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Assessing the efficacy of sanitizer sprays during brush or polyvinyl chloride (PVC) roller treatment to reduce *Salmonella* populations on whole mangoes

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ABSTRACT

Sanitizer spray and brush roller treatments have been documented as an effective means of reducing *Salmonella* on the surface of produce. The purpose of this study was to evaluate the efficacy of chlorine (NaOCl), peroxyacetic acid (PAA), and chlorine dioxide (ClO_2) sprays to reduce *Salmonella* populations on the surface of mangoes during washing with brush or polyvinyl chloride (PVC) rollers. Whole mangoes were spot inoculated with 100 μL of a rifampicin-resistant *Salmonella* (8 log CFU/mL) cocktail at the equator and dried for 1 h. Mangoes were washed with a lab-scale roller system with either ground water (control), or sanitizers (100 ppm NaOCl, 80 ppm PAA, or 5 ppm ClO_2) for 0, 5, 15, 30, or 60 s (n = 15 mangoes). Dey/Engley buffer (100 mL) was used to rinse mangoes before plating on media supplemented with rifampicin. NaOCl, PAA, and ClO_2 spray (except for ClO_2 at 30 s) had significantly higher reduction on *Salmonella* population than water spray at all treatment times ($P \leq 0.05$) when brush rollers were used. All tested sanitizers also achieved a significantly higher reduction than water at 5 s when PVC rollers were used ($P \leq 0.05$). *Salmonella* reductions achieved by brush and PVC rollers was not statistically different ($P > 0.05$). After a 5 s treatment on brush and PVC rollers, NaOCl, PAA, and ClO_2 spray had ca. 3.03 and 3.45 log, 3.96 and 3.28 log, and 2.54 and 2.00 log CFU/mango reductions, respectively, whereas water spray achieved 1.75 and 0.98 log CFU/mango reduction. Addition of sanitizers to spray water used during brush or PVC washing in mango packinghouses can reduce *Salmonella* on mango surfaces.

1. Introduction

Imported fresh mangoes have been associated with five documented outbreaks of *Salmonella* from serotypes Oranienburg, Newport, Saintpaul, Braenderup, and Worthington in North America (Beatty et al., 2004; CDC, 2006; CDC, 2012; PHAC, 1998; Sivapalasingam et al., 2003). A limited number of studies have reported the incidence of *Salmonella* on mangoes. In Brazil, Bordini et al., (2007) sampled 100 Tommy Atkins mangoes from domestic or export warehouses with unknown prior processing; *Salmonella* was identified in 2 % of samples. In Mexico, Godínez-Oviedo et al., (2022) evaluated 300 Atafulo mangoes (reported as piece of mango ca. 170 to 350 g) from supermarkets and fresh markets in three states, with unknown prior processing; *Salmonella* was identified on four mangoes (1.3 % positive). *Salmonella* was enumerated from three of the mangos in this study, with concentrations ranging from < − 2.0 to − 1.6 log MPN/g. Also in Mexico, Ragazzo-Sanchez et al., (2009); sampled five Tommy Atkins or Atafulo mangoes at one packinghouse, from each reception, after washing, after hydrothermal and hydrocooling treatments, and during caliber selection and packaging, and at the distribution center; *Salmonell*a was detected on two mangoes (8 % positive), one of each variety, both sampled after washing. This higher incidence of *Salmonella* (8 %) is likely due to contamination during washing, as *Salmonella* was also isolated from the tap water of the packing operation. After contamination, survival of *Salmonella* on whole and fresh-cut mangoes has been reported under various storage conditions (Kroft et al., 2022; Luciano et al., 2022; Mathew et al., 2018a; Strawn & Danyluk, 2010: Saha et al., 2023).

The United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) has required a hot water immersion quarantine treatment for imported mangoes since 1987 to prevent importation of fruit flies to the U.S. (Mitcham & Yahia, 2009). The immersion time in the hot water treatment depends on the shape and weight of the fruits. While hot water treatment is beneficial in maintaining a mango's firmness and color (Djioua et al., 2010), it may

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increase food safety risks due to the potential for pathogen infiltration when the heat-treated mangoes are cooled in water.

Chlorine, often in the form of NaOCl, is commonly used in packinghouses as a sanitizer to kill pathogens in water and prevent cross-contamination (Banach et al., 2015). Alternative sanitizers, including chlorine dioxide (ClO_2) and peroxyacetic acid (PAA,) are also used (CFR, 2010a; CFR, 2010b; EPA, 2006). The use of overhead spray sanitizers, brush roller, and the combination of the both, is an effective means of reducing *Salmonella* populations in packinghouses on the surface of cantaloupe (Saucedo-Alderete et al., 2018), tomatoes (Balaguero et al., 2015; Beuchat et al., 2001; Chang & Schneider, 2012; Pao et al., 2009; Pao et al., 2012), oranges (Pao et al., 1999), grapefruit (Danyluk et al., 2019), and Jalapeno peppers (Pao et al., 2012).

Limited published research assesses methods to control *Salmonella* populations that may be present on the surface of mangoes prior to hot water treatment. Fernandes et al. (2014) treated 1 x 1 cm pieces of mango peel by submersion in different sanitizers and surfactant mixtures for 10 min. They concluded that the adhesion of *Salmonella* Typhimurium to mangoes is a multifactorial process, in which the roughness and hydrophobicity of the fruit surface did not affect the efficiency of sanitation treatments. A 10 min submersion does not represent a practical packinghouse treatment to remove pathogens from the surface of mangoes. Mathew et al., (2018b) report the efficacy of chlorine and peroxyacetic acid is greater than of chlorine dioxide to reduce *Salmonella* populations on mangoes in simulated dump tank washes for up to 2 min. This study was conducted to evaluate the reduction of *Salmonella* populations on the surface of mangoes during an overhead sanitizer spray on brush or PVC rollers. A pilot scale overhead spray with brush or PVC rollers was used with three different sanitizers, NaOCl, ClO_2, and PAA. The data generated in this study are designed to help mango packinghouses understand the efficacy of their overhead spray and roller systems to mitigate food safety risks.

2. Material and methods

2.1. Mangoes

Mature but not ripe mangoes (*Mangifera indica* L. var. Tommy Atkins), similar to those packed at commercial mango packinghouses, that had undergone an APHIS Hot Water Treatment (USDA, 2016) were either sourced from the National Mango Board (NMB) or bought from local supermarkets (Auburndale and Haines City, FL). All mangoes were imported (Ecuador, Peru, Nicaragua, and Guatemala). Mangoes were stored at 12 °C for up to 60 days and appropriate numbers of fruits were brought to ambient temperature (ca. 23 °C) prior to the experiment. All fruits were individually inspected for defects (e.g., breaks in the peel, bruises, or microbiological spoilage) prior to each experiment; any defective mangoes were discarded.

2.2. Salmonella cultures

Salmonella serotypes included *Salmonella* Montevideo (isolate from tomato outbreak, human feces; Beuchat et al., 2001; Zhuang et al., 1995), *Salmonella* Michigan (isolate from cantaloupe outbreak, human feces; Beuchat et al., 2001), *Salmonella* Muenchen (isolate from orange juice outbreak, human feces; CDC, 1999; Kenney and Beuchat, 2020), *Salmonella* Saintpaul (isolate from orange surface; Jain et al., 2009), and *Salmonella* Newport (MDD314; isolate from tomato outbreak, environmental; Greene et al., 2008). All cultures were resistant to 80 μg/mL rifampicin (Rif) to allow easy identification of the inoculated strains in the presence of a background microflora.

2.3. Inoculum preparation and mango inoculation

Each of the five strains of *Salmonella*, stored at −80 °C, were streaked onto tryptic soy agar supplemented with 80 μg/mL Rif (TSAR; Difco, Becton Dickinson, Sparks, MD). After incubation at 35 ± 2 °C for 18 ± 2 h, one isolated colony from each strain was transferred into 10 mL of tryptic soy broth supplemented with 80 μg/mL Rif (TSBR; Fluka, Sigma-Aldrich Co., St. Louis, MO). TSBR tubes were incubated for 24 h at 35 ± 2 °C. One loopful (10 μL) of each culture was transferred into new TSBR (10 mL) and incubated for 24 h at 35 ± 2 °C. The cells were then centrifuged at 3,000 × *g* for 10 min (Allegra X-12, Beckman Coulter, Fullerton, CA) and washed by suspending the cell pellet in 9 mL of 0.1 % peptone (Difco, Becton Dickinson, Sparks, MD). Cells were centrifuged three times and washed two times before suspending in 5 mL of 0.1 % peptone water. Each bacterial strain was combined in equal volume (1 mL) to produce the five-strain *Salmonella* cocktail. The cocktail was serially diluted to reach a final concentration of ca. 10^8 CFU/mL. The inoculum cocktail was stored at 4 ± 2 °C for up to 2 h before use.

'Tommy Atkins' mangoes were inoculated by spotting 100 μL (10 spots of ca. 10 μL) of the five-strain *Salmonella* cocktail onto the appropriately marked mango equator, resulting ca. 10^7 CFU/mango. Inoculated mangoes were dried on stabilizers (plastic rings) on the benchtop for 1 h before any treatments. Post-drying, concentrations were ca. 10^6 CFU/mango (data not shown).

2.4. Sanitizer solution preparation

Ground water, meeting the microbiological standards for potable water (Florida Department of Environmental Protection, 2020), from a well (Lake Alfred, FL) was used for all experiments. Three different overhead spray sanitizer solutions were prepared in ground water, including: 100 ppm NaOCl (pH = 7.0), 80 ppm peroxyacetic acid (PAA), and 5 ppm chlorine dioxide (ClO_2); a ground water control was also evaluated. These sanitizers are labeled for the postharvest washing of fruits and vegetables and are highlighted in the "Mango Postharvest Best Management Practices Manual" (Brecht et al., 2014). NaOCl's highlighted concentration range is between 50 and 200 ppm (Brecht et al., 2014), while PPA and ClO_2 cannot exceed permissible limits of 80 and 5 ppm (resulting in ClO_2 residue concentration of 3 ppm) in wash water, respectively (US CFR, 2010a; US CFR, 2010b).

The NaOCl solution was prepared by combining 9 mL of 10 % NaOCl (Freshguard 71; Armchem, FL) with 9.5 L ground water at room temperature to achieve a final concentration of 100 ppm. When required, the solution was buffered with dilute hydrochloric acid (HCl, Fisher, Fair Lawn, NJ) to reach pH (pH test strips; Ricca Chemical Co., Arlington, TX) of 7.0. The PAA solution was prepared by mixing 4.3 mL of 15 % PAA (VigorOx Citrus XA-15, PeroxyChem, LLC, Philadelphia, PA) with 9.45 L ground water at room temperature to achieve a final concentration of 80 ppm. Aqueous ClO_2 was made by adding 2 L of tap water into a Selectrocide® 2L500 (Selective Micro Technologies, Dublin, OH) packet at room temperature. After 2 h reaction of Selectrocide® with water, 500 ppm of active ClO_2 concentration was verified by using wide range chlorine dioxide test strips (Selective Micro Technologies, Canal Winchester, OH). Once the concentration was confirmed, 500 ppm solution was diluted with the appropriate amount of ground water according to the manufacturer's instructions, to achieve final concentration of 5 ppm (one part 500 ppm solution to 99 parts water).

The concentration of sanitizer solutions was confirmed with a colorimeter for NaOCl (Hach, Loveland, CO) and strips for PAA and ClO_2 (Limitless Clean, Dublin, OH) at the beginning and at the end of each experiment. Additional volumes (~1 mL of 10 % NaOCl and 1 mL of 15 % PAA) were added to adjust for the aerosolization from the spray nozzles that lowered concentrations, when necessary.

2.5. Overhead spray and roller treatment

A lab-scale overhead spray and brush or polyvinyl chloride (PVC) roller system was used in all experiment replications. The spray and brush roller system was designed by Chang & Schneider (2012) and manufactured by Agri Machinery Inc. (Orlando, FL). Two rotating nylon

brush or PVC rollers (46 cm long and 12 cm diameter) were set in a box measuring 46 cm by 34 cm, rotated in the same direction at 180 rpm. Three mangoes were placed in the groove between the two rollers and treated together at a time. Mangoes revolved on their axis in a direction depending on their shape. Three spray nozzles (Spraying Systems Co., Wheaton, IL), 13 cm above rollers released a cone shaped spray at 13 psi and 21.4 mL/s. A 20 L bucket was connected to the machine through a spigot and pipes to feed into the machine. At least 6 L of solution had to be in the bucket at all times in order to submerge the spigot and maintain the pressure of the system.

One experiment replication for each sanitizer and the water control was divided into three groups: a negative control group of uninoculated treated mangoes; a positive control group of inoculated, untreated; and experimental groups, of inoculated mangoes treated with overhead spray and roller system for 0, 5, 15, 30, or 60 s. Three mangoes were treated at a time per sanitizer per time in each replication, and the experiment was replicated five times (n = 15) (NACMCF, 2010). The negative control group was always treated first to detect any potential cross-contamination from the equipment.

2.6. Enumeration

Following treatment, Dey/Engley buffer (DE; 100 mL; Remel, Thermo Scientific, Lenexa, KS) was added to whole mangoes in sterile bags to remove *Salmonella* or background microflora (total aerobic mesophyll bacteria) from the surface following a rub (30 s) – shake (30 s) – rub (30 s) protocol modified from Beuchat et al (2001). DE also neutralized the sanitizer to prevent any further reductions in bacterial load. After the rub-shake-rub, serially diluted samples (10-fold) were plated (0.1 ml) in duplicate onto non-selective (TSAR) and bismuth sulfite agar as selective media supplemented with 80 μg/mL Rif (BSAR; Difco, Becton Dickinson, Sparks, MD) to enumerate *Salmonella*, or plate count agar to enumerate for background microflora (PCA; Difco, Becton Dickinson, Sparks, MD). To increase the limit of detection to 2 log CFU/mango, an additional 1 mL of the lowest dilution was plated onto four plates each (0.25 mL/plate) of non-selective (TSAR) and selective media (BSAR).

2.7. Enrichment

When there was no *Salmonella* growth at the lowest dilution, samples were enriched following U.S. FDA Bacteriological Analytical Manual (FDA BAM) with minor adjustments (FDA, 2016). Briefly, 100 mL of double strength lactose broth (Difco, Becton Dickinson, Sparks, MD) was added to sample bags containing the mango and 100 mL DE and incubated for 24 h at 35 ± 2 °C. One mL of the overnight pre-enrichment was transferred into tetrathionate (TT; Difco, Becton Dickinson, Sparks, MD) broth and incubated for 24 h at 35 ± 2 °C. One loopful (10 μL) of the enrichment was streaked onto Hektoen enteric agar with Rif (HER; Difco, Becton Dickinson, Sparks, MD), xylose lysine deoxycholate agar with Rif (XLDR; Difco, Becton Dickinson, Sparks, MD), and BSAR, and incubated for 24 h at 35 ± 2 °C. When typical *Salmonella* colonies were observed on enrichment, the population was recorded as < 2 log CFU/mango. When no *Salmonella* was detected upon enrichment, <0 log CFU/mango was recorded.

2.8. Statistical analysis

Data were statistically evaluated by analysis of variance (ANOVA) among sanitizers and water control, selective media (TSAR and BSAR), treatment times (0, 5, 15, 30, 60 s), and roller types (brush rollers and PVC rollers). If no significant difference was found between TSAR and BSAR, then TSAR data alone was further analyzed. A mean separation was performed with Tukey's-HSD. The software used to perform ANOVA was RStudio (version 3.3.1; Rstudio, Inc. Boston, MA). Differences were considered significant at $P \leq 0.05$.

3. Results

Average populations of *Salmonella* inoculated onto mangoes and enumerated on BSAR and TSAR media, respectively, were 6.94 ± 0.05 and 7.13 ± 0.15 log CFU/mango initially upon inoculation, and 5.78 ± 0.22 and 6.39 ± 0.30 log CFU/mango with following 1 h drying (Data not shown; n = 15). *Salmonella* populations decreased between 0.66 and 1.23 log CFU/mango during the 1 h drying period post inoculation on mangoes.

There was no colony growth on either TSAR or BSAR media from the negative control mangoes, indicating that no cross-contamination occurred during experiments and the background microflora on mangoes was not resistant to rifampicin. *Salmonella* populations recovered from TSAR and BSAR within treatment groups were not significantly different ($P > 0.05$); TSAR results only are presented for all subsequent *Salmonella* results.

3.1. Sanitizer efficacy on brush rollers

The efficacy of NaOCl (100 ppm), PAA (80 ppm), and ClO_2 (5 ppm) to reduce *Salmonella* populations on mangoes following overhead treatment on brush rollers is shown in Table 1. Significant differences were observed between NaOCl and water at treatment times of 5, 15, 30, 60 s ($P \leq 0.05$). The largest *Salmonella* population reduction using the NaOCl treatment, was from 6.37 ± 0.24 to < 2.54 ± 0.65 log CFU/mango after 15 s. The equivalent treatment with water alone resulted in a *Salmonella* population decrease from 6.39 ± 0.30 to 4.05 ± 0.45 log CFU/mango. Following a 60 s NaOCl treatment, *Salmonella* populations were < 1.94 ± 0.91 log CFU/mango, whereas *Salmonella* populations on mangoes receiving only water treatment were 3.12 ± 0.50 log CFU/mango. The impact of NaOCl treatment time on *Salmonella* populations reductions was also evaluated. There were significant differences between treatment times of 0 and all other times, 5 and 30 s, and 5 and 60 s ($P \leq 0.05$); there was no significant difference between 5 and 15 s, and 15, 30, and 60 s ($P > 0.05$).

PAA treatment reduced *Salmonella* populations significantly more than water at all treatment times ($P \leq 0.05$). The largest difference was after 5 s, where PAA reduced populations from 6.30 ± 0.17 log to < 2.34 ± 0.57 CFU/mango, while water reduced populations from 6.39 ± 0.30 to 4.64 ± 0.73 log CFU/mango. Following 60 s of PAA treatment, populations were reduced to < 1.23 ± 1.05 log CFU/mango. Evaluating the impact of time on the reduction *Salmonella* populations by PAA on brush rollers, there were significant differences in *Salmonella* reduction between 0 and other treatment times, 5 and 60 s ($P \leq 0.05$); there was no significant difference between 5, 15, and 30 s, and 15, 30, and 60 s ($P > 0.05$).

There was no significant difference between ClO_2 and water at 30 s ($P > 0.05$). Significant differences ($P \leq 0.05$) were found between water and ClO_2 at treatment times of 5, 15, and 60 s. At 60 s, ClO_2 reduced *Salmonella* populations from 5.99 ± 0.18 to < 2.18 ± 0.29 log CFU/mango. Evaluating the impact of time on the reduction of *Salmonella* populations by ClO_2 on brush rollers, treatment had a significant impact on *Salmonella* populations between 0 s and all the other treatment times, as well as 5 and 60 s ($P \leq 0.05$). There was no significant difference on *Salmonella* population reductions between 5, 15, and 30 s, and 15, 30, and 60 s ($P > 0.05$).

There is no significant ($P > 0.05$) difference in *Salmonella* populations at any time following treatment with NaOCl and ClO_2. Significantly lower ($P \leq 0.05$) *Salmonella* populations were recovered from PAA than from ClO_2 at all treatment times except for 15 s. The efficacy of NaOCl and PAA was not significantly different at 15, 30, 60 s, but PAA significantly reduced *Salmonella* population by at least 1 additional log CFU/mango when compared to NaOCl following the 5 s treatment. *Salmonella* populations were not significantly different using any of the tested sanitizers after treatment on brush rollers for 15 s. Significant ($P > 0.05$) reductions, beyond what had already been achieved, were not

Table 1
Average log CFU/mango populations and log reductions of *Salmonella* after overhead sanitizers and water spray with brush roller treatment of inoculated mangoes recovered from TSAR media[a].

Time	Water (control)		NaOCl (100 ppm)		PAA (80 ppm)		ClO_2 (5 ppm)	
(s)	Population	Reduction	Population	Reduction	Population	Reduction	Population	Reduction
0	6.39 ± 0.30 Aa	0	6.37 ± 0.24 Aa	0	6.30 ± 0.17 Aa	0	5.99 ± 0.18 Aa	0
5	4.64 ± 0.73 Ba	1.75	3.34 ± 0.90 Bb	>3.03	<2.34 ± 0.57 Bc	>3.96	<3.45 ± 0.83 Bb	>2.54
15	4.05 ± 0.45 BCa	2.34	<2.54 ± 0.65[b] BCb	>3.83	<1.98 ± 0.60 BCb	>4.32	<2.80 ± 0.64 BCb	>3.19
30	3.55 ± 0.45 CDa	2.84	<2.44 ± 0.69 Cbc	>3.93	<1.62 ± 0.84 BCc	>4.68	<2.78 ± 0.60 BCab	>3.21
60	3.12 ± 0.50 Da	3.27	<1.94 ± 0.91 Cbc	>4.43	<1.23 ± 1.05 Cc	>5.07	<2.18 ± 0.29 Cb	>3.81

[a] Values are mean ± standard deviation of five replicates of experiment of 3 mangoes each (n = 15). Means with same letter in the same column (ABCD) or in the same row (abc) are not statistically different ($P \leq 0.05$).
[b] < represents there were one or more replicate(s) that was (were) below the limit of detection among the 15 replicates; < 2 log CFU/mango was used for the positive results of *Salmonella* enrichment, < 0 log CFU/mango was used for the negative results of *Salmonella* enrichment.

observed for any sanitizer by treating for more than 15 s.

The efficacy of NaOCl (100 ppm), PAA (80 ppm), and ClO_2 (5 ppm) to reduce background microflora on mangoes following overhead treatment on brush rollers is shown in Table 2. Addition of sanitizers to spray on brush rollers did not increase reductions of background microflora populations over water at any treatment time ($P > 0.05$) (Table 2).

3.2. *Sanitizer efficacy on PVC rollers*

The efficacy of NaOCl (100 ppm), PAA (80 ppm), and ClO_2 (5 ppm) to reduce *Salmonella* populations on mangoes following overhead treatment on PVC rollers is shown in Table 3. Reductions of *Salmonella* populations following treatment with NaOCl were significantly higher ($P \leq 0.05$) than water at all treatment times. After mangoes were treated for 5 s, NaOCl treatment reduced *Salmonella* populations from 6.06 ± 0.21 to < 2.61 ± 0.89 log CFU/mango, while water only decreased from 6.22 ± 0.23 to 5.24 ± 0.97 log CFU/mango. Although NaOCl had an initial log CFU/mango reduction of around 3.5 log at 5 s, subsequent washing for up to 60 s only decreased *Salmonella* populations by one additional log.

PAA treatment resulted in significantly higher *Salmonella* population reduction than water treatment following 5 to 15 s of treatment ($P \leq 0.05$). Similar to the results seen with NaOCl, the most significant reduction by PAA occurred in the first 5 s of (from 5.94 ± 0.16 to < 2.66 ± 0.72 log CFU/mango reduction) ($P \leq 0.05$); no further significant reductions were observed ($P > 0.05$), with populations only decreasing by about one more log from 5 s to 60 s (Table 3).

ClO_2 and water achieved similar *Salmonella* reduction ($P > 0.05$) at all treatment times except for 5 s ($P \leq 0.05$) (Table 3). ClO_2 reduced *Salmonella* population from 6.11 ± 0.21 to 4.11 ± 0.70 log CFU/mango after 5 s treatment where water achieved a 1-log reduction. There were significantly higher differences of CFU/mango *Salmonella* reduction from time 0 to 15 s on ClO_2 ($P \leq 0.05$). There was no significant difference between 15, 30, and 60 s, where ClO_2 only achieved a 0.73 log CFU/mango reduction from 15 to 60 s ($P > 0.05$).

The efficacy of NaOCl (100 ppm), PAA (80 ppm), and ClO_2 (5 ppm) to reduce background populations on mangoes following overhead treatment on PVC rollers are shown in Table 4. Addition of sanitizers to spray on brush rollers did occasionally result in significantly higher reductions ($P \leq 0.05$) than water alone (eg. NaOCl at all treatment times). In general, sanitizers had a limited impact on reductions of background populations naturally found on mangoes, with no sanitizer exceeding a 0.5 log reduction regardless of treatment time.

3.3. *Comparison of brush and PVC rollers*

There was no significant difference in *Salmonella* population reductions following treatments with NaOCl, PAA, and ClO2 spray, at any treatment time, between brush and PVC rollers ($P > 0.05$).

4. Discussion

During mango packing, mangoes are transferred from harvesting containers onto the packing line using a dump tank to gently transfer the fruit onto the line while preventing damage. Following submersion in the dump tank they proceed onto the packing line and are washed on under a spray bar (Brecht et al., 2014). The primary goal of washing is to remove dirt and grit on fruit and vegetable surfaces; sanitizing chemicals are added to postharvest wash water to prevent cross-contamination between contaminated and uncontaminated fruits and vegetables (Banach et al., 2015). Outbreaks from fresh fruits and vegetables (US FDA, 2011; US FDA, 2012; US FDA, 2019), including mangoes (Sivapalasingam et al., 2013), have been linked to post harvest water mismanagement. The addition of sanitizers to wash water not only prevents cross-contamination but can have the added benefit of reducing the population of pathogens on contaminated fruits and vegetables.

The sanitizers chlorine (200 ppm), peroxyacetic acid (80 ppm) and chlorine dioxide (5 ppm) reduced *Salmonella* populations in wash water, prevented cross-contamination and internalization of *Salmonella* into submerged mangoes under simulated packinghouse dump tanks where mangoes are submerged (Mathew et al., 2018b). Overhead spray washing, may be advantageous over submersion as the mechanical

Table 2
Average log populations and log reductions of background microflora after overhead sanitizers and water spray with brush roller treatment of not inoculated mangoes recovered from PCA media[a].

Time	Water (control)		NaOCl (100 ppm)		PAA (80 ppm)		ClO_2 (5 ppm)	
(s)	Population	Reduction	Population	Reduction	Population	Reduction	Population	Reduction
0	4.93 ± 0.65 Aab	0	4.93 ± 0.65 Aab	0	4.23 ± 0.66 Ab	0	3.24 ± 0.57 Ac	0
5	5.36 ± 0.51 Aa	−0.43	5.36 ± 0.51 Aa	0.60	4.22 ± 0.85 Ab	0.01	3.20 ± 0.77 Ac	0.04
15	5.22 ± 0.62 Aa	−0.29	5.22 ± 0.62 Aa	0.74	3.96 ± 0.92 Ab	0.27	3.00 ± 0.48 Ac	0.24
30	5.08 ± 0.37 Aa	−0.15	5.08 ± 0.37 Aa	0.54	3.91 ± 0.85 Ab	0.32	2.72 ± 0.52 Ac	0.52
60	5.06 ± 0.46 Aa	−0.13	5.06 ± 0.46 Aa	0.70	3.53 ± 1.04 Ab	0.70	2.99 ± 0.83 Ab	0.25

Means with same letter in the same column (A) or in the same row (abc) are not statistically different ($P \leq 0.05$).
[a] Values are mean ± standard deviation of five replicates of experiment of 3 mangoes each (n = 15).

Table 3
Average log CFU/mango populations and log reductions of *Salmonella* after overhead sanitizers and water spray with PVC roller treatment of inoculated mangoes recovered from TSAR media[a].

Time	Water (control)		NaOCl (100 ppm)		PAA (80 ppm)		ClO_2 (5 ppm)	
(s)	Population	Reduction	Population	Reduction	Population	Reduction	Population	Reduction
0	6.22 ± 0.23 Aa	0	6.06 ± 0.21 Aa	0	5.94 ± 0.16 Aa	0	6.11 ± 0.21 Aa	0
5	5.24 ± 0.97 Ba	0.98	<2.61 ± 0.89[b] Bb	>3.45	<2.66 ± 0.72 Bb	>3.28	4.11 ± 0.70 Bc	2
15	3.78 ± 0.87 Ca	2.44	<2.05 ± 1.40 BCb	>4.01	<2.14 ± 0.76 Bbc	>3.8	3.07 ± 0.81 Cac	3.04
30	2.84 ± 0.71 CDa	3.38	<1.81 ± 0.77 BCb	>4.25	<1.95 ± 0.59 Bab	>3.99	2.77 ± 0.60 Cab	3.34
60	2.56 ± 0.61 Da	3.66	<1.60 ± 0.83 Cb	>4.46	<1.74 ± 0.93 Bab	>4.2	<2.34 ± 0.41 Cab	>3.77

[a] Values are mean ± standard deviation of five replicates of experiment of 3 mangoes each (n = 15). Means with same letter in the same column (ABCD) or in the same row (abc) are not statistically different ($P \leq 0.05$).
[b] < represents there were one or more replicate(s) that was (were) below the limit of detection among the 15 replicates; < 2 log CFU/mango was used for the positive results of *Salmonella* enrichment, < 0 log CFU/mango was used for the negative results of *Salmonella* enrichment.

Table 4
Average log CFU/mango populations and log reductions of background microflora after overhead sanitizers and water spray with PVC roller treatment of not inoculated mangoes recovered from PCA media[a].

Time	Water (control)		NaOCl (100 ppm)		PAA (80 ppm)		ClO_2 (5 ppm)	
(s)	Population	Reduction	Population	Reduction	Population	Reduction	Population	Reduction
0	4.41 ± 1.42 Aa	0	3.07 ± 0.31 Ab	0	3.68 ± 0.71 Aab	0	3.58 ± 0.96 Aab	0
5	4.88 ± 1.21 Aa	−0.47	3.21 ± 0.61 Ac	−0.14	3.32 ± 0.96 Abc	0.36	3.79 ± 0.85 Aabc	−0.21
15	4.47 ± 1.25 Aa	−0.06	2.90 ± 0.48 Ab	0.17	3.43 ± 1.05 Aab	0.25	3.92 ± 0.74 Aab	−0.34
30	4.38 ± 1.15 Aa	0.03	2.80 ± 0.59 Ab	0.27	3.18 ± 1.01 Aab	0.50	3.96 ± 0.81 Aa	−0.38
60	4.27 ± 1.05 Aa	0.14	2.71 ± 0.45 Ab	0.36	3.19 ± 1.02 Aab	0.49	3.63 ± 0.91 Aab	−0.05

Means with same letter in the same column (A) or in the same row (abc) are not statistically different ($P \leq 0.05$).
[a] Values are mean ± standard deviation of five replicates of experiment of 3 mangoes each (n = 15).

action of the rollers can remove more pathogen contamination and that in not submerging fruit into water you prevent the potential for pathogen infiltration. Washing tomatoes with an overhead sanitizer spray on brush rollers achieved at least a 3-log CFU/mL reduction of *Salmonella*. This reduction was significantly higher than those achieved by water alone, or in a model tomato dump/flume wash after 15 to 60 s (Chang & Schneider, 2012). In the present study, a previously developed (Chang & Schneider, 2012) overhead sanitizer spray on brush or PVC roller system was used to evaluate the efficacy of sanitizers common in mango packinghouses to reduce *Salmonella* populations on mangoes during simulated overhead spray washing.

With the same overhead spray and brush roller system, the efficacy of 25, 50, and 100 ppm NaOCl at reducing *Salmonella* populations was evaluated on inoculated tomatoes by Chang & Schneider (2012). The *Salmonella* reductions on mangoes reported here consistent with the previously reported results on tomatoes, where a 100 ppm NaOCl treatment resulted in a ca. 3.5 log reduction at 15 s; 100 ppm NaOCl was the only evaluated concentration of sanitizer with significantly higher ($P \leq 0.05$) reduction than water spray results at 15 (2.3 ± 0.4 log CFU/mL) on tomatoes (Chang & Schneider, 2012). Higher *Salmonella* reductions were observed after a 5 s NaOCl treatment of mangoes than those seen on tomatoes. This difference between the studies on the same spray and brush roller system could be due to the use of different type of fruit, *Salmonella* strains, inoculum levels, drying time, and fruit surface properties affecting pathogen attachment.

Addition of 80 ppm of PAA to the overhead spray reduced *Salmonella* populations by significantly more than water alone ($P \leq 0.05$). Similar to NaOCl, we report a greater *Salmonella* reduction over the first 5 s (ca. 4 log CFU/mango) than that was reported by Chang & Schneider, (2012) using the same spray and brush and roller system on tomatoes. *Enterococcus faecium* NRRL B- 2354 has been proven as an acceptable *Salmonella* surrogate for various food processes on low-moisture foods (Ahmad et al., 2022), nuts (Brar & Danyluk, 2019), and produce (Chen & Meng, 2021). PAA use with spray-bar brush bed systems achieved limited reductions of *E. faecium*, up to 2.2 log, on different types of fresh apples (Shen et al., 2020; Zhu et al., 2021). Potential factors leading to the differences between commodities high standard deviation may include shape, morphology and surface properties of the different produce. Although Fernandes et al. (2014) stated that the roughness and hydrophobicity of the fruit surfaces did not affect the efficacy of sanitizing solution on reduction of *Salmonella* Typhimurium during immersion sanitation of mangoes, the overhead spray and brush roller system may help increase contact with sunken areas of fruit surfaces due to mechanical and shear forces.

ClO_2 spray over brush rollers resulted in significantly higher log reductions than water alone ($P \leq 0.05$) except for the treatment time at 30 s ($P > 0.05$). Pao et al. (2009) compared the efficiency of ClO_2 (5 ppm) and water during spray washing of tomatoes inoculated with *Salmonella* at low and high flow rates (5.0 and 9.3 mL/s per fruit, respectively); the different flow rates did not significantly influence the sanitizing effect of ClO_2 treatments. They report higher log reductions on tomatoes after ClO_2 treatment than we report on mangoes, with spray washing with ClO_2 for 10 to 60 s reduced *Salmonella* populations by 4.4 to 5.2-log CFU/cm^2 on tomato surfaces (Pao et al., 2009). Beyond the use of a different treatment system, this may be do to differences in shapes, surface structures, and firmness of the fruits. That NaOCl and PAA overhead spray treatments resulted in higher *Salmonella* reduction on mangoes than an overhead spray with ClO_2 is consistent with the finding of Mathew et al. (2018b) in the submersion washing of mangoes, where *Salmonella* populations were higher when ClO_2 was used as a sanitizer. Similar log reductions seen between the brush and dump tank washing between this work and Mathew et al., (2018b) maybe due to the sources of the chemicals used for the experiments; our sources were commercially available sanitizers, while Mathew et al., (2018b) sourced their NaOCl and PAA from Fisher Scientific and ClO_2 from Wisconsin Pharmacal Company LLC.

No significant differences were seen between brush and PVC rollers. Pao et al., (2012) report similar results, where no significant differences ($P > 0.05$) in *Salmonella* log CFU/cm^2 reduction were found between roller and brush washing for 60 s on Jalapeno peppers and Roma. These combined results suggested that the mechanical effect of brush rollers play a minor role in reducing *Salmonella* on the surface of produce. The trends in log reductions reported here are also comparable to those of Mathew et al. (2018b), where no mechanical mango washing was

applied.

The majority of log CFU/mango reductions achieved during the full 60 s of washing were seen during the initial 5 s for all sanitizers evaluated; beyond 15 s, NaOCl, PAA, and ClO_2, did not reduce *Salmonella* significantly more. This implies that wash times with overhead sanitizer sprays on brush or PVC rollers in commercial packing environments would not provide further reduction of *Salmonella* on the surface of mangoes by extending the washing time beyond 15 s. In terms of type of sanitizers present in the overhead spray, results from the current study are similar to those on tomatoes Chang & Schneider (2012), where NaOCl and ClO_2 performed similar at all treatment times ($P \leq 0.05$); and PAA had a significantly higher *Salmonella* reduction compared to other sanitizers and water at 5 s ($P > 0.05$).

Negative control groups demonstrate the background microflora levels on mango surfaces, and the difficulty of removing them. The reduction of the native microbiota was significantly less than the reduction of inoculated *Salmonella* on mango surfaces. This may be due to a weaker attachment during the 1 h drying time of *Salmonella* on the mango surface. Mango surfaces support a wide range of microorganisms, including various species of bacteria, filamentous fungi, and yeasts (Jha et al., 2010). Mangoes in this study had lower indigenous microbial populations than previously documented (Jha et al., 2010; 3.0 to 5.7-log CFU/mango in this study compared to 5.0 to 8.0-log CFU/ml). This difference occurred might be because of cultivation conditions and locations affecting composition of indigenous microorganisms and microbial load.

5. Conclusions

The objective of current study was to evaluate the effectiveness of postharvest overhead washing using different sanitizers and roller types on *Salmonella* reduction on mango surfaces. Brush and PVC rollers were found to have similar efficacy in reducing *Salmonella* populations. Sanitizers, including 100 ppm NaOCl, 80 ppm PAA, and 5 ppm ClO_2 sprays were more effective than water spray in removing *Salmonella*, achieving at least a 2 to 4 log CFU/mango reduction, compare to a 1 to 2 log reduction reached by water spray after 5 s treatment. PAA was the most effective sanitizer evaluated, resulting in a 5-log *Salmonella* reduction with brush rollers at 60 s. The addition of any of the evaluated sanitizers NaOCl, PAA, or ClO_2 into spray water on either roller type was more effective than water alone. Inclusion of a sanitizer spray over PVC or brush rollers in postharvest mango operations should help minimize contamination on the surfaces of mangoes entering a hot water immersion quarantine treatment where infiltration into the flesh is possible.

CRediT authorship contribution statement

Xinyue Wang: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Zeynal Topalcengiz:** Writing – review & editing, Data curation. **Michelle D. Danyluk:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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