ENVIROTECH

CASE STUDY

The Effectiveness of Water, Sodium Hypochlorite Bleach, and Peroxyacetic Acid (PAA) in Eradicating *Salmonella Typhimurium* from the Surface of Tomatoes

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Background

The contamination of tomatoes by *Salmonella* bacteria has had a devastating impact upon the industry. Fast-food restaurants and supermarkets temporarily suspended serving and selling this popular and nutritious family-favorite to their customers. This prompted us to consider whether there was a simple safeguard available to both the consumer and the producer that could improve current practices used to eliminate or reduce the infective risks. A very simple experiment was devised: The surfaces of store-bought vine-ripened tomatoes (the only ones available at the local supermarket) were inoculated with a laboratory culture of *Salmonella* by spraying a suspension of the culture onto the tomatoes and allowing to dry. Then the tomatoes were rinsed with either (1) plain city water, (2) a solution of sodium hypochlorite bleach, or (3) a solution of peroxyacetic acid. The amount of viable *Salmonella* bacteria rinsed from the surface of the tomatoes was measured by plating the respective rinse solutions. Recognizing that it is not the amount of bacteria in the rinse solutions that pose a menace to the public, rather it is the amount of bacteria still resident on the tomato surface that is critical, the surfaces were swabbed for bacteria after the respective rinses in order to determine the effectiveness.

The tomatoes were exposed to each rinsing program for two different contact times. A one-minute contact time was used to simulate the exposure time of tomatoes being sprayed on a conveyor belt, or to simulate the amount of time a consumer may take to wash their produce in the kitchen. A five-minute contact time was employed to simulate the amount of time to which the tomatoes would be exposed in a typical hydrocooler during processing.

Materials and Methods

a) One-minute Contact Time Study

Salmonella typhimurium bacteria (ATCC 14028, MicroBioLogics, St. Cloud, MN) were cultured in nutrient broth (Sigma, St. Louis, MS) by incubating for two days at 35 oC. The bacteria were separated from the nutrient broth by centrifugation, and resuspended in 150 ml of city water contained in a spray bottle. A determination of the amount of *Salmonella* bacteria in suspension was performed through common plating techniques, (described later) but was found to be Too-Numerous-To-Count (TNTC).

The tomatoes used in the study were the vine-ripened variety and were the only ones available at the local supermarket due to the unavailability of round reds and Roma varieties. Each was around 7.5 inches in circumference having a surface area of approximately 18 square inches. Thirty-five tomatoes were evenly spaced apart on an aluminum foil sheet. Holding the spray bottle about 6 inches from the tomatoes, the surfaces were doused with a fine spray of the *Salmonella* suspension. Care was taken to ensure that the tomatoes were covered evenly. The tomatoes were left to dry overnight, whereupon a second and third *Salmonella* inoculation was applied. When the tomatoes were dry, they were randomly sorted into three groups of ten. Each group of ten tomatoes was placed in a one-gallon Ziploc bag for contact with one of the following rinse solutions:

(1) 500 ml of Modesto city water *(control)*

(2) 500 ml of Modesto city water containing 40 ppm *(as Cl2)* of sodium hypochlorite bleach.

(3) 500 ml of Modesto city water containing 40 ppm of peroxyacetic acid *(PAA)* from BioSide HS15%®.

The tomatoes were then gently tumbled in the rinse solution for exactly one minute, after which they were removed. The rinse solution was immediately processed by a microbiology technician who was unaware of its identity. Each rinse solution was plated onto 3M Enterobacteriace petrifilms at a 100 and a 102 dilution in Butterfield's buffer.

Each set of ten wet tomatoes were then assigned to another microbiology technician who was also unaware which rinsing solution had been used on which set of tomatoes. The technician was instructed to randomly select five tomatoes from the group of ten for surface swabbing with 3M Quickswabs. Using gloved hands to hold the tomato, the technician swabbed half of the surface of the still-wet tomato with a Q-tip-like swab. No area of the surface tomato was contacted more than once with the swab, which was rotated slightly between swabbing strokes for uniform distribution of bacteria on the swab. This was followed by vortexing the swab into the 1 ml of nutrient broth that came with the Quickswab in order to dislodge the bacteria from the swab and into the aqueous phase. Another swab was then used to swab the other half of the tomato in identical fashion. Thus, two fresh Quickswabs were used for each tomato. The nutrient broth containing viable bacteria swabbed from the tomatoes were subsequently serially diluted (100 and 102) using Butterfield's buffer, and plated onto 3M Enterobacteriace petrifilms.

b) Five-minute Contact Time Study

The above test was repeated to simulate the exposure time and temperature of rinse solutions used in a typical hydrocooler operation during processing of the tomatoes. A five-minute contact time was selected. This testing was performed on a separate day, with a fresh culture of *Salmonella typhimurium* and a newly-purchased set of vine-ripened tomatoes. The only other change being that the water used to prepare the rinse solutions was chilled to 40º F.

Results and Discussion

Microbiological Quality of the Rinse Waters

The results of measuring the viability of *Salmonella* bacteria washed into the aqueous phase have important ramifications in commercial processing operations where wash and rinse water is recycled and reused. The more effective a chemical program is in eradicating aqueous phase bacteria, the less likely that rinsed tomatoes will be recontaminated by redeposition of viable bacteria present in the recycled water.

Table I records the $\log_{10}$ CFU/ml of the *Salmonella* bacteria in the 500 ml of water used to rinse ten tomatoes whose surfaces had been inoculated with *Salmonella*. Data for both the one-minute and five minute intervals are presented.

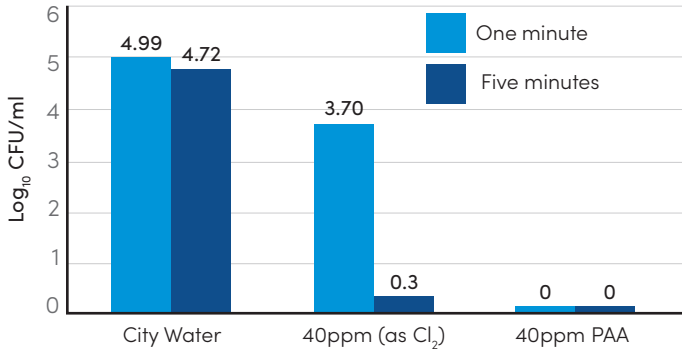
TABLE I $\log_{10}$ CFU/ml *Salmonella* Bacteria in the Respective Rinse Waters

	$\log_{10}$ CFU/ml *(One minute)*	$\log_{10}$ CFU/ml *(Five minutes)*
City water *(control)*	4.99	4.72
40 ppm *(as Cl_2)* NaOCl	3.70	0.3
40 ppm PAA	0	0

FIGURE 1

$\log_{10}$ CFU/ml of Viable *Salmonella* Bacteria in the Rinse Waters

FIGURE 1 is a graphical representation of the data reported in Table I.



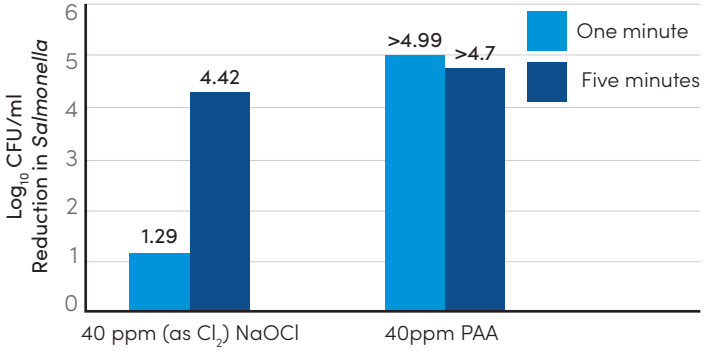
It can be seen that at both the one-minute and five-minute contact times, PAA has a dramatic impact upon the *Salmonella* bacteria washed into the aqueous phase, as zero surviving colonies were detected. *(Note: the graphic display for PAA in Figure 1 is an extrapolation for comparative purposes only, since the log10 of zero cannot be calculated.)* Sodium hypochlorite, on the other hand, has a relatively minor impact on the *Salmonella* bacteria at the one-minute contact time, but improves as the contact time is extended to five minutes.

FIGURE 2:

$\log_{10}$ CFU/ml Reduction for the Rinses

(Compared to city water control)

FIGURE 2 expresses the data of Figure1 in terms of the $\log_{10}$ CFU/ml reduction for the two chemical treatment programs compared to the untreated city water control rinse.



As evidenced by the log10 CFU/ml for the city water control, the data Figure 1 and 2 show that considerable amounts of viable *Salmonella* bacteria can be washed from the surface of the tomatoes with a simple rinse of water containing no sanitizing chemicals. When the rinse water contains either NaOCl or PAA, the amount of viable bacteria in the aqueous phase is measurably lower. At the one-minute interval, compared to the city water control, the NaOCl treatment has afforded a log10 reduction of 1.29 CFU/ml, which corresponds to a 94.9% reduction in aqueous phase *Salmonella* bacteria, whereas the PAA treatment totally eradicated aqueous phase bacteria, representing a **100% reduction.** The same held true for the PAA treatment after a five-minute rinse, where NaOCl performed much better and yielded a log10 reduction of 4.42 CFU/ml, corresponding to a **99.996% reduction of *Salmonella*** in the aqueous phase.

Microbiological Quality of the Rinsed Tomatoes

It is obvious that it is not the amount of bacteria in the rinse solutions that pose a threat to the public; rather it is the amount of bacteria still resident on the tomato surface after the rinsing step that is the important public health issue. Therefore, after the respective rinses, the surfaces of the tomatoes were swabbed in order to determine the relative effectiveness of each rinsing/sanitizing program.

Table II reports the log10 CFU/tomato of the *Salmonella* bacteria remaining on the surface of the tomato after the respective rinse treatment at the one-minute and five minute intervals. The numbers in parenthesis represent the standard deviation from a data set consisting of swabbing five randomly selected from the ten tomatoes rinsed.

TABLE II: Log_{10} CFU/tomato of *Salmonella* Bacteria Remaining on the Surface Subsequent to the Respective Rinses

	Mean Log_{10} CFU /tomato *(One minute)* *(± standard deviation)*	Mean Log_{10} CFU /tomato *(Five minutes)* *(± standard deviation)*
City water *(control)*	5.66 *(±0.11)*	4.70 *(±0.25)*
40 ppm *(as Cl_2)* NaOCl	5.58 *(±0.17)*	3.96 *(±0.74)*
40 ppm PAA	4.34 *(±1.27)*	3.30 *(±1.39)*

FIGURE 3

Log_{10} Surface *Salmonella* CFU/tomato Remaining after the Respective Rinses

FIGURE 3 is a graphical representation of the data shown in Table II

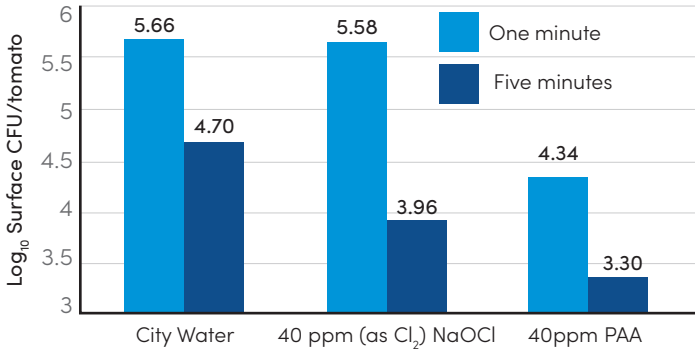


Table III reports the data of Table II and Figure 2 in terms of log10 reduction in surfaceassociated *Salmonella* bacteria compared to the amount removed by the city water control rinse. The figures in parentheses express the results in terms of the % reduction in surface-associated bacteria over and above what was achieved with just the city water control rinse.

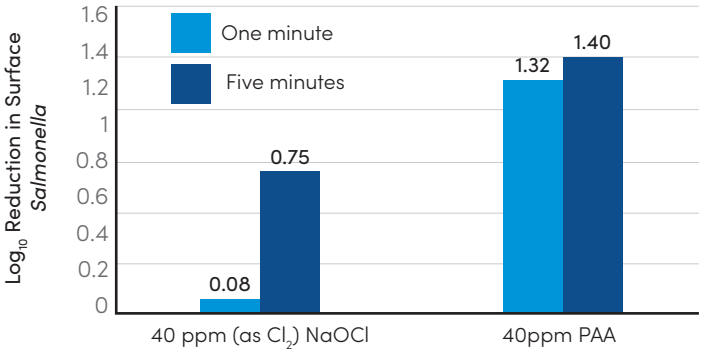
TABLE IIII: Log10 and % Reduction in *Salmonella* Bacteria for NaOCl and PAA Solutions Compared to the City Water Rinse

	(One minute) Mean Log_{10} Reduction CFU/tomato *(% Reduction vs. city water rinse)*	*(Five minutes)* Mean Log_{10} Reduction CFU/tomato *(% Reduction vs. city water rinse)*
40 ppm *(as Cl_2)* NaOCl	0.08 *(16.8%)*	0.75 *(81.8%)*
40 ppm PAA	1.27 *(94.6%)*	1.40 *(95.9%)*

FIGURE 3 plots the data of Table III in terms of the mean log10 reduction in *Salmonella* CFU/tomato.

FIGURE 4

Log_{10} Reduction in Surface *Salmonella* Compared to the City Water Control Rinse /CFU/tomato



It can be seen that rinsing the tomatoes with 40 ppm NaOCl solution for one minute affords only an additional log10 0.08 CFU/tomato reduction in surface-associated *Salmonella* compared to rinsing the tomatoes in water alone. When expressed in terms of the % reduction, compared to the water wash alone, this amounts to only 16.8%. The 40 ppm NaOCl solution performs better when the contact time is extended to 5 minutes, affording a log10 0.75 CFU/tomato reduction in surface-associated *Salmonella* compared to the city water rinse. This corresponds to an 81.8% greater reduction compared to rinsing the tomatoes in water alone.

Contrast this with the performance of the 40 ppm PAA rinse solution. At the one-minute contact time, PAA yields an additional log10 1.32 CFU/tomato reduction in surfaceassociated *Salmonella*, compared to rinsing the tomatoes in water alone. When expressed in terms of the % reduction, compared to the water wash alone, this amounts to 94.6%. The performance of the 40 ppm PAA rinse solution is essentially the same at the fiveminute contact time as it was at the one-minute contact time.

The difference of performance between the NaOCl bleach solution and the PAA solution has important implications in both kitchen and commercial processing environments. Certainly, the PAA rinse outperforms NaOCl bleach in short contact times. These shorter time scenarios would certainly represent the results a consumer may take to rinse their produce in a kitchen, or the short contact time typically encountered when the tomatoes are dipped in water or sprayed on a conveyor belt during commercial processing.

At the longer five-minute contact time, the performance of PAA and NaOCl bleach converge, with PAA eradicating 95.9% more surface associated *Salmonella*, and NaOCl eliminating 81.8% more surface-associated *Salmonella* compared to the city water rinse. In a kitchen or commercial environment, the consumer is unlikely to have the patience or time to rinse their produce for five minutes. Therefore, much stronger concentrations of NaOCl bleach, possibly up to 200 ppm as Cl2, may be required to equal the efficacy of BioSide HS 15% (PAA) in relation to improving public health concerns about *Salmonella* contamination.

How Much Surface-Associated *Salmonella typhimurium* is Removed by Simple Rinsing in City Water ?

Up to now the discussion has centered on the performance enhancements of NaOCl and PAA solutions in comparison to the rinse with city water alone. But the question remains:

How well does a simple city water rinse remove the surface-associated Salmonella typhimurium?

This number can be calculated from the data already presented viz.

From Table I, it can be seen that washing ten tomatoes with 500 ml of city water for oneminute yields a plate count of $\log_{10}$ 4.99 CFU/ml in the aqueous phase. Since 104.99 is 9.77×10^4, multiplying this number by 500 equals 4.88×10^7 Total CFU per ten tomatoes.

Divide this number by 10 to get 4.88×10^6 CFU of *Salmonella* bacteria that are rinsed off each tomato and into the aqueous phase. From Table II, at the one-minute interval, swabbing of the rinsed tomato surface revealed there to be log10 5.66 CFU/tomato still resident on the surface after the rinse. Since 105.66 is 4.57×10^5, adding this number to 4.88×10^6 (the amount of *Salmonella* washed from each tomato) yields 5.34×10^6 total *Salmonella* bacteria per tomato. Using the formula:

$$\%\ \text{removal by water wash} = \frac{\text{Number } \textit{Salmonella} \text{ washed from each tomato}}{\text{Total } \textit{Salmonella} \text{ per tomato}} \times 100$$

$$= \frac{4.88 \times 10^6}{5.34 \times 10^6} \times 100$$

$$= 91.4\%$$

TABLE IV: Table IV: Relative *Salmonella* Removal/Eradication Effectiveness of the Respective Rinses after One Minute

	City Water	40 ppm *(as Cl_2)* NaOCl	40 ppm PAA
% Removal/eradication from tomato surface	91.4	92.9	92.9

Conclusions

For both one-minute and five minute contact times, a 40 ppm solution of PAA is measurably more effective than a 40 ppm NaOCl solution in removing and eradicating *Salmonella* bacteria from the surface of contaminated tomatoes.

• A one-minute rinse of *Salmonella*-contaminated tomatoes in city water containing no sanitizing chemical dislodges over 91.4% of the bacteria from the surface and into the aqueous phase. A sodium hypochlorite bleach rinse (40 ppm as Cl2) over the same contact time provides a 16.8% greater reduction in bacteria compared to the city water rinse. A one-minute peroxyacetic acid rinse (40 ppm as PAA) effects a 94.6% greater reduction in bacteria compared to the city water rinse. Expressing these results on an absolute basis, the % relative effectiveness in eradication/removal of surface-associated *Salmonella* bacteria from the tomatoes is: city water 91.4%; 40 ppm NaOCl (as Cl2) bleach (92.9%); and 40 ppm PAA (99.6%).

• At the longer five-minute contact time, the performance of PAA and NaOCl bleach converge, with PAA eradicating 95.9% more surface-associated *Salmonella*, and NaOCl eliminating 81.8% more surface-associated *Salmonella* than rinsing with city water alone. In a kitchen environment, the consumer is unlikely to have the patience to rinse their produce for five minutes, and so stronger concentrations of NaOCl bleach, possibly up to 200 ppm as Cl2 or more should be considered in order to achieve equivalent efficacy of BioSide HS 15% (PAA).

• The data strongly highlights the need for PAA to be used as a consumer or commercial product for food safety purposes. A 40-80 ppm PAA rinse solution could be sold in a Ready-to-Use spray bottle and applied to the produce just before consumption. No further rinsing would be required because at this low concentration, PAA is well below detection by smell or taste. In addition, there are no hazardous by-products or toxicology or dietary concerns (as contrasted with sodium hypochlorite bleach).

• The results of measuring the viability of *Salmonella* bacteria washed into the aqueous phase have important ramifications in processing operations where wash and rinse water is recycled and reused. The more efficient a chemical program is in eradicating aqueous phase bacteria, the less likely that rinsed tomatoes will be recontaminated by redeposition of viable bacteria present in the recycled water. At the one-minute interval, compared to the city water control, the NaOCl treatment affords a log10 reduction of 1.29 CFU/ml that corresponds to a 94.9% reduction in aqueous phase *Salmonella* bacteria, whereas the PAA treatment totally eradicated aqueous phase bacteria for a 100% reduction in the same time frame. The same conclusion holds true for the PAA treatment for the five-minute rinse. NaOCl performed much better for a log10 reduction of 4.42 CFU/ml corresponding to a 99.996% reduction of *Salmonella* in the aqueous phase for the 5 minute exposure, which is an unrealistic time frame for produce to be exposed to an antimicrobial process for typical commercial or consumer-based preparation methods.

7th July 2008

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