CPS 2022 RFP – FINAL PROJECT REPORT

Project Title:

Control of *Salmonella* and *Listeria monocytogenes* on peaches through spray-bar brush bed sanitizer intervention

Project Period:

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Objectives:

1. Validate the efficacies of selected sanitizers against *Salmonella* and *Listeria monocytogenes* on peaches.
2. Verify the selected sanitizer interventions in the representative commercial peach packing lines in California.

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FINAL REPORT

Summary of Findings and Recommendations

This study comprehensively evaluated the efficacy of chlorine and chlorine dioxide applied through spray-bar brush bed systems in laboratory, pilot-scale, and commercial packing environments for controlling *Listeria monocytogenes* and *Salmonella* on stone fruit using yellow-flesh peaches. *Enterococcus faecium* NRRL B-2354 was validated as a reliable surrogate for these pathogens in sanitizer efficacy studies. Chlorine (50–150 ppm free chlorine) and chlorine dioxide (2.5–5.0 ppm) effectively reduced *L. monocytogenes* and *Salmonella* in a dose-dependent manner. In-facility testing using *E. faecium* NRRL B-2354 demonstrated up to a 2.9-log reduction with 100 ppm free chlorine and a 2.6-log reduction with 5 ppm chlorine dioxide within 30 seconds. In comparison, water alone, when applied through a spray-bar brush bed, achieved reductions of 0.8–1.4 log CFU/peach. Incorporating EpiClean (pH-neutral cleaner) with the selected sanitizers further enhanced pathogen reduction, particularly at 50 ppm free chlorine or 5 ppm chlorine dioxide. While sanitizer spray treatments significantly reduced cross-contamination, their effectiveness varied across different facilities, likely due to variations in equipment design, spray-bar configurations, and operational practices. Despite these interventions, complete elimination of *L. monocytogenes* on fresh peaches remains challenging, highlighting the need for preventive measures throughout production and packing. The findings underscore the importance of optimizing sanitizer concentrations, spray-bar parameters, and handling procedures to maximize microbial reduction and minimize cross-contamination in commercial stone fruit packing operations.

Abstract

Stone fruits, including peaches, are a critical global commodity, with California being the leading state of the production, accounting for about 76% of U.S. production. However, recent multistate outbreaks of *Salmonella* and *Listeria monocytogenes* linked to peach and stone fruit consumption have caused substantial economic losses to the stone fruit industry. These incidents emphasize the need for effective preventive controls to mitigate foodborne pathogens in peaches and other stone fruits. While the incorporation of sanitizing agents into peach spray wash water is a common industry practice, limited information is available on the practical efficacy of these antimicrobial interventions against foodborne pathogens under commercial packing conditions. The objectives of study were to evaluate operational parameters for practical and Generally Recognized as Safe (GRAS) sanitizers against *Salmonella* and *L. monocytogenes*, and further verify their efficacy in-plant. The study tested chlorine and chlorine dioxide with or without fruit cleaner for their ability to reduce *Salmonella* and *L. monocytogenes* on peaches and further validated these interventions on three representative commercial stone fruit packing lines in California, using the surrogate strain *Enterococcus faecium* NRRL B-2354. The findings provide science-based recommendations to the stone fruit industry, aiding in the optimization of process parameters and supporting compliance with the Food Safety Modernization Act (FSMA) Preventive Controls requirements.

Background

Stone fruits such as peaches are an important commodity in the United States. California produced 468,000 tons of peaches in 2020, which is ~76% of peaches produced in the United States (NASS, 2021). The 2020 multistate outbreak of *Salmonella* associated with peaches led to 101 illnesses and 28 hospitalizations (CDC, 2020). This high-profile *Salmonella* outbreak along with recent *L. monocytogenes* outbreaks (Chen et al., 2016; Jackson et al., 2015) linked to peaches and stone fruit consumption identified

peaches as a potential vehicle for foodborne pathogens, highlighting the need for controlling these pathogens in stone fruits.

Peaches are routinely sorted, washed, and packed in packing facilities for further distribution and marketing. Stone fruits can be contaminated by pathogenic microorganisms along with the production continuum. It is generally held that the postharvest handling and packing environment can be a source of contamination of produce. *Listeria* spp. was detected in a stone fruit cold storage room (Duvenage and Korsten, 2017). The *L. monocytogenes* level on contaminated peaches can be as high as 3.5 $\log_{10}$ CFU/fruits (Chen et al., 2016). *L. monocytogenes* artificially introduced to peaches and nectarines remain stable on peaches and nectarines during simulated stone-fruit packinghouse unloading, 18 hours of staging temperature conditions, and holding following fruit waxing under different holding conditions, regardless of inoculation level and fruit wax coating (Kuttappan et al., 2021). Similarly, *L. monocytogenes* artificially inoculated on stone fruits at practical contamination loads can survive on stone fruit surfaces during 26 days of refrigerator storage; the survival of *L. monocytogenes* was not impacted by strain/serotype (4b and 1/2b) and fruit type (commercially processed peach or nectarine) (De Jesus et al., 2020). These studies demonstrated that foodborne pathogens could survive and persist on peaches and other stone fruits, highlighting the need for effective control methods.

Although the addition of sanitizing agents in peach wash water is a standard industry practice, specific practices and process designs are highly variable among industry operations. Therefore, there is a general lack of knowledge regarding the practical efficacy of brush bed spray wash against foodborne pathogens including *Salmonella and L. monocytogenes* on stone fruits received from the orchard. Peaches are commonly washed with chlorinated water; however, its effectiveness varies depending on the washing conditions and line design. There is also safety concern about the production of carcinogenic halogenated by-products resulting from chlorinated organic compounds (Parish et al., 2003). Chlorine dioxide has been used as an alternative sanitizer (Mahmoud et al., 2008), and one log reduction of *Salmonella* was achieved when peaches were treated with 1.4 ppm chlorine dioxide for 5 min (Sy et al., 2005). Ozone is a strong oxidizing agent and provides a broad-spectrum antimicrobial effect (Khadre et al., 2001). However, due to practical experience and economic issues associated with the use of aqueous ozone in commercial stone fruit packing operations, this project did not include ozone. Peroxyacetic acid (PAA) is FDA approved to be used at 80 ppm as a wash water processing aid on fresh produce, without further rinse requirements. PAA at 80 ppm has better efficacy against *L. monocytogenes* on fresh apples than chlorine at 100 ppm free chlorine (FC) (Shen et al., 2019). Its efficacy against foodborne pathogens was less influenced by organic matter (Ruiz-Cruz et al., 2007). However, PAA is less commonly used in stone fruit packing due to concerns about its potential impact on fruit quality.

Testing antimicrobial interventions under commercial packing conditions requires a reliable non-pathogenic surrogate to assess the response or predict the fate of target foodborne pathogens in these facilities. Since all *Salmonella* serotypes/strains are pathogens, a non-pathogenic surrogate is essential. *Enterococcus faecium* NRRL B-2354 is a commonly used surrogate of *Salmonella* in validations involving low-moisture foods (ABC, 2014; Ceylan and Bautista, 2015; Jeong et al., 2011; Kopit et al., 2014; Zhu et al., 2021). *E. faecium* NRRL B-2354 was reported as a suitable surrogate of *Salmonella* during PAA-based sanitizer treatment of flax and chia seeds (Hylton et al., 2019) and gaseous chlorine dioxide treatment of almonds (Rane et al., 2021). Additionally, *E. faecium* NRRL B-2354 is an appropriate surrogate of *L. monocytogenes* for chlorine and PAA-based sanitizer interventions on apples (Sheng et al., 2020). In this study, its potential as a non-pathogenic surrogate for both *Salmonella* and *L. monocytogenes* for peach antimicrobial treatments was further evaluated. The overall goal of the project was to assess and validate critical operating parameters for commercially used practical and GRAS sanitizers against *L. monocytogenes*, *Salmonella*, and their surrogate and to further seek to verify this efficacy on multiple commercial peach packing lines.

Research Methods and Results

A. Research Methods

Objective 1: Validate the efficacies of selected sanitizers against *Salmonella* and *Listeria monocytogenes* on peaches.

1. Strains

   Salmonella: Three outbreak serotypes/strains, including *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Salmonella* Newport were used to prepare a 3-strain cocktail inoculum.
   L. monocytogenes: A panel of three outbreak strains consisting of serotype 1/2a (cantaloupe outbreak), 1/2b (stone fruit outbreak), 4b (stone fruit or apple outbreak) was used to prepare a 3-strain cocktail inoculum.
   These strains were kept in a stock solution of trypticase soy broth supplemented with 0.6% (w/v) yeast extract (TSBYE) and 20% *(v/v)* glycerol at -80°C until used.

2. Peach

   Yellow-flesh peaches, harvested at commercial maturity, were obtained from a commercial peach packing facility in Fresno, California. All fruit were maintained in a walk-in cooler (1°C) until use. Before inoculation, the fruit was transferred to room temperature (22 ± 1°C, RT) for tempering before use.

3. Inoculum preparation

   Each strain was growth-phase synchronized twice in TSBYE broth by consecutively culturing at 37°C for 24 h, then pelletized by centrifugation and re-suspended in 0.1% peptone water to achieve the target population. To prepare a 3-strain *L. monocytogenes* or *Salmonella* inoculum cocktail, each respective strain suspension was mixed in a 1:1:1 ratio.

4. Inoculation of fruits

   Unwaxed fruit was individually and separately inoculated to establish 1×10^6 CFU/peach of *L. monocytogenes* or *Salmonella* per our established method. After inoculation, fruits were held at RT for 24 h to let inoculated bacteria attach to the fruit surface. Before each intervention, 12 fruits of each inoculation batch were randomly sampled immediately and 24 h after inoculation to confirm pathogen load and the uniformity of the inoculum on peaches. Fruits were randomly sampled prior to inoculation to assess initial levels of natural microbiota.

5. Antimicrobial intervention

   Inoculated or uninoculated peaches (24 h post-inoculation or post-wash, respectively) were subjected to hypochlorite (50, 100, and 150 ppm FC, pH 6.8, from sodium hypochlorite), chlorine dioxide (2.5 and 5.0 ppm), and with or without EpiClean (pH-neutral cleaner for fruits) for different durations (30 and 120 sec) on lab-scale and pilot brush bed spray wash systems following a standard commercial practice. Water was used as a control to show the bacterial reduction due to mechanical forces and factors other than antimicrobial activities. The effectiveness in controlling cross-contamination was further tested by passing inoculated and uninoculated fruits. The temperature of the wash solution was at ~22°C following standard commercial sanitizer spray-bar practice. All studies were repeated three times independently.

6. Microbial enumeration

   To enumerate the microbial populations on inoculated and uninoculated peaches, 10 mL of neutralizing buffer was added to each stomacher bag containing a peach, which was then hand rubbed for 120 s. The detached bacterial suspension was serially diluted and plated onto duplicate TSAYE (TSBYE with 1.5% agar) plates overlaid with Modified Oxford Agar (MOX) for *L. monocytogenes* and

Xylose Lysine Deoxycholate Agar (XLD) for *Salmonella*. The plates were then incubated at 37°C for 48 h. The residual bacterial populations in spent wash solutions were enumerated following the above-mentioned quantitative method. Additionally, the membrane filtration method was used for determining the residual bacterial populations in spent wash solutions, where 100 mL of spent chlorine solution was filtrated through a 0.2-µm analytical test filter funnel and followed by a rinse with neutralizing buffer. The membrane was then placed on TSAYE plates overlaid with MOX for *L. monocytogenes* and XLD for *Salmonella*, and incubated at 37°C for 48 h. For enrichment, 1 mL of neutralized spent wash solution was transferred into 9 mL of buffered *Listeria* enrichment broth and Rappaport Vassiliadis *Salmonella* enrichment broth for *L. monocytogenes* and *Salmonella*, respectively. The enrichments were then incubated at 30°C for 48 h for *L. monocytogenes* and 42°C for 48 h for *Salmonella*.

The rub solutions of uninoculated peaches after their respective chlorine intervention were serially diluted and the appropriate dilution was plated onto TSAYE plates for total plate counts and onto potato dextrose agar (PDA) plates to determine the yeast and mold counts. The TSAYE plates were incubated at 37°C for 24–48 h. The PDA plates were incubated at RT for 5 days.

7. Statistical analysis

   The data were reported as means ± standard error mean (SEM) averaged from three independent experiments with 10 peaches per treatment or two water samples per treatment in each independent study, unless specified. Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons using IBM SPSS version 20 (Chicago, IL, USA), and p-values that were equal to or less than 0.05 were considered statistically significant.

Objective 2: Verify the selected sanitizer interventions in representative commercial peach packing lines in California.

2.1: Validate *E. faecium* NRRL B-2354 as a suitable surrogate of *L. monocytogenes* and *Salmonella* for antimicrobial intervention in pilot wash line

1. Strains

   L. monocytogenes and Salmonella: The same *L. monocytogenes* and *Salmonella* strains were used as described in Objective 1.

   E. faecium NRRL B-2354: *E. faecium* NRRL B-2354 culture with rifampicin resistance that was previously obtained from Dr. Trevor Suslow (University of California, Davis) was used.

2. Peach

   Yellow-flesh peaches at commercial maturity were used.

3. Inoculum preparation and inoculation

   Inoculum preparation was conducted as described in the Objective 1 studies. Unwaxed peaches were individually and separately inoculated to establish 1×10^6 CFU/fruit of a 3-strain cocktail of *L. monocytogenes* or *Salmonella*, or *E. faecium* NRRL B-2354.

4. Antimicrobial intervention

   The antimicrobial intervention was conducted as described in the Objective 1 studies. The bactericidal effects were compared between *E. faecium* NRRL B-2354, *L. monocytogenes*, and *Salmonella*. All studies were repeated three times independently.

5. Intervention at the pilot mini spray-bar brush bed

   The most effective sanitizer treatment(s) identified from lab sanitizer intervention studies were further evaluated in a pilot spray washing line in the PI's lab (Figure 1) using *E. faecium* NRRL B-2354 as a non-

pathogenic surrogate. The mini spray-bar and brush bed system is equipped with two spray bars, and a flat brush bed with electronic control panel (Figure 1). During each trial, fruits inoculated with *E. faecium* NRRL B-2354 underwent spray-bar intervention of the selected sanitizer treatments. The experiment was independently repeated three times.

6. Survival enumeration

   The survival enumeration of *Salmonella* and *L. monocytogenes* was conducted as described in Objective 1. For *E. faecium* NRRL B-2354 enumeration, appropriate dilutions were plated on TSAYE plates with 40 μg/ml of rifampicin and incubated at 35°C for 48 h. If *E. faecium* NRRL B-2354 survival was below the limit of detection, the microbial suspension was enumerated for Presence/Absence after 48 h of enrichment in Enterococcosel broth. The enrichment positive samples were streaked onto both Enterococcosel agar and TSAYE with rifampicin plates. Presumptive positive colonies were further confirmed using PCR by targeting the *vanB* gene per our established method (Shen et al., 2020).

7. Statistical analysis

   The data were reported as means ± standard error mean (SEM) averaged from three independent experiments. Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons using IBM SPSS version 20 (Chicago, IL, USA), and p-values that were equal to or less than 0.05 were considered statistically significant.

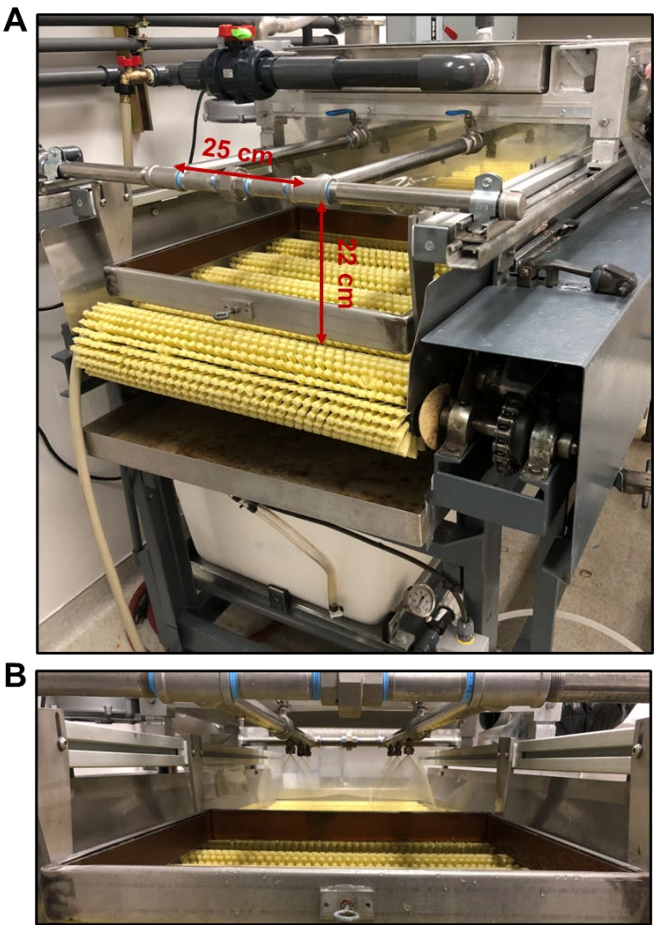

Figure 1. Schematic layout of the pilot mini spray-bar intervention at the WSU pilot processing facility. A. Overview of the pilot spray bar and brush bed. B. Spray bar with the loading tray. The flow rate of spray bar is 0.258 gallon/min and the brush bed rotating speed is 27 n/min.

2.2: Verify the selected sanitizer interventions in representative commercial stone fruit packing lines in California

1. Strain and inoculum preparation

   E. faecium NRRL B-2354 culture with rifampicin resistance was used for in-plant testing. *E. faecium* NRRL B-2354 inoculum preparation followed the procedures described in Objective 2.1.

2. Peach

   Yellow-flesh peaches at commercial maturity were used for testing.

3. Inoculation

   Unwaxed peaches were individually and separately inoculated to establish 1×10^5 CFU/peach of *E. faecium* NRRL B-2354 as described in the Objective 2.1 studies.

4. In-plant antimicrobial intervention

Commercial packing facilities selected in the study: Three commercial stone fruit packing facilities (Facilities A–C) with spray-bar brush bed systems were recruited for the validation study (Figure 2). The spray-bar system in the selected packing facilities all include a flat brush bed, and chlorine and/or chlorine dioxide spray-bar system. Each packing facility has its unique setting in terms of the distance between nozzles of the selected spray bar, the distance between the spray bars (Figure 2). The specific parameters of the respective packing facilities are outlined in Figure 2. The distance between spray bars ranged from 30–100 cm. The distance between nozzles was 25–34 cm. The heights from the spray bar nozzle to the brush bed were 25 cm for all three facilities. The flow rate, brush rotating speeds, water pH, temperature, and contact time are shown in Table 3.

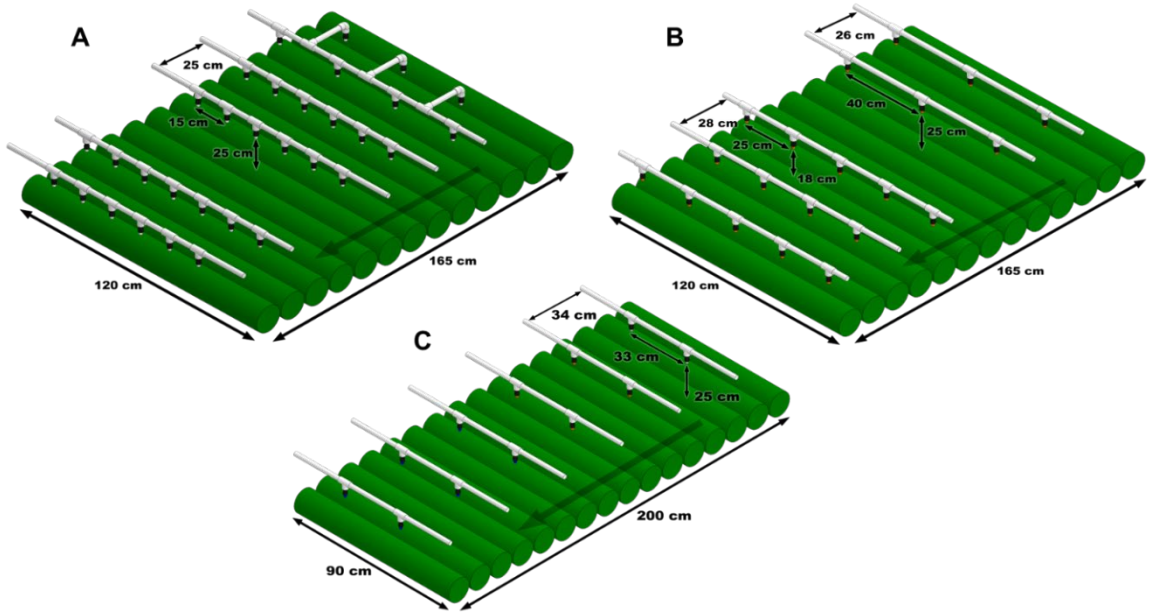

Figure 2. Schematic diagrams illustrating the layout of three commercial packing lines in California used for in-plant testing. A. Facility A, B. Facility B, C. Facility C.

Peach processing in the packinghouse: In each packing facility, 30–40 yellow flesh peaches inoculated with *E. faecium* NRRL B-2354 underwent the spray-bar sanitizer intervention. During the spray-bar intervention, peaches were exposed to a sanitizer treatment at the spray bar for the standard contact time (30 sec). Within each trial, 120–160 non-inoculated peaches were introduced with inoculated fruit at 1:4 to test for quantitative (enumeration) and qualitative (enrichment and qPCR confirmation) detection of cross-contamination. The concentrations of the sanitizer, as well as the temperature of the water and sanitizer solutions, were measured prior to each treatment. The brush bed was sanitized with chlorine between spray-bar sanitizer treatments. Brush beds were swabbed before and after sanitizer disinfection.

Peaches were sampled 1) before inoculation to document initial levels of natural microbiota, 2) after inoculation and 24-h hold-time, and 3) after each brush bed sanitizer treatment to evaluate reductions from water spray alone and different sanitizer spray washes. Peaches were chilled to ~4°C and transported to the local laboratory for microbial survival analyses. All peaches were processed within 24 h after collecting microbiological analyses per our established method (Shen et al., 2024) . The food contact surfaces in spray beds were also swabbed before and after each intervention. Surface swab samples were analyzed for a viable inoculated *E. faecium* NRRL B-2354 strain using standard quantitative and qualitative (enrichment and qPCR) per our established method (Shen et al., 2020).

5. Survival enumeration

   Microbial survival, as log-reduction, on inoculated peaches and transfers to uninoculated peaches, spray bed surfaces were analyzed per our established methods. Appropriate dilutions were plated on TSAYE plates with 40 μg/ml of rifampicin and enumerated after 48 h incubation at 35°C. If bacterial survival was below the limit of detection, the microbial suspension was enumerated for Presence/Absence after 48 h of enrichment in Enterococcosel broth. The enrichment positive samples were streaked onto both Enterococcosel agar and TSAYE with rifampicin plates. Presumptive positive colonies were further confirmed using PCR by targeting the *vanB* gene per our established method (Shen et al., 2020).

6. Statistical analysis

   The data were reported as means ± standard error mean (SEM). Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons using IBM SPSS version 20 (Chicago, IL, USA), and p-values that equal to or less than 0.05 were considered statistically significant.

B. Results

1. Evaluating the efficacy of chlorine sanitization for controlling *L. monocytogenes* and *Salmonella* on fresh peaches

1.1. Efficacy of chlorine intervention against *L. monocytogenes*

The initial inoculation level of *L. monocytogenes* on peaches was 6.3 $\log_{10}$ CFU/peach (Figure 3A). Following a 24h attachment at RT, the count of *L. monocytogenes* decreased to 5.7 $\log_{10}$ CFU/peach ($p < 0.05$) (Figure 3A). The tap water wash resulted in ~0.2 $\log_{10}$ CFU/peach reductions after 30 s or 2 min of contact (Figure 3B). A 30 s exposure to chlorine at 50 ppm FC resulted in a reduction in *L. monocytogenes* by 0.9 $\log_{10}$ CFU/peach (Figure 3B). The efficacy of chlorine improved with an increasing concentration ($p < 0.05$); FC at 100 and 150 ppm caused 1.61 and 1.79 $\log_{10}$ CFU/peach reductions in *L. monocytogenes* after 30 s (Figure 3B). Extending the contact time from 30 s to 2 min resulted in a slight but significant improvement in the antimicrobial efficacy of chlorine against *L. monocytogenes* at their respective concentrations (Figure 3, $p < 0.05$).

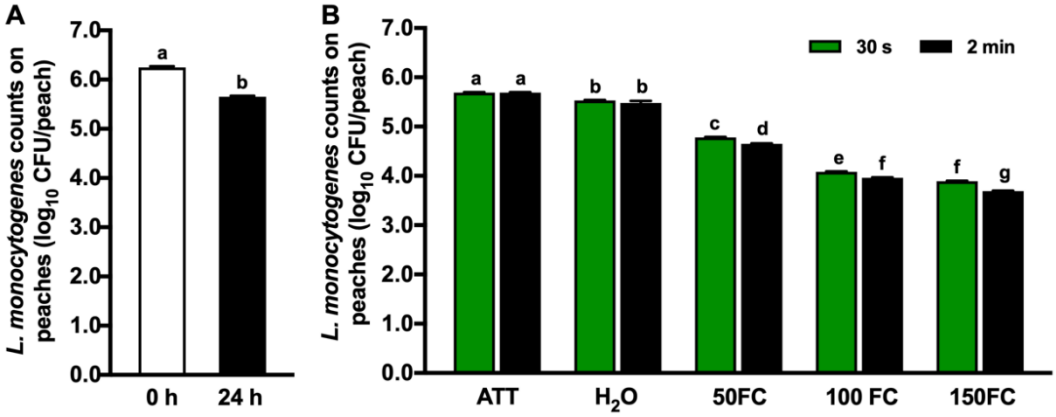

Figure 3. Efficacy of chlorine against *L. monocytogenes* on peaches with 30 s or 2 min contact. (A) *L. monocytogenes* counts on peaches immediately after inoculation (0 h) and 24 h post-inoculation (24 h). (B) *L. monocytogenes* recovered on peaches after 30 s or 2 min contact in the respective wash solution. ATT: peaches 24 h post-inoculation. H_2O: peaches washed with tap water. FC: free chlorine. 50 FC, 100 FC, or 150 FC: peaches washed with chlorine solution at 50 mg/L FC, 100 mg/L FC, or 150 mg/L FC. Mean ± SEM averaged from three independent studies with 10 peaches/treatment in each independent study. Histogram bars without common letters differ significantly ($p < 0.05$).

1.2. Efficacy of chlorine intervention against *Salmonella*

The inoculation level of *Salmonella* on peaches and the counts 24 h after inoculation were comparable to those of *L. monocytogenes* (Figure 4A). Likewise, tap water wash had limited efficacy in reducing *Salmonella* on peaches, regardless of the contact time (Figure 4B). Chlorine led to a dose-dependent reduction in *Salmonella* on peaches ($p < 0.05$) (Figure 4B). After 30 s of contact with 50, 100, and 150 ppm FC, the viable count of *Salmonella* on peaches was reduced by 0.9, 1.5, and 1.7 $\log_{10}$ CFU/peach, respectively (Figure 4B). Noticeably, the magnitude of pathogen reduction became smaller after the FC reached 100 ppm, irrespective of the pathogen type. Increasing the contact time from 30 s to 2 min resulted in an additional reduction in *Salmonella* on peaches by ~ 0.2 $\log_{10}$ CFU/peach for the chlorine intervention with 50–150 ppm FC, respectively ($p < 0.05$, Figure 4B). Additionally, chlorine at the tested concentrations showed comparable efficacy against *L. monocytogenes* and *Salmonella* at each concentration ($p > 0.05$, Figure 3B and 4B).

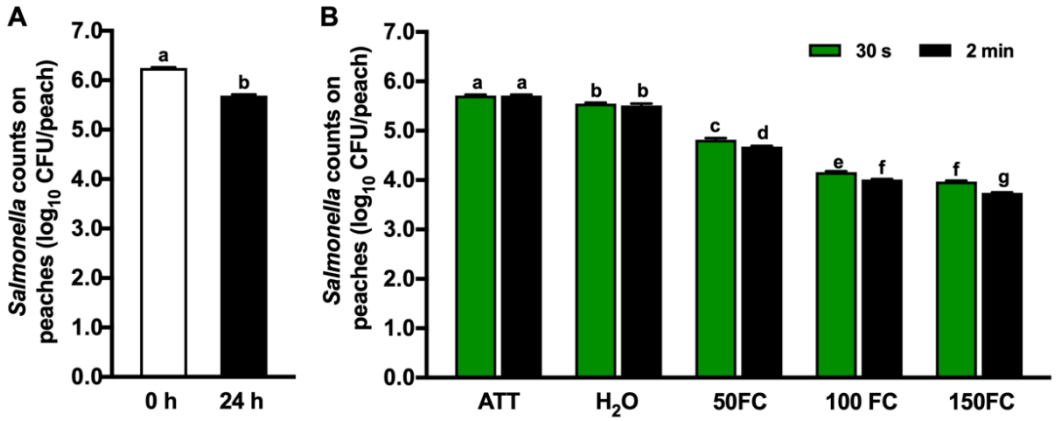

Figure 4. Efficacy of chlorine against *Salmonella* on peaches with 30 s or 2 min contact. (A) *Salmonella* counts on peaches immediately after inoculation (0 h) and 24 h post-inoculation (24 h). (B) *Salmonella* counts recovered on peaches after 30 s or 2 min contact. ATT: peaches 24 h post-inoculation. H_2O: peaches washed with tap water. FC: free chlorine. 50 FC, 100 FC, or 150 FC: peaches washed with chlorine solution at 50, 100, or 150 ppm free chlorine (FC). Mean ± SEM averaged from three independent studies with 10 peaches/treatment in each independent study. Histogram bars without common letters differ significantly ($p < 0.05$).

1.3. Efficacy of chlorine intervention in the prevention of cross-contamination

During the 30 s to 2 min exposure in tap water, *L. monocytogenes* transferred from inoculated peaches to uninoculated peaches and water by ~ 2.9 $\log_{10}$ CFU/peach and 1.7–1.9 $\log_{10}$ CFU/mL, respectively (Table 1). A higher rate of *Salmonella* transfer from inoculated fruits to uninoculated fruits (3.0 $\log_{10}$ CFU/peach) and wash water (2.3–2.4 $\log_{10}$ CFU/mL) was observed in tap water without sanitizers (Table 2). The transfer of bacteria to uninoculated fruit and water decreased with the increased chlorine concentration, regardless of the contact time (Tables 1 and 2). *L. monocytogenes* was transferred from inoculated peaches to uninoculated peaches by ~2.7, ~2.5, and 2.0–2.1 $\log_{10}$ CFU/peach in chlorinated water with 50, 100, and 150 ppm FC, respectively, after up to 2 min of contact time. The levels recovered in the spent wash water were ~0.7 $\log_{10}$ CFU/mL, 1.0–1.2 $\log_{10}$ CFU/100 mL, and ~1 $\log_{10}$ CFU/100 mL, respectively (Table 1). The efficacy of chlorine in controlling the cross-contamination of *Salmonella* from inoculated fruits to uninoculated fruits was not different from that of *L. monocytogenes* ($p > 0.05$) (Tables 1 and 2). However, the counts of *Salmonella* recovered in the spent wash solution were lower than those of *L. monocytogenes* (Tables 1 and 2).

Table 1. Efficacy of chlorine intervention in preventing the cross-contamination of *Listeria monocytogenes* from inoculated peaches to uninoculated peaches and washing solutions.

Contact Time	Treatment	Inoculated Fruit Reduction (log_{10} CFU/Peach)	Uninoculated Fruit Recovery (log_{10} CFU/Peach)	Recovered in Spent Wash Solution	
				Plating (log_{10} CFU/mL)	MF (log_{10} CFU/100 mL)
30 s	Tap water	0.12 ± 0.03 [a]	2.87 ± 0.01 [a]	1.73 ± 0.04 [a]	/
	50 ppm FC	0.94 ± 0.02 [b]	2.65 ± 0.06 [b]	0.73 ± 0.09 [b]	/
	100 ppm FC	1.68 ± 0.01 [d]	2.52 ± 0.01 [c]	/	1.17 ± 0.04 [a]
	150 ppm FC	1.83 ± 0.02 [e]	2.14 ± 0.11 [d]	/	0.98 ± 0.09 [a]
2 min	Tap water	0.17 ± 0.04 [a]	2.91 ± 0.01 [a]	1.91 ± 0.01 [c]	/
	50 ppm FC	1.06 ± 0.02 [c]	2.67 ± 0.04 [b]	0.68 ± 0.10 [b]	/
	100 ppm FC	1.76 ± 0.02 [e]	2.45 ± 0.06 [c]	/	1.00 ± 0.08 [a]
	150 ppm FC	2.00 ± 0.02 [f]	2.00 ± 0.12 [d]	/	0.99 ± 0.07 [a]

MF: membrane filtration. FC: free chlorine. Each intervention was independently repeated three times. Means ± SEM, n = 9 for inoculated peaches, n = 36 for uninoculated peaches, and n = 6 for wash water samples. [a–f] means within a column with different letters differ significantly ($p < 0.05$).

Table 2. Efficacy of chlorine intervention in preventing the cross-contamination of *Salmonella* from inoculated peaches to uninoculated peaches and washing solutions.

Contact Time	Treatment	Inoculated Fruit Reduction (log_{10} CFU/Peach)	Uninoculated Fruit Recovery (log_{10} CFU/Peach)	Recovered in Spent Wash Solution	
				Plating (log_{10} CFU/mL)	MF (log_{10} CFU/100 mL)
30 s	Tap water	0.15 ± 0.02 [a]	3.01 ± 0.06 [a]	2.41 ± 0.02 [a]	/
	50 ppm FC	0.99 ± 0.02 [b]	2.72 ± 0.04 [b]	/	0.57 ± 0.17 [a]
	100 ppm FC	1.67 ± 0.01 [d]	2.67 ± 0.06 [bc]	/	0.18 ± 0.11 [b]
	150 ppm FC	1.86 ± 0.01 [f]	2.56 ± 0.08 [bc]	/	0.00 ± 0.00 [b]
2 min	Tap water	0.21 ± 0.04 [a]	3.01 ± 0.04 [a]	2.31 ± 0.13 [a]	/
	50 ppm FC	1.11 ± 0.01 [c]	2.71 ± 0.09 [bc]	/	0.15 ± 0.07 [b]
	100 ppm FC	1.79 ± 0.01 [e]	2.56 ± 0.03 [c]	/	0.26 ± 0.18 [b]
	Tap water	2.03 ± 0.02 [g]	2.38 ± 0.08 [d]	/	0.23 ± 0.13 [b]

MF: membrane filtration. FC: free chlorine. Each intervention was independently repeated three times. Means ± SEM, n = 9 for inoculated peaches, n = 36 for uninoculated peaches, and n = 6 for wash water samples. [a–f] means within a column with different letters differ significantly ($p < 0.05$).

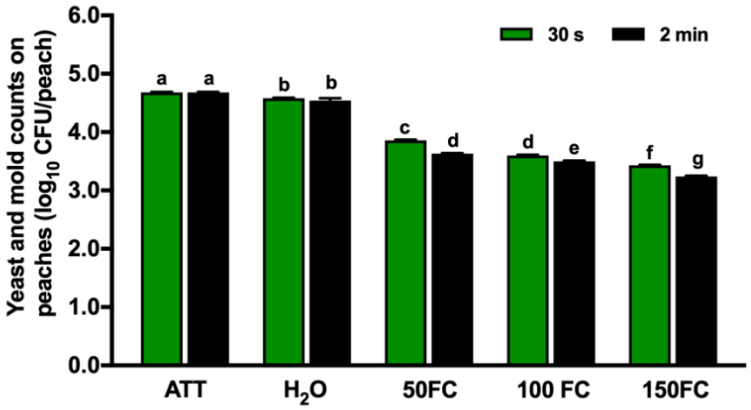

Figure 5. Efficacy of chlorine against yeasts and molds on uninoculated peaches after 30 s or 2 min contact. H_2O: peaches washed with tap water. 50 FC, 100 FC, or 150 FC: peaches washed with chlorine solution at 50, 100, or 150 ppm free chlorine. Mean ± SEM, n=30. Histogram bars without common letters differ significantly ($p < 0.05$).

1.4 Efficacy of chlorine interventions against yeast and mold on peaches

The yeast and mold count on peaches was 4.7 $\log_{10}$ CFU/peach. A tap water wash of up to 2 min had a limited ability to remove yeasts and molds, achieving only a ~0.1 $\log_{10}$ CFU/peach reduction (Figure 5). The application of chlorine and the extension of the contact time at the respective chlorine concentrations improved ($p < 0.05$) the removal of yeasts and molds. Exposure to 50, 100, and 150 ppm FC for 30 s to 2 min reduced yeasts and molds by 0.8–1.1, 1.1–1.2, and 1.2–1.4 $\log_{10}$ CFU/peach, respectively (Figure 5).

2. Evaluating the efficacy of chlorine dioxide against *L. monocytogenes* and *Salmonella* on peaches

2.1. Efficacy of chlorine dioxide against *L. monocytogenes* and *Salmonella*

Chlorine dioxide led to a dose-dependent reduction in *L. monocytogenes* and *Salmonella* on peaches ($p < 0.05$) (Figure 6). Chlorine dioxide at the tested concentrations showed comparable efficacy against *L. monocytogenes* and *Salmonella* at each concentration ($p > 0.05$, Figure 6), regardless of contact time. A 30 s exposure to 2.5 ppm and 5.0 ppm chlorine dioxide led to 0.7 and 1.1 $\log_{10}$ CFU/peach reductions, respectively (Figure 6A). Extending the contact time to 2 min resulted in an additional ~0.2 $\log_{10}$ CFU/peach reduction at 2.5–5.0 ppm chlorine dioxide ($p < 0.05$, Figure 6B).

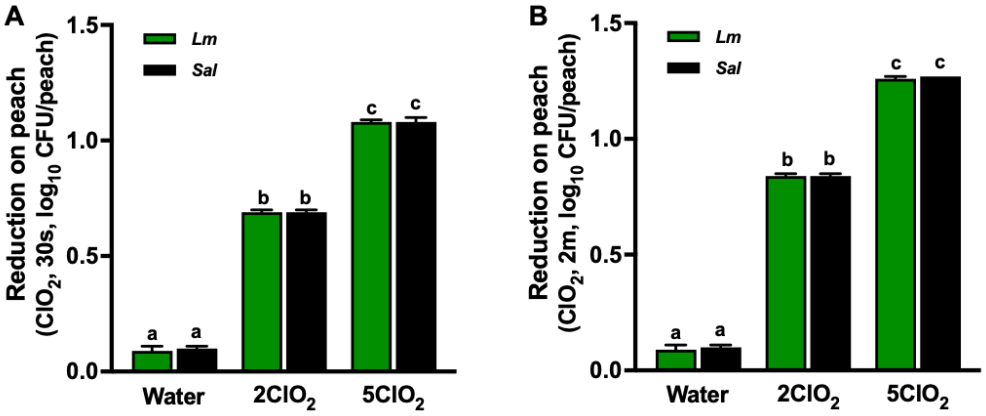

Figure 6. Efficacy of chlorine against *L. monocytogenes* (*Lm*) and *Salmonella* (*Sal*) on peaches with 30 s or 2 min contact. (A) 30 s. (B) 2 min. $2ClO_2$: 2.5 ppm chlorine dioxide. $5ClO_2$: 5.0 ppm chlorine dioxide. Mean ± SEM averaged from three independent studies with 10 peaches/treatment in each independent study. Histogram bars without common letters differ significantly ($p < 0.05$).

2.2. Efficacy of chlorine dioxide treatment in the prevention of cross-contamination

During a 30 s exposure in tap water, *L. monocytogenes* transferred from inoculated to uninoculated peaches and wash water by ~ 2.9 $\log_{10}$ CFU/peach and 1.8 $\log_{10}$ CFU/mL, respectively. *Salmonella* exhibited a higher transfer rate to wash water (2.5 $\log_{10}$ CFU/mL) in the absence of sanitizers. The transfer of bacteria to uninoculated fruit and water decreased with the increased chlorine dioxide concentration, regardless of strain. In a water system with 2.5 and 5.0 ppm chlorine dioxide, *L. monocytogenes* transferred to uninoculated peaches at ~2.7 and 2.6 $\log_{10}$ CFU/peach, with 1.2 and 0.8 $\log_{10}$ CFU/mL recovered in wash water, respectively. The levels recovered in the spent wash water were 1.2 and 0.8 $\log_{10}$ CFU/mL, respectively. *Salmonella* was transferred from inoculated peaches to uninoculated peaches by ~ 2.3 and 2.2 $\log_{10}$ CFU/peach in a water system with 2.5 and 5.0 ppm chlorine dioxide, respectively. The counts of *Salmonella* recovered in the spent wash solution were lower than those of *L. monocytogenes* ($p < 0.05$)

3. Evaluate the efficacy of the selected sanitizer in the pilot line

3.1 The suitability of *E. faecium* NRRL B-2354 as a surrogate for *L. monocytogenes* and *Salmonella* in sanitizer intervention

To validate sanitizer efficacy in commercial packing lines, a non-pathogenic surrogate is essential. We compared the bactericidal effects of chlorine and chlorine dioxide on *E. faecium* NRRL B-2354 relative to *L.*

monocytogenes and *Salmonella*. Results showed that chlorine and chlorine dioxide achieved comparable reductions across all tested organisms. A 30 s exposure to 50 ppm and 150 ppm FC resulted in 0.8–0.9 and 1.7 $\log_{10}$ reductions, respectively. Similarly, a 30 s treatment with 2.5 ppm and 5.0 ppm chlorine dioxide led to 0.9 and 1.1 $\log_{10}$ reductions in *E. faecium* NRRL B-2354, mirroring reductions observed for *L. monocytogenes* and *Salmonella*. Further, when chlorine and chlorine dioxide were combined with EpiClean, a pH-neutral cleaner commonly used in stone fruit packing, additional reductions were observed, with *E. faecium* NRRL B-2354 exhibiting responses similar to those of the pathogens. These findings collectively support the use of *E. faecium* NRRL B-2354 as a suitable surrogate for validating chlorine-based sanitizer efficacy in both pilot line and commercial packing lines.

3.2 Evaluation of sanitizer effectiveness in the pilot line using *E. faecium* NRRL B-2354 as a surrogate

The antimicrobial efficacy of chlorine against *E. faecium* NRRL B-2354 on peaches was evaluated using a pilot-scale spray-bar and brush bed system at WSU (Figure 1). A 30 s spray bar application of 50 ppm, 100 ppm, and 150 ppm FC resulted in *E. faecium* NRRL B-2354 reductions of 1.1, 2.0, and 2.2 $\log_{10}$ CFU/peach, respectively. In comparison, a tap water wash achieved a 0.6 $\log_{10}$ CFU/peach reduction, which was significantly greater than reductions observed in submerged interventions, likely due to the mechanical action of the brush bed. Consistent with lab-scale findings, incorporating EpiClean at the manufacturer's recommended concentration significantly enhanced chlorine efficacy. The addition of EpiClean to 50 ppm, 100 ppm, and 150 ppm FC treatments resulted in additional reductions of 1.1, 0.6, and 0.8 $\log_{10}$ CFU/peach, respectively.

4. Evaluate the efficacy of chlorine and chlorine dioxide sanitizer in three commercial stone fruit packing facilities using *E. faecium* NRRL B-2354

We further validated the efficacy of selected sanitizer treatments in three commercial stone fruit packing facilities (Facilities A–C) using spray bars and brush bed systems (Figure 2). The antimicrobial efficacy of chlorine at 50 ppm and 100 ppm FC and chlorine dioxide at 5 ppm, with and without EpiClean, was evaluated on fresh peaches during commercial packing. A water spray wash was included to assess bacterial reduction due to mechanical factors rather than antimicrobial activity. In-plant testing across the three facilities revealed that a 30 s water spray wash alone achieved an *E. faecium* NRRL B-2354 reduction of 0.8–1.3 $\log_{10}$ CFU/peach. Treatment with chlorine at 50 ppm and 100 ppm FC at ambient temperature for 30 s resulted in *E. faecium* reductions of 2.2–2.3 $\log_{10}$ and 2.4–2.9 $\log_{10}$ CFU/peach, respectively, on inoculated fruit across all facilities. Incorporating EpiClean into the chlorine spray wash further enhanced reductions by 0.6 $\log_{10}$ CFU/peach for 50 ppm FC and 0.1 $\log_{10}$ CFU/peach for 100 ppm FC. Similarly, a 30 s exposure to 5 ppm chlorine dioxide reduced *E. faecium* NRRL B-2354 by 2.4, 2.6, and 2.4 $\log_{10}$ CFU/peach in Facilities A, B, and C, respectively. The addition of EpiClean to the chlorine dioxide treatment further improved reductions by 0.3 $\log_{10}$ CFU/peach. Differences in *E. faecium* reduction across facilities might be attributed to variations in spray bar and brush bed layouts, design, and contact time (Figure 2 and Table 3). A 30 s water spray bar intervention resulted in the transfer of *E. faecium* NRRL B-2354 to uninoculated fruit at a 1:4 cross-contamination ratio, with 2.3–2.5 $\log_{10}$ CFU/peach transferred. Sanitizer spray treatments significantly reduced cross-contamination; however, the extent of reduction varied among facilities. These differences may be attributed to variations in spray and brush system parameters, handling practices, and sanitizer concentration.

Table 3. Spray bar and brush bed parameters in three commercial stone fruit packing lines.

Facility	Brush speed (rpm)	Water pressure (psi)	Water temperature (°C)	Water pH	Flow rate (L/min)	Contact time (s)
A	60	40	21.6 ± 0.2	6.8± 0.1	1-1.6	20-30
B	60	32	22.0 ± 0.1	6.9 ± 0.1	1.1-2.1	26-34
C	60	30	22.0 ± 0.2	7.0 ± 0.1	2.0	25-31

Outcomes and Accomplishments

1. *E. faecium* NRRL B-2354 was shown to be a reliable surrogate for *L. monocytogenes* and *Salmonella* in chlorine and chlorine dioxide spray-bar interventions, making it suitable for sanitizer efficacy evaluations in commercial settings.
2. Spray-bar water wash, when applied in brush bed system, led to reductions of 0.8 to 1.4 log CFU/peach higher than observed in the submersion interventions, indicating the mechanical action of the brush bed aiding bacterial removal from peaches.
3. Chlorine (50–150 ppm free chlorine) and chlorine dioxide (2.5–5.0 ppm) effectively reduced *L. monocytogenes* and *Salmonella* on peaches in a dose-dependent manner.
4. Chlorine at 100 ppm FC was more effective than 5 ppm chlorine dioxide, achieving up to 2.9 log reduction within 30 s.
5. Chlorine at 50 ppm FC was slightly less efficient than 5 ppm chlorine dioxide, with reductions of 2.2–2.3 log versus 2.4–2.6 log, respectively.
6. Incorporating EpiClean into the sanitizer solutions significantly enhanced pathogen reduction, particularly for 50 ppm FC and 5 ppm chlorine dioxide.
7. Significant transfer of pathogens to uninoculated peaches occurred during the spray water brush bed intervention. Adding sanitizers, regardless of type and concentration, reduced cross-contamination; however, the effectiveness varied among facilities, likely due to differences in equipment design and operational practices.

APPENDICES

Publications and Presentations

1. Shen, X., Hang, M., Su, Y., de Avila, J.M., Zhu, M.J., 2024. Evaluating chlorine sanitization at practical concentrations for controlling *Listeria monocytogenes* and *Salmonella* on fresh peaches. Foods 13:3344.
2. Three additional manuscripts are currently in preparation for submission to scientific journals.
3. In addition to poster presentations made at the annual CPS Research Symposium in 2023 and 2024, a couple of meeting abstracts and presentations will be presented to 2025 Washington Association for Food Protection (WAFFP).

Budget Summary

This project was awarded $398,154 in research funds. All grant funds awarded were spent by the end of the project period in executing the planned and modified objectives associated with this project.

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