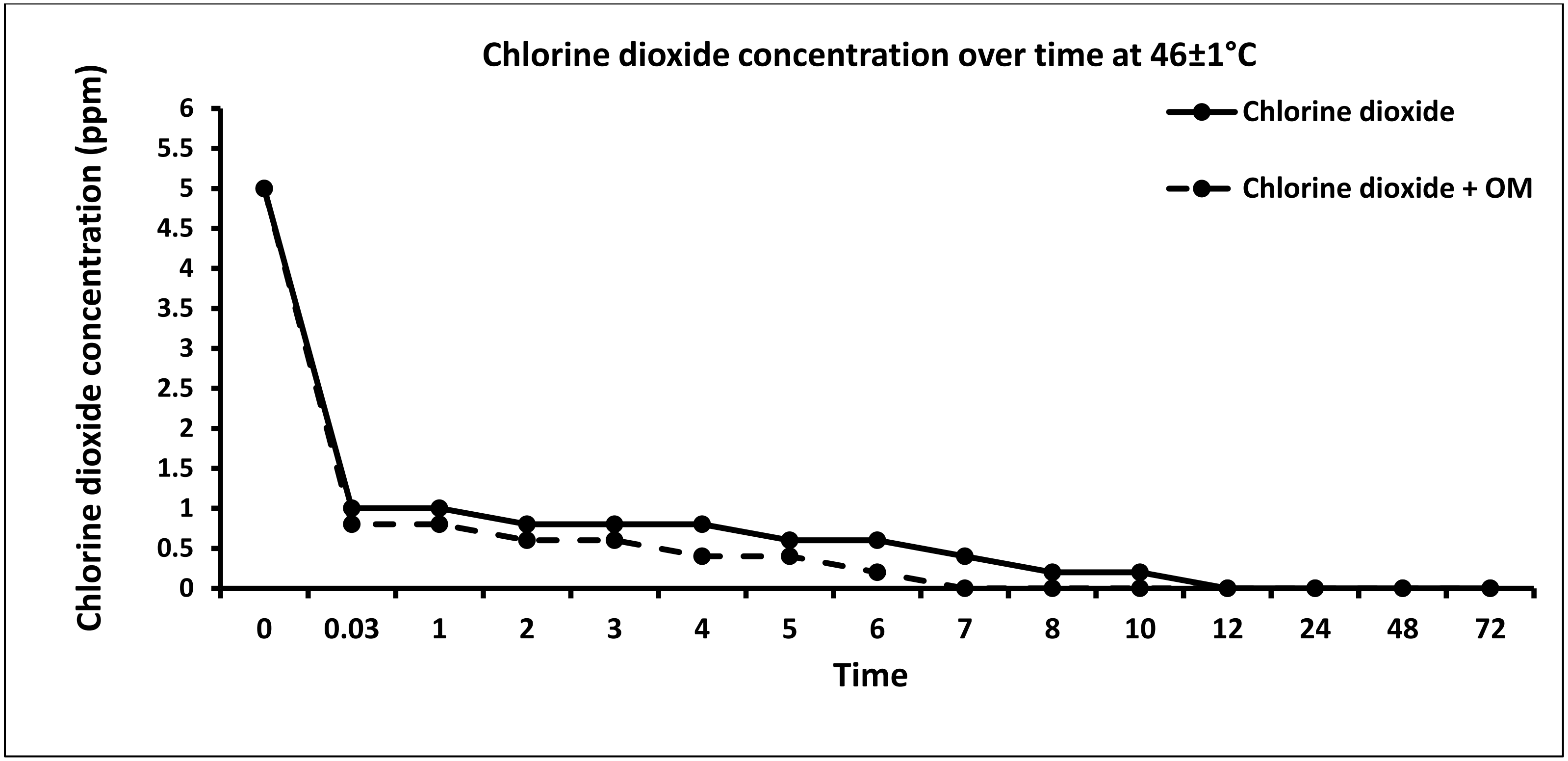

PAA concentration over time at 22±2°C
PAA
PAA + OM
PAA concentration (ppm)
Time
0
10
20
30
40
50
60
70
80
90
100
0
0.03
1
2
3
4
5
6
7
8
10
12
24
48
72


Chlorine dioxide concentration over time at 22±2°C
Chlorine dioxide concentration (ppm)
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
0.03
1
2
3
4
5
6
7
8
10
12
24
48
72
Time
Chlorine dioxide
Chlorine dioxide + OM

B. Hot water tank




PAA concentration over time at 46±1°C
PAA
PAA + OM
PAA concentration (ppm)
100
90
80
70
60
50
40
30
20
10
0
0
0.03
1
2
3
4
5
6
7
8
10
12
24
48
72
Time


Chlorine dioxide concentration over time at 46±1°C
Chlorine dioxide concentration (ppm)
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
0.03
1
2
3
4
5
6
7
8
10
12
24
48
72
Time
Chlorine dioxide
Chlorine dioxide + OM

Objective 5: To determine the effect of disinfectant treatment on mango color

A. Dump tank wash

Table 1: Effect of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide on Ataulfo mango color following dump tank wash (2 minutes)

Treatment	Ataulfo color (b*value – Yellow color)	
	Before wash	After wash
Sterile DW	32.19±0.46	32.54±0.39
Chlorine (200 ppm)	33.82±0.59	34.46±0.55
PAA (80 ppm)	33.45±0.64	34.24±0.67
Chlorine dioxide (5 ppm)	34.34±0.38	35.42±0.38

Table 2: Effect of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide on Tommy Atkins mango color following dump tank wash (2 minutes)

Treatment	Tommy Atkins color (a*value – Red color)	
	Before wash	After wash
Sterile DW	21.35±0.65	21.80±0.65
Chlorine (200 ppm)	24.03±0.61	24.22±0.61
PAA (80 ppm)	24.25±0.59	25.28±0.65
Chlorine dioxide (5 ppm)	23.96±0.76	24.42±0.38

B. Hot water treatment

Table 3: Effect of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide on Ataulfo mango color following hot water treatment (75 minutes)

Treatment	Ataulfo color (b*value – Yellow color)	
	Before wash	After wash
Sterile DW	33.56±0.49	34.41±0.51
Chlorine (200 ppm)	31.97±0.63	33.62±0.64
PAA (80 ppm)	32.62±0.69	33.47±0.56
Chlorine dioxide (5 ppm)	32.06±0.59	33.50±0.62

Table 4: Effect of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide on Tommy Atkins mango color following hot water treatment (110 minutes)

Treatment	Tommy Atkins color (a*value – Red color)	
	Before wash	After wash
Sterile DW	22.33±0.74	23.52±0.75
Chlorine (200 ppm)	24.10±0.79	25.47±0.85
PAA (80 ppm)	24.70±0.54	25.94±0.57
Chlorine dioxide (5 ppm)	23.02±0.72	24.25±0.80

C. Hydrocooling

Table 5: Effect of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide on Ataulfo mango color following hydrocooling (30 minutes)

Treatment	Ataulfo color (b*value – Yellow color)	
	Before wash	After wash
Sterile DW	31.29±0.58	32.59±0.57
Chlorine (200 ppm)	33.23±0.62	34.13±0.61
PAA (80 ppm)	33.35±0.66	33.96±0.62
Chlorine dioxide (5 ppm)	32.68±0.70	33.19±0.63

Table 6: Effect of 200 ppm chlorine, 80 ppm PAA and 5 ppm chlorine dioxide on Tommy Atkins mango color following hydrocooling (30 minutes)

Treatment	Tommy Atkins color (a*value – Red color)	
	Before wash	After wash
Sterile DW	23.82±0.72	24.88±0.64
Chlorine (200 ppm)	23.27±0.97	23.81±0.95
PAA (80 ppm)	23.60±0.60	24.93±0.64
Chlorine dioxide (5 ppm)	22.91±0.68	23.55±0.61