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Research article

Evaluating the efficacy of peroxyacetic acid in preventing *Salmonella* cross-contamination on tomatoes in a model flume system

Christopher R. Pabst [a], Karuna Kharel [a], Jaysankar De [a,b], Cameron A. Bardsley [a,c], Bruna Bertoldi [a], Keith R. Schneider [a,*]

[a] *Department of Food Science and Human Nutrition, University of Florida, Gainesville, FL, 32611, USA*
[b] *Department of Microbiology & Cell Science, University of Florida, Gainesville, FL, 32611, USA*
[c] *USDA-ARS Southeastern Fruit and Tree Nut Research Station, Byron, GA, 31008, USA*

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ABSTRACT

The use of flume tanks for tomato processing has been identified as a potential source of cross-contamination, which could result in foodborne illness. This study's objective was to assess the efficacy of peroxyacetic acid (PAA) at a concentration of ≤80 mg/L in preventing *Salmonella enterica* cross-contamination under various organic loads in a benchtop model tomato flume tank. The stability of 80 mg/L PAA at different chemical oxygen demand (COD) levels was also tested. Tomatoes were spot inoculated with a five-serovar rifampin-resistant (rif+) *Salmonella* cocktail (10^6 or 10^8 colony forming unit (CFU)/tomato). Inoculated (n = 3) and uninoculated (n = 9) tomatoes were introduced into the flume system containing 0–80 mg/L PAA and 0 or 300 mg/L COD. After washing for 30, 60, or 120 s, uninoculated tomatoes were sampled and analyzed for cross-contamination. All experiments were conducted in triplicate. Increasing the organic load (measured as COD) affected the stability of PAA in water with significantly faster dissociation when exposed to 300 mg/L COD. The concentration of PAA, inoculum level, COD levels, and time intervals were all significant factors that affected cross-contamination. Cross-contamination occurred at the high inoculum level (10^8 CFU/tomato) even when 80 mg/L PAA was present in the model flume tank, regardless of the organic load level. When the tomatoes were contaminated at a level of 10^6 CFU/tomato, concentrations as low as 5 mg/L of PAA were effective in preventing cross-contamination at 0 mg/L COD; however, 100 % tomatoes (9/9) were positive when the organic load increased to 300 mg/L COD. When the PAA concentration was increased to 10 mg/L, it effectively prevented cross-contamination in the tank, regardless of the presence of organic load. These results suggest that using PAA at concentrations below the maximum limit remains effective in limiting bacterial cross-contamination and offers a more environment-friendly option for tomato packinghouse operators.

1. Introduction

Even with recent sanitary advancements, the risk of cross-contamination of pathogens in tomato wash water may occur if sanitizer

* Corresponding author.
E-mail address: keiths29@ufl.edu (K.R. Schneider).

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levels and water quality are poorly maintained [1]. It has been shown that effective levels of sanitizers are vital in preventing the transfer of human microbial pathogens in tomato wash water [2–5]. A major concern surrounding sanitizer efficacy is maintaining water quality during packinghouse operations. The accumulation of soil and other debris during the recirculation of the flume water can decrease the sanitizer efficacy [4,6,7]. In extreme cases, the overuse of sanitizing chemicals can lead to equipment corrosion and, in excessive quantities, may pose health-related issues for workers [8]. Therefore, research on optimizing sanitizer concentration is paramount.

In the past, fresh tomatoes, typically consumed raw, have been associated with several *Salmonella* outbreaks [9]. Tomatoes can be exposed to microbial contamination at several stages during production. In many packing operations, post-harvest tomatoes are dumped from field bins into water-filled flume tanks containing sanitizers. This water is often recirculated to lower sanitizer costs, reduce the environmental impact of excessive water use, and minimize the impact of sanitizer-laden wash water disposal [10]. Consequently, the longer a packinghouse reuses wash water, the potential for microbial cross-contamination increases. This loss of sanitizer efficacy occurs due to the build-up of organic compounds and accumulating debris in the water. Organic matter build-up in the flume water, as measured by chemical oxygen demand (COD), creates additional demand for the sanitizers, reducing their availability to inactivate microbes. This deteriorating water quality increases the potential for cross-contamination due to the resulting decrease in the efficacy of antimicrobial sanitizers [3,4,10].

Sanitizing agents such as sodium hypochlorite (NaClO; measured as free chlorine; $HOCl/OCl^-$) and peroxyacetic acid (also known as peracetic acid; PAA) are the most commonly used disinfectants in dump tanks and flumes in the fresh produce industry [11] to prevent/reduce the potential of cross-contamination. The application of PAA has been successful in various industries for its high oxidation capability and antimicrobial activity [12]. PAA has been shown to be an acceptable sanitizer to reduce cross-contamination in postharvest wash water [2,13–15]. PAA works by attacking bacterial membranes with hydroxyl radicals, rendering them inactive [13,16]. PAA has also been shown to have a variety of benefits over the more commonly used Cl-based sanitizers. It degrades into fewer harmful by-products and has greater stability in the presence of decaying organic matter [17,18]. Historically, PAA has been used at the Environmental Protection Agency's (EPA) maximum allowable concentration of 80 mg/L [19,20] and has been allowed by the US Food and Drug Administration (FDA) as per 21 Code of Federal Regulations (CFR) 173.315 for its use not exceeding 80 ppm in produce wash water [15]. However, little research exists on the efficacy of PAA in preventing cross-contamination at lower concentrations.

Tomato packers have indicated their interest in exploring whether reducing sanitizer use in their fluming operations (personal communication) might be beneficial in reducing costs while not compromising food safety. The use of 80 mg/L both as the minimum and maximum limits for PAA puts packers into a situation where going under this critical limit would lead to food being considered adulterated, when likely, the tomatoes could be safe for consumers. Conversely, exceeding the EPA allowable limit for a minimum PAA level leaves no room for fluctuation.

Despite the evidence to support PAA as a preferred antimicrobial sanitizer used in postharvest wash water, there has not been enough attention given to determining the efficacy at lower concentrations and their ability to prevent cross-contamination [1,2,21]. With many operators considering switching from Cl-based sanitizers to PAA, more research is needed on its efficacy, especially at concentrations <80 mg/L and under various organic loads in wash water. This study evaluated COD's influence on PAA's efficacy and the potential for *Salmonella* cross-contamination on tomatoes in a model flume system. Additionally, the stability of PAA in the presence of varying levels of COD in wash water was also studied.

2. Materials and methods

2.1. Model flume system

A laboratory model flume system consisting of a $58 \times 34 \times 24$ cm^3 water bath (model 2845, Thermo Scientific, Waltham, MA) was used for the study. The model flume tank was filled with 15 L of DI water at 25 °C (ambient laboratory temperature). Each experiment started by filling the 15 L water bath, followed by the addition of chemicals (PAA and soil-tea as COD) and/or inoculated and un-inoculated tomatoes per the requirement of the specific experiment.

2.2. Soil-tea preparation for simulating organic load (measured as COD)

To obtain the desired oxidant demand (measured as COD) level in the flume system, a previously described method by Gereffi, Sreedharan and Schneider (2015) [2] was used with slight modifications. Briefly, ca. 1.5 kg of autoclaved topsoil (Scotts® Premium Topsoil, The Scotts Miracle-Grow Company, Marysville, OH) was distributed into two filter bags (1 L; 0.33 mm mesh) (Nasco, Fort Atkinson, WI), placed into 15 L of DI water and allowed to steep overnight. The next day, any remaining liquid was pressed through the sample bag filtering mesh. The resulting filtered soil-water mixture, described as 'soil-tea,' was used as a COD stock solution for the model flume experiments. After determining the COD of the stock solution, the soil-tea was added to the flume in the appropriate amount to reach the desired COD levels (0, 100, or 300 mg/L). During a typical tomato packing shift (operated for ~8 h), a maximum COD of 300 mg/L was observed in tomato packinghouses in Florida [4] and, therefore, was selected as the maximum limit for this study. For measuring COD, a 0.2 mL wash water sample was placed in Orion COD test vials (Thermo Scientific, Chelmsford, MA), inverted several times for proper mixing, and then placed in a TR125 Reactor chemical digestion heating block (Orbeco-Hellige Inc., Sarasota, FL) and digested at 150 °C for 2 h. Following digestion, the vials were allowed to cool to room temperature in dark conditions for 45 min, and COD measurements were taken using AQUAfast II Orion AQ2040 COD Colorimeter (Thermo Scientific, Center Beverly, MA).

2.3. Peroxyacetic acid preparation

Peroxyacetic acid solutions were prepared less than 1 h before each experiment. PAA concentrations in the model flume systems were prepared by adding sufficient amounts of the 15.2 % commercial PAA stock solution (Tsunami® 100, Ecolab, St. Paul, MN) to the model flume system containing deionized water to achieve the target concentrations. PAA concentrations were measured using a portable DR/890 colorimeter (Hach, Loveland, CO) before and after each experiment.

2.4. Assessing the stability of PAA under different COD levels

This study monitored the concentration of two main components, peroxyacetic acid and hydrogen peroxide (H_2O_2), in a commercial PAA solution. The initial concentration of PAA was 80 mg/L in the model flume system, and residual concentration was monitored for 48 h under varying organic loads (0, 100, and 300 mg/L COD). An aquarium pump (VicTsing 300 L/H submersible water pump) was used to circulate the solution during the trials to ensure mixing. Both PAA and H_2O_2 were measured using a portable DR/890 colorimeter at 0, 0.25, 0.5, 1, 2, 4, 8, 24, and 48 h. At each targeted COD level, COD was also monitored immediately after PAA was mixed in using the colorimeter as described in section 2.2. All experiments were conducted in triplicate. The rate of change in concentration (mg/L/h), referred to as the degradation rate for PAA, was calculated as the slope by fitting a linear regression over the concentration-versus-time graph plotted for PAA using Microsoft® Excel (Version 16.83, Redmond, Washington).

2.5. Inoculum preparation

A cocktail of five *Salmonella enterica* serovars (200 mg/L rifampin-resistant), consisting of Saintpaul (tomato/pepper; MDD 32), Montevideo (tomato; MDD 22), Newport (tomato; MDD 314), Poona (cantaloupe; MDD237) and Typhimurium (outbreak strain (unknown source); ATCC 13311) were used in the study. Each strain was streaked onto tryptic soy agar (TSA) (Difco, Sparks, MD) slants with 200 mg/L rifampin (TSAR-200), incubated at 37 °C for 24 h, and stored at 4 °C until further use. To obtain a working 5-serovar cocktail of *Salmonella*, inoculum from five slants were transferred separately with a sterile loop (10 μL) to five separate tubes of 10 mL tryptic soy broth (TSB) (Beckton Dickinson, Sparks, MD) supplemented with 200 mg/L of rif (TSBR-200) and grown at 37 °C for 24 h. Following incubation, 10 μL of the overnight culture was transferred to 10 mL TSBR-200 tubes and incubated at 37 °C for 24 h. A total of three transfers were conducted before the final inoculum preparation. After three consecutive transfers, the five serovars of *Salmonella* (10 mL each) were transferred into individual 50 mL TSBR-200 containing flasks and incubated for ca. 18 h. The next day, 100 mL (20 mL of each serovar) of the 5-serovar cocktail was harvested and combined into a sterilized centrifuge tube (Fisher Scientific, Fair Lawn, NJ). The cocktail was vortexed and then centrifuged for 10 min at 3,000 rpm (985×*g*) (MR23i; Jouan SA, Saint-Herblain, France). The supernatant was decanted, and cell pellets were washed with 0.1 % buffered peptone water (BPW; Beckton Dickinson, Sparks, MD). The same procedure of washing the cell pellet was conducted two additional times. Finally, cell pellets were resuspended in 10 mL of BPW, resulting in a cocktail of a final concentration of approximately 10^9 CFU/mL. The enumeration of the final inoculum cocktail was carried out by 10-fold serial dilution with BPW and spread plating in xylose lysine deoxycholate agar (XLD) agar. The plates were incubated at 37 °C for 24 h, and the readings were expressed as CFU/mL.

2.6. Inoculation of tomatoes

Harvested mature green round tomatoes (*Solanum lycopersicum*) obtained from a Florida grower were placed on top of aluminum rings on a sanitized tray. Aluminum rings helped prevent the tomatoes from rolling off the tray when transported. Before inoculation, the inoculation sites on the tomato surfaces were marked with a black permanent marker for better visibility. Ten spots were marked in a circle around the blossom scar. A total of 100 μL of the 5-serovar *Salmonella* cocktail was spot inoculated to the surface around the blossom scar (10 μL placed on each spot) of the tomato. The study used dilutions of the *Salmonella* stock solutions to achieve inoculation rates of approximately 10^8 and 10^6 CFU/tomato. After inoculation, tomatoes were dried for 2 h in a biosafety hood to promote bacterial cell attachment to the surface.

2.7. Salmonella cross-contamination

Potential cross-contamination of *Salmonella* was studied in the model flume system at varying PAA concentrations and organic loads. Tomatoes inoculated with *Salmonella* at 10^6 CFU/tomato were exposed to 0, 5, 10, 20, 40, or 80 mg/L of PAA at either 0 or 300 mg/L COD. Tomatoes with *Salmonella* inoculated at 10^8 CFU/tomato were exposed to 0, 20, 40, or 80 mg/L of PAA at either 0 or 300 mg/L COD. To evaluate the cross-contamination of *Salmonella* in the model flume system, the desired levels of PAA and COD were added to the flume and allowed to mix for 60 s. Once thoroughly mixed, three inoculated and nine uninoculated tomatoes were introduced into the model flume. Individual tomatoes (uninoculated) were then sampled for the presence or absence of *Salmonella* after specific immersion time intervals of 30, 60, and 120 s. Three inoculated tomatoes were retained (unwashed) to be used as positive controls, and three uninoculated tomatoes were retained (unwashed) to be used as negative controls.

2.8. Salmonella recovery and enumeration

After each sample point, three uninoculated tomatoes were randomly withdrawn and individually placed into sterile Stomacher®

bags (Fisher Scientific, Springfield, NJ). Each bag contained 100 mL of TSB supplemented with 80 mg/L of rif. Each sample was then massaged and shaken by hand for 90 s. The bags were incubated at 37 °C for up to 48 h to test for the presence or absence of *Salmonella*. Following incubation, 10 μl of the enrichment was streaked onto TSAR-80 and XLD. TSAR-80 and XLD plates were incubated at 37 °C for 24 h for confirmation. Negative controls were examined to ensure the absence of naturally occurring rif + organisms present on the

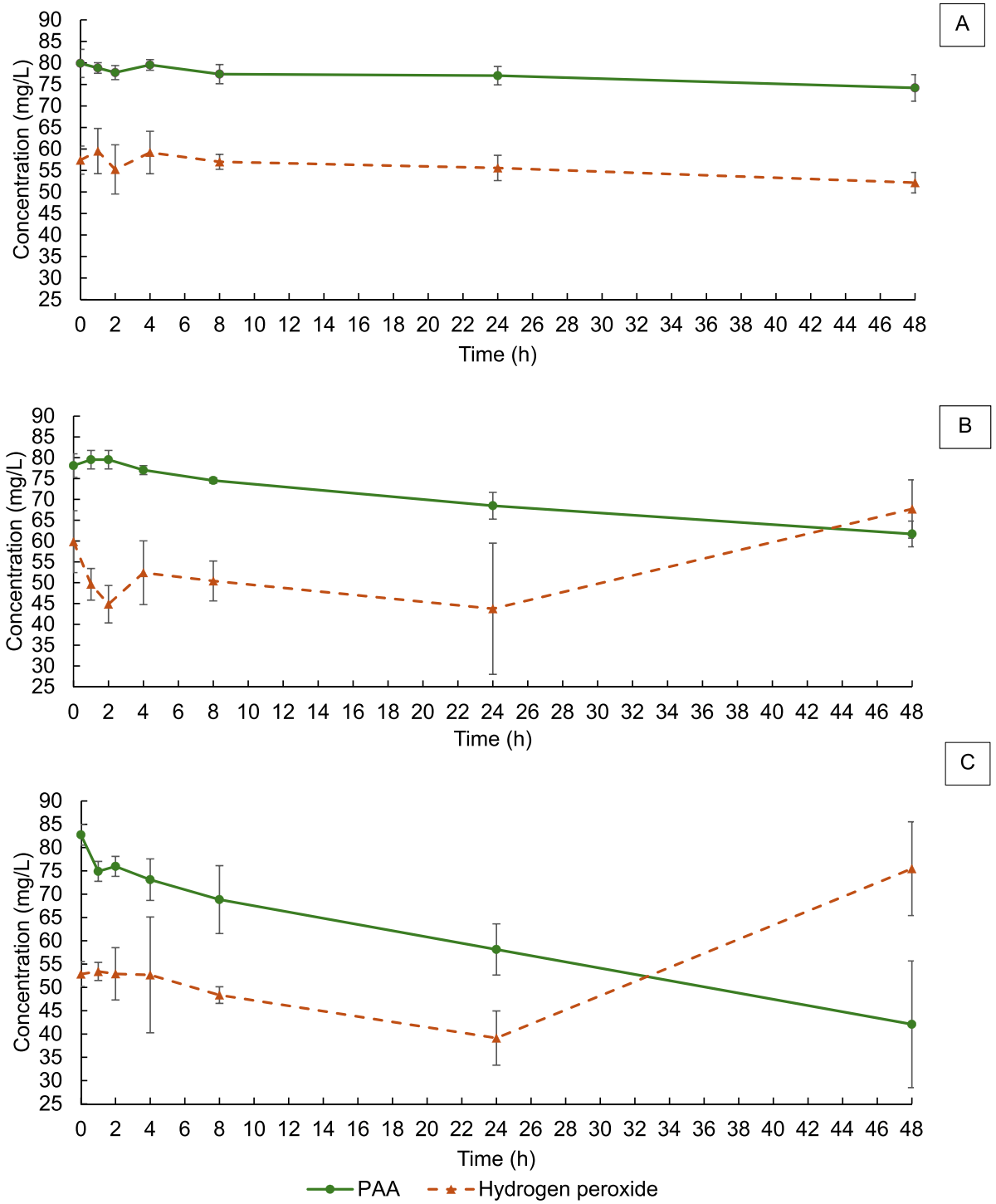

Fig. 1. Residual concentration of components of an 80 mg/L peroxyacetic acid (PAA): peroxyacetic acid and hydrogen peroxide over 48 h at three different chemical oxygen demand (COD) levels: (A) 0, (B) 100, and (C) 300 mg/L in the module flume tank. The experiment was conducted in triplicate (n = 3).

tomatoes.

2.9. Statistical analysis

A total of three trials were performed for each experiment. For each trial, triplicate samples were analyzed (n = 9). Unpaired *t*-test was used to identify significant differences in PAA degradation rates among various COD levels. One-way ANOVA followed by Tukey-Kramer Honest Significant Difference (HSD) was used to evaluate differences in PAA and COD concentrations before and after 48 h run ($P \leq 0.05$) using JMP® Pro 17.0.0 (SAS, Cary, NC). Binomial regression was used to model the effect of independent variables (inoculum, COD, PAA, and time) on the transfer of *Salmonella* (presence/absence). A subsequent chi-square test or Fisher's exact test (when expected observations were <5) was used to evaluate the significance of these variables in influencing *Salmonella* cross-contamination. When a significant difference was observed, a Fisher's Exact *post-hoc* pairwise comparison was used to identify differences ($\alpha = 0.05$). All statistical analyses, unless otherwise specified, were performed at the confidence level $\alpha = 0.05$ using GraphPad Prism 10.0.2 (GraphPad Software, Boston, MA).

3. Results and discussion

3.1. Stability of PAA under different COD levels

The residual concentration of PAA and H_2O_2 at different COD levels during 48 h and PAA degradation rate are presented in Fig. 1 (A–C) and Table 1, respectively. The presence of COD in wash water had a significant effect ($P \leq 0.05$) on the degradation rate of PAA (Table 1). In the absence of COD in the model flume tank, PAA concentrations declined at rates of -0.09 ± 0.08 mg/L/h (Table 1). Increasing the COD level to 100 mg/L in the tank showed no significant effect on their degradation rate. However, when the tank was maintained at 300 mg/L COD, PAA significantly declined ($P \leq 0.05$) at a rate of -0.76 ± 0.24 mg/L/h (Table 1). At the end of 48 h, the PAA concentration in the model flume tank significantly decreased ($P < 0.05$) from ~80 mg/L to a final concentration of 74.19 ± 3.09, 61.70 ± 3.09, and 42.09 ± 13.59 mg/L at 0, 100 and 300 mg/L COD, respectively (Table 2). The results confirm the previous findings that PAA is both statistically significant and positively correlated to COD levels in the water [22], indicating faster degradation in the presence of organic matter. Additionally, the introduction of PAA sanitizer into the system led to elevated levels of COD in the water, regardless of soil-tea addition. This was evidenced by higher levels of measured COD than that targeted in the water (Table 2 and Table S1). Even when no soil-tea was added as organic load in the model flume system (0 mg/L COD), a COD of 338.0 ± 11.9 mg/L was observed (Table 2). This underscores the potential of PAA alone to introduce and increase organic load in the water. Although chlorine and chlorine-based sanitizers are widely used in water treatment, they generate harmful by-products toxic to the environment and human health. PAA has stronger oxidation potential, higher disinfection efficiency, and lower toxicity than chlorine-based sanitizers [13,23]. The decomposition of PAA was found to follow a first-order kinetics indicating continuous decay, irrespective of organic load in the water, resulting in a reduction of its active concentration available for disinfection [24]. Consistent with these findings, this study also demonstrated a gradual decline in PAA concentration over time. Moreover, it was also observed that higher organic loads accelerated the PAA sanitizer depletion process in the water.

In addition to the peracetic acid, H_2O_2 is another component of the PAA sanitizer solution whose fate in the wash water has not been well documented. With no COD in the model flume tank, H_2O_2 concentration declined slowly over time (Fig. 1(A)). However, when the organic load in the water was increased, a depletion followed by an increase in its concentration was observed in Fig. 1(B and C). Several studies have researched the selective nature of oxidation mechanisms of organic compounds by PAA [13,25]. PAA in a solution is consumed by spontaneous decomposition or hydrolysis that ultimately, through several intermediate steps, gets converted to H_2O_2, acetic acid, and hydroxonium ions (H_3O^+) [11]. Generally, the H_2O_2 formed during the hydrolysis step further decomposes into water and oxygen. However, if the PAA hydrolysis rate is higher than the H_2O_2 decomposition rate, H_2O_2 could accumulate in the flume tank. As observed in Fig. 1(A–C) and Table 2, in the presence of higher organic load, significant depletion of PAA was observed over time. This reaction could be one of the reasons for the increase of H_2O_2 levels after 24 h observed in studies with 100 and 300 mg/L COD [Fig. 1(A–C)]. However, only one data point (at 48 h) to observe the increase in H_2O_2 may not be conclusive therefore,

Table 1
Degradation rates (mg/L/h) of peroxyacetic acid (PAA) over 48 h period under different chemical oxygen demand (COD) concentrations.[a]

COD (mgL)	Degradation rate	R^2
0	$-0.09 \pm 0.08_{\ b}$	0.84
100	$-0.37 \pm 0.09_{\ ab}$	0.97
300	$-0.76 \pm 0.24_{\ a}$	0.96

Values (means ± SD) with different small letters indicate a significant difference (n = 3).
Negative values indicate a decrease in concentration.
Degradation rate is obtained from the slope of the PAA concentration over 48 h data set using linear regression.
R^2 (coefficient of determination) indicates goodness of fit of the curve.

Table 2
Initial and final peroxyacetic acid (PAA) concentrations and actual chemical oxygen demand (COD) values in the flume tank maintained at 80 mg/L PAA concentration and varied COD levels.

Target COD (mg/L)	Initial PAA (mg/L)	PAA after 48 h (mg/L)	Initial measured COD (mg/L)
0	$79.89 \pm 3.27_{aA}$	$74.19 \pm 3.09_{aA}$	$338.0 \pm 11.9_{B}$
100	$78.11 \pm 2.83_{aA}$	$61.70 \pm 3.09_{bAB}$	$413.7 \pm 42.8_{B}$
300	$82.75 \pm 2.23_{aA}$	$42.09 \pm 13.59_{bB}$	$494.0 \pm 28.0_{A}$

Values are the means ± standard deviations ($n = 3$).
Different small letters across rows and different capital letters within columns indicate a significant difference, ($P < 0.05$).

future studies need to be conducted to evaluate and verify the trend. In the PAA mixture, H_2O_2 is also an effective disinfectant that contributes to overall disinfection power, and both PAA and H_2O_2 are effective at inactivating a wide spectrum of microorganisms at low concentrations [13,26,27]. Despite reports that PAA was less affected by organic load [17,18], results seen in this study showed a higher decline rate as COD increased (Table 2). One issue that needs to be accounted for is that the addition of PAA increased the total COD measured in the system (Table 2). Therefore, future studies may need to focus on alternative methods to measure organic load in the system or have a separate control in place to cancel out the effect of PAA on measured COD. Conversely, H_2O_2 concentrations showed an initial decline followed by a rise in its levels with increasing COD in the water [Fig. 1(A–C)], which warrants further studies to verify the trend. When viewing increasing COD in wash water, faster depletion of PAA was observed than H_2O_2 [Fig. 1(A–C)], which concurs with the previous study performed in wastewater [28].

3.2. Effect of PAA concentration, contact time, COD, and inoculum level on cross-contamination of Salmonella on tomatoes

Based on the binomial regression analysis, all the variables i.e., PAA concentration (0, 5, 10, 20, 40, and 80 mg/L), contact time (30, 60, and 120 s), COD (0 and 300 mg/L), and inoculum level (10^8 and 10^6 CFU/tomato) significantly ($P \leq 0.05$) influenced the cross-contamination of *Salmonella* on tomatoes in the model flume system. Tomatoes introduced into the system with 0 mg/L PAA resulted in all tomatoes being positive for cross-contamination regardless of COD, wash time, and inoculation level (Tables 3 and 4). This trial simulated a scenario where no sanitizer was present in the flume water. Cross-contamination was reduced when the sanitizer level was maintained at 80 mg/L PAA but never prevented it from occurring at the 10^8 CFU/tomato inoculation level (Table 3). At a lower inoculum level of 10^6 CFU/tomato, concentrations lower than 80 mg/L effectively prevented cross-contamination even when organic load was present (Table 4). The significant factors affecting the *Salmonella* cross-contamination at each inoculum level were also tested using binomial regression. For the higher inoculum level (10^8 CFU/tomato), contact time and PAA concentration were significant ($P \leq 0.05$) factors affecting cross-contamination, but COD had no significant effect (Table 3). As we get closer to the realistic levels of bacterial contamination on the produce in the natural environment, i.e., for the lower inoculum level (10^6 CFU/tomato), COD and PAA concentrations affected ($P \leq 0.05$) the cross-contamination but contact time had no significant effect (Table 4).

PAA is known for being effective at inactivating *Salmonella* and other foodborne pathogens [2,3], where most of the scientific literature has considered PAA efficacious at 80 mg/L [2]. It is thought that the maximum contamination levels observed on fruit and/or leaf debris would not exceed approximately 10^4 CFU/tomato [29], thus if PAA could prevent cross-contamination at these higher inoculation levels used in the study, this would ensure a sufficient margin of safety. The US Food and Drug Administration (FDA), per 21 CFR 173.315, allows the use of PAA not exceeding 80 ppm in produce wash water [15]. Furthermore, the United Fresh Commodity Specific Food Safety Guidelines for the Fresh Tomato Supply Chain does not currently have a PAA level recommendation and recommends following manufacturer's label instructions for tomato washing pending published data on PAA efficacy in tomato wash systems [11].

As evident from these experiments, while sanitizers are not required for recirculated water systems under the Produce Safety Rule regulation (21 CFR 112 subpart E – Agricultural Water) [30], cross-contamination can occur in their absence. As observed with 10^8 CFU/tomato inoculation level and no organic load, these results suggest that even in the presence of PAA at a maximum use level of 80 mg/L, if the initial contamination is too high, *Salmonella* cross-contamination can still occur (Table 3). These results align with the

Table 3
Transfer of *Salmonella* from tomatoes contaminated at 10^8 CFU/tomato in a model flume system with varying peroxyacetic acid (PAA) and chemical oxygen demand (COD) concentrations.

Time (s)	0 mg/L PAA		20 mg/L PAA		40 mg/L PAA		80 mg/L PAA	
	0 mg/L[a]	300 mg/L	0 mg/L	300 mg/L	0 mg/L	300 mg/L	0 mg/L	300 mg/L
30	[b] $9/9_a$	$9/9_a$	$9/9_a$	$9/9_a$	$9/9_a$	$8/9_a$	$6/9_a$	$6/9_a$
60	$9/9_a$	$9/9_a$	$5/9_{ab}$	$9/9_a$	$4/9_b$	$4/9_{ab}$	$1/9_b$	$0/9_b$
120	$9/9_a$	$9/9_a$	$2/9_b$	$7/9_a$	$2/9_b$	$1/9_b$	$0/9_b$	$2/9_{ab}$

Values with different small letters within the same column indicate significant difference (Fisher's exact *post hoc* pairwise comparison, significance level at 0.05).
No significant difference was observed across rows within the same PAA level (Fisher's exact *post hoc* pairwise comparison, significance level at 0.05).
[a] COD concentration (mg/L) in the flume system.
[b] Number of tomatoes positive for cross-contamination of *Salmonella* of the total tomatoes collected at 30, 60, and 120 s (n = 9).

Table 4
Transfer of *Salmonella* from tomatoes contaminated at 10^6 CFU/tomato in a model flume system with varying peroxyacetic acid (PAA) and chemical oxygen demand (COD) concentrations.

Time (s)	0 mg/L PAA		5 mg/L PAA		10 mg/L PAA		20 mg/L PAA		40 mg/L PAA		80 mg/L PAA	
	0 mg/L[a]	300 mg/L	0 mg/L	300 mg/L	0 mg/L	300 mg/L	0 mg/L	300 mg/L	0 mg/L	300 mg/L	0 mg/L	300 mg/L
30	[b] 9/9	9/9	0/9	9/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9
60	9/9	9/9	0/9	9/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9
120	9/9	9/9	0/9	9/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9

No significant difference was observed within values in the same column (Fisher's exact *post hoc* pairwise comparison, significance level at 0.05). Except in 5 mg/L concentration, no significant difference was observed across rows within the same PAA level (Fisher's exact *post hoc* pairwise comparison, significance level at 0.05).

[a] COD concentration in the flume system.

[b] Number of tomatoes positive for cross-contamination of *Salmonella* of the total tomatoes collected at 30, 60, and 120 s (n = 9).

study by Banach et al. (2020), where *E. coli* was recovered from lettuce leaf punches even after being washed with 60 or 80 mg/L PAA wash water concentrations for 2 min in systems with 0–750 mg/L organic load [31]. The lettuce leaves were inoculated with ~6.0 log CFU/punch, and a 2.0–3.9 log CFU/punch reduction was observed [31]. While higher inoculation levels would be atypical in the natural environment, inactivation of ~4.0 log would not be adequate to guarantee that cross-contamination would not occur. Several studies have evaluated the efficacy of PAA in reducing bacterial contamination on tomato surfaces. For example, when tomatoes were surface inoculated with *Salmonella* (10^7 CFU/tomato), PAA reduced the microbial load by up to 4.0 log CFU/tomato when washed for 60–120 s [32]. Similarly, on tomatoes inoculated with ~10^8 CFU/tomato, when exposed to PAA at 45 mg/L, *Salmonella* was reduced by 4.4 log CFU; however, complete inactivation was never achieved, as evident by further enrichment of samples [33]. While previous studies have found PAA effective in reducing bacterial contamination from tomatoes, the present study observed that tomatoes with high inoculum levels (10^8 CFU/tomato) were positive for *Salmonella* even after being washed for 120 s with water containing 80 mg/L of PAA. Therefore, although PAA at concentrations ≤80 mg/L has been found to be effective in reducing the microbial load on tomatoes and wash water, a 4–5 log CFU reduction would not guarantee no cross-contamination would occur.

Unlike the experiments tested at the higher inoculation level, for experiments utilizing *Salmonella* at an inoculation level of 10^6 CFU/tomato exposed to 10, 20, 40, and 80 mg/L PAA, the results showed that all the uninoculated tomatoes tested negative for contamination regardless of the COD level (Table 4). This demonstrates that PAA levels as low as 10 mg/L can prevent cross-contamination with contamination levels as high as 10^6 log CFU/tomato. Tomatoes inoculated at 10^6 CFU/tomato tested against 5 and 10 mg/L PAA, with 0 mg/L COD, all tested negative for cross-contamination. Even with 300 mg/L COD added to the model system, the tomatoes exposed to 10 mg/L PAA all tested negative for cross-contamination. At lower concentrations of PAA (5 mg/L), COD had a significant ($P \leq 0.05$) negative influence on the effectiveness of the sanitizer in preventing cross-contamination, with all tomatoes being positive for cross-contamination when COD levels were increased to 300 mg/L. Based on these results, even with the added 300 mg/L COD, having a critical limit as low as 10 mg/L PAA may be able to prevent cross-contamination in a model flume system. Results from the 10^6 CFU/tomato inoculation studies indicate that at this level of *Salmonella* contamination, any cells that might have washed off the surface of an inoculated tomato were inactivated in the water before they had a chance to contaminate the uninoculated tomato. Even at 10^6 CFU/tomato and under conditions with high organic load, PAA effectively prevented cross-contamination. This opens the possibility of lowering the critical limits for PAA. This is in stark contrast to the results seen at the 10^8 CFU/tomato experiments, which showed that cross-contamination could occur even at the maximum allowable concentration of PAA. However, this contamination level of 10^8 CFU/tomato represents the worst-case scenario and not one commonly encountered in a commercial packing operation. The findings are in parallel with a similar study demonstrating the efficacy of free chlorine in preventing cross-contamination at much lower concentrations than that recommended for tomato flume tanks [5]. Bertoldi et al. (2022) observed that 25 mg/L free chlorine (measured as hypochlorous acid or HOCl) was effective in preventing cross-contamination when tomatoes were washed for ≥30 s in chlorinated water [5]. In this study, PAA as low as 10 mg/L showed similar efficacy, suggesting that PAA could be an effective alternative to HOCl. PAA, H_2O_2, and water are the major components of any commercial PAA-based sanitizer, and the two main active components work synergistically for higher inactivation efficacy [34].

These findings help corroborate previous studies that show PAA can reduce cross-contamination in postharvest wash water [1,21,35]. PAA has been considered an effective alternative to chlorine-based sanitizers [2,13,16,36,37]. PAA, as with other oxidizing agents, acts on the outer cell membrane of vegetative bacterial cells and oxidizes important membrane proteins in microorganisms by disrupting the disulfide (S–S) and sulfhydryl bond (—SH), thereby causing inactivation [13,16]. A better understanding of the efficacy of PAA will aid in determining concentrations necessary to prevent cross-contamination in fluming operations. Its benefits over chlorine-based sanitizers have been well documented [18]. Benefits such as producing less harmful disinfection by-products, having less environmental impact, and can be used in organic operations [17]. Additionally, it has greater stability in the presence of organic matter, which means it can be used longer in wash cycles without having to replace the water or replenish sanitizers [17,18]. Laboratory results may not transfer to commercial operations, which operate on a different scale as they run for hours and can result in the depletion of sanitizer in a fluming operation.

4. Conclusion

In these experiments, the stability of 80 mg/L PAA was tested against a maximum of 300 mg/L COD. PAA rapidly degraded in the presence of high organic load in the water but also contributed to overall levels of COD measured in the system. There was a decline in the sanitizer level by 50 % after 48 h, but it could still be effective in preventing cross-contamination at an inoculation level of 10^6 CFU/tomato. As low as 10 mg/L PAA in wash water was effective in preventing cross-contamination at all tested conditions under 10^6 CFU/tomato inoculum load. When incoming tomatoes were heavily contaminated (10^8 CFU/tomato), an 80 mg/L PAA concentration was insufficient to prevent cross-contamination under all testing conditions. However, this high level of contamination is very unlikely to occur in the environment. Lower levels of PAA were not tested for a long duration to determine their stability and degradation. Thus, more work on the reliability and stability of using lower concentrations of PAA is necessary. If proven successful, using less sanitizer would be of great interest to tomato packers and other produce packers in general. The use of more environmentally and economically friendly sanitizer options that still prevent *Salmonella* cross-contamination in flume water is an area that warrants further research.

Data availability statement

Data will be made available upon request.

CRediT authorship contribution statement

Christopher R. Pabst: Writing – original draft, Validation, Investigation, Formal analysis. **Karuna Kharel:** Writing – review & editing, Formal analysis. **Jaysankar De:** Writing – review & editing, Formal analysis. **Cameron A. Bardsley:** Writing – review & editing, Formal analysis. **Bruna Bertoldi:** Investigation. **Keith R. Schneider:** Writing – review & editing, Supervision, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31521.

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