The Effectiveness of Water, Sodium Hypochlorite Bleach, and Peroxyacetic Acid (PAA) in Eradicating *Listeria monocytogenes* from the Surface of Cantaloupes

Tina Rodrigues, B.S.
Jonathan Howarth Ph.D
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Enviro Tech Chemical Services
Modesto CA 95358

Background

The outside surfaces of raw cantaloupes are rarely washed with antimicrobial solutions at processing facilities because only the inside of the fruit is eaten. On the other hand, when the cantaloupe is cut into pieces, the knife can act as a vector to translocate surface-associated bacteria from the outside to the inside fruit. *Listeria monocytogenes* is a virulent food-borne pathogenic bacterium, naturally-occurring in the environment and is often found in the soil, precisely where cantaloupes are grown.

A *Listeria monocytogenes* outbreak traced to tainted cantaloupes from Jensen Farms and Frontera (shipped July 29 through September 10) to at least 24 states has claimed the lives of at least 23 people and sickened 123 in 25 states so far, becoming the most deadly food-borne illness outbreak in United States history. 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 cantaloupe peel slices were inoculated with a laboratory culture of *Listeria monocytogenes* by spraying a suspension of the culture onto the cantaloupe peels and allowing to dry. Then the cantaloupe peels were rinsed with one of the following: (1) plain city water, (2) a solution of sodium hypochlorite bleach, or (3) a solution of peroxyacetic acid. The amount of viable *Listeria* bacteria rinsed from the surface of the cantaloupes 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 cantaloupe that is critical, the surfaces were swabbed for bacteria after the respective rinses in order to determine the relative effectiveness.

Materials and Methods

Listeria monocytogenes bacteria (ATCC 13932) were cultured in nutrient broth (Sigma, St. Louis, MS) by incubating for two days at 35 ^{o}C. The bacteria were separated from the nutrient broth by centrifugation, and resuspended in 150 ml of sterile Butterfield's Buffer contained in a spray bottle. A determination of the amount of *Listeria* bacteria in suspension was performed through common plating techniques (described later), and was found to be $\log_{10}$ 7.64 CFU/ml (4.4 x 10^7).

Two cantaloupes used in the study were purchased at a local supermarket. Each cantaloupe was sliced into 8 equal pieces and the peel was separated from the fruit. Twelve of the cantaloupe peels were used in this study and can be seen in Image 1. Holding the spray bottle about 6 inches from the cantaloupe peels, the surfaces were doused with a fine spray of the *Listeria* suspension. Care was taken to ensure that the peels were covered evenly, see Image 2. They were then left to dry for one hour.

Image 1: Cantaloupe peels used in this study

Image 2: *Listeria* inoculation

When the cantaloupe peels were dry, they were randomly sorted into two groups of five and one group of two. The two groups of five were each used as test groups and the remaining two were used as the control group which was simply a test using Modesto city water. Each group of cantaloupe peels were placed in sterile poultry rinse bags for contact with one of the following rinse solutions:

(1) 200 ml of Modesto city water (control), adjusted to pH 7.
(2) 500 ml of Modesto city water containing 60 ppm (as Cl_2) of sodium hypochlorite bleach (NaOCl), adjusted to pH 7.
(3) 500 ml of Modesto city water containing 60 ppm of peroxyacetic acid (PAA) from BioSide HS15%®.

The cantaloupe peels were then gently tumbled in the rinse solution for exactly two minutes, after which a 10% solution of erythorbic acid was added to each bag to neutralize the remaining hydrogen peroxide and PAA (3.0 g) or bleach (0.71 g). Each rinse solution was serially diluted using Butterfield's Buffer and plated onto 3M Environmental Listeria Petrifilms.

Each set of wet cantaloupe peels were then assigned to a microbiology technician who was unaware which rinsing solution had been used on which set of cantaloupe peels. Using gloved hands to hold the cantaloupe peel, the technician swabbed half of the surface of the still-wet peel with a Q-tip-like swab. No area of the surface was contacted more than once with the swab, which was rotated slightly between swabbing strokes for uniform distribution of bacteria on to the swab. This was followed by vortexing the swab into the 1 ml buffer 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 peel in identical fashion. Thus, two fresh Quickswabs were used for each cantaloupe peel. The buffers containing viable bacteria swabbed from the cantaloupes were subsequently serially diluted using Butterfield's buffer, and plated onto 3M Environmental Listeria Petrifilms. The Petrifilms were then incubated for 28 hours at 35°C, upon which they were enumerated.

Results and Discussion

Microbiological Quality of the Rinse Waters

The results of measuring the viability of *Listeria* 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 fruit will be recontaminated by redeposition of viable bacteria present in the recycled water.

Table 1 records the $\log_{10}$ CFU/ml of the *Listeria* bacteria in the water used to rinse the cantaloupe peels whose surfaces had been inoculated with *Listeria*. As evidenced by the $\log_{10}$ CFU/ml for the city water control, the data in Table 1 shows that considerable amounts of viable *Listeria* bacteria can be washed from the surface of the cantaloupes with a simple rinse of water containing no sanitizing chemicals. When the rinse water contains either NaOCl or PAA, the amount of viable bacteria remaining in the aqueous phase is measurably lower. It can be seen that at the two minute contact time, both chemical treatments at 60 ppm have a dramatic impact upon the *Listeria* bacteria washed into the aqueous phase compared to the untreated control as there was a 99.24% reduction for PAA and 98.62% reduction in *Listeria* bacteria using NaOCl bleach.

Table 1: Log_{10} CFU/ml *Listeria* Bacteria in the Respective Rinse Waters

	Log_{10} CFU/ml Remaining	Log_{10} Reduction (% reduction)
City water (control)	6.16	N/A
60 ppm (as Cl_2) NaOCl	4.30	1.86 (98.62%)
60 ppm PAA	4.04	2.12 (99.24%)

Microbiological Quality of the Rinsed Cantaloupes

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 fruit surface *after* the rinsing step that is the important public health issue. Therefore, after the respective rinses, the surfaces of the cantaloupe peels were swabbed in order to determine the relative effectiveness of each rinsing/sanitizing program.

Tables 2-4 report the $\log_{10}$ CFU/cantaloupe peel of the *Listeria* bacteria remaining on the surface of the peel after the respective rinse treatment.

It can be seen that rinsing the cantaloupe peels with 60 ppm NaOCl solution for two minutes affords an additional $\log_{10}$ 1.47 CFU/cantaloupe peel reduction in surface-associated *Listeria* compared to rinsing the cantaloupes in water alone. When expressed in terms of the % reduction, compared to the water wash alone, this amounts to 96.61%. Contrast this with the performance of the 60 ppm PAA rinse solution. PAA yields an additional $\log_{10}$ 2.08 CFU/cantaloupe peel reduction in surface-associated *Listeria,* compared to rinsing the cantaloupe peels in water alone. When expressed in terms of the

% reduction, compared to the water wash alone, this amounts to 99.17%. A graphical representation of the data reported in Tables 2-4 can be seen in Figure 1.

Table 2: Log_{10} CFU/cantaloupe peel of *Listeria* Bacteria Remaining on the Surface using Modesto City Water

Description	log_{10} (Canteloupe Peel)
Control Cantaloupe Sample 1	5.49
Control Cantaloupe Sample 2	5.22
Average:	5.36
Standard deviation:	0.191

Table 3: Log_{10} CFU/cantaloupe peel of *Listeria* Bacteria Remaining on the Surface using 60 ppm Cl_2 (from bleach)

Description	log_{10} (Cantaloupe Peel)
Bleach Cantaloupe Sample 1	3.76
Bleach Cantaloupe Sample 2	3.80
Bleach Cantaloupe Sample 3	3.57
Bleach Cantaloupe Sample 4	4.10
Bleach Cantaloupe Sample 5	4.23
Average:	3.89
Standard deviation:	0.268
log_{10} reduction:	1.47
Percent reduction:	96.61%

Table 4: Log_{10} CFU/cantaloupe peel of *Listeria* Bacteria Remaining on the Surface using 60 ppm PAA

Description	log_{10} (Cantaloupe Peel)
PAA Cantaloupe Sample 1	3.34
PAA Cantaloupe Sample 2	3.37
PAA Cantaloupe Sample 3	3.39
PAA Cantaloupe Sample 4	3.04
PAA Cantaloupe Sample 5	3.27
Average:	3.28
Standard deviation:	0.143
log_{10} reduction:	2.08
Percent reduction:	99.17%

Figure 1: A graphical representation of the log10 CFU/cantaloupe peel **remaining**.

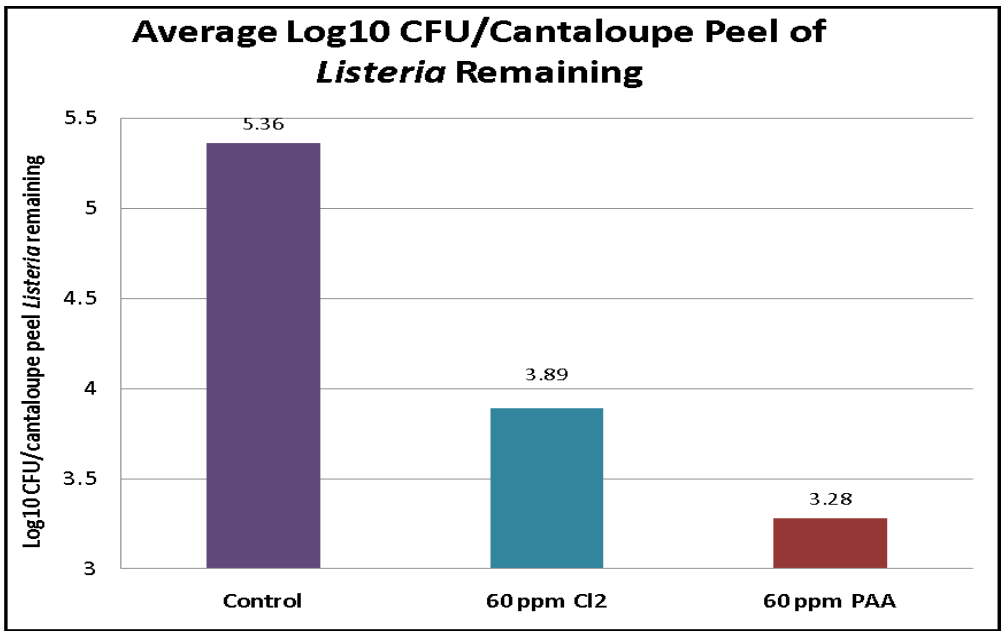
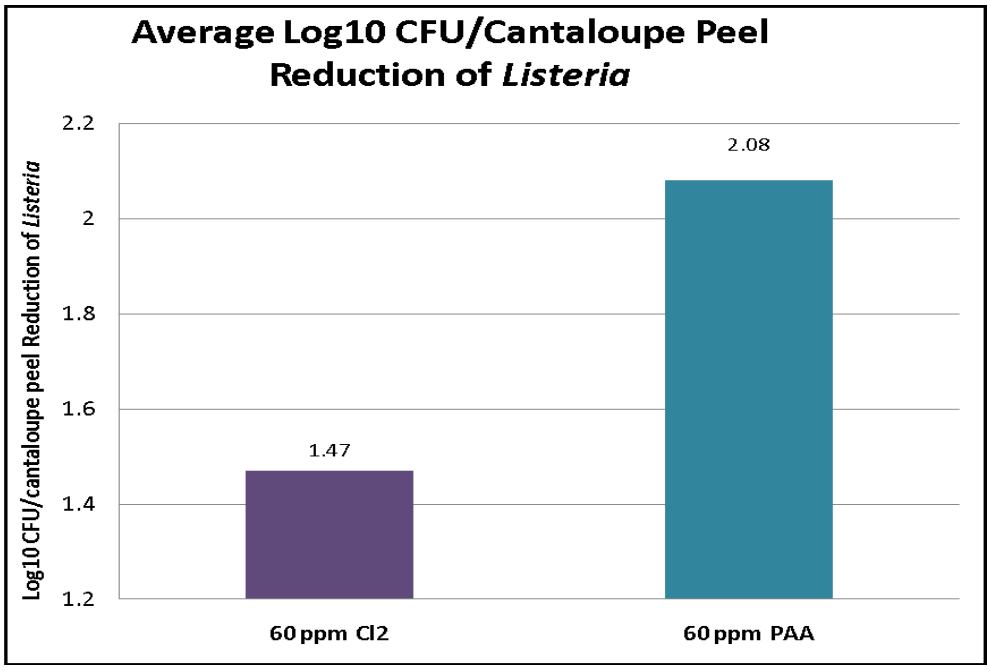


Figure 2: A graphical representation of the log10 CFU/cantaloupe peel **reduction**.



Conclusions

- The purpose of this study was to determine whether peroxyacetic acid or sodium hypochlorite bleach is effective in eradicating *Listeria* contaminated cantaloupes.

- After the two minute contact time, compared to the city water control, the NaOCl treatment affords a $\log_{10}$ reduction of 1.86 CFU/ml that corresponds to a 98.62% reduction in aqueous phase *Listeria* bacteria. Over the same time period, the PAA treatment had a $\log_{10}$ reduction of 2.12 (99.24%) in *Listeria* bacteria that were washed from the fruit and into the aqueous phase.

- A two-minute rinse of *Listeria*-contaminated cantaloupes using sodium hypochlorite bleach rinse (60 ppm as Cl_2) over two minutes provides a 96.61% reduction in surface-associated bacteria compared to the city water rinse. A two-minute peroxyacetic acid rinse (60 ppm as PAA) provides a 99.17% reduction in surface-associated bacteria compared to the city water rinse.

- Although sodium hypochlorite displays a good reduction on cantelope surfaces associated *Listeria* bacteria, the reduction for PAA is superior. On the other hand the melons purchased for this study had previously been water washed to remove soil and debris. Had sodium hypochlorite or PAA been used in the rinse water, this disparity in performance would be expected to widen further. This is because it is known that the performance of chlorinated products is compromised by the presence of organic nitrogen present in the soils and on organic surfaces to form stable N-chloro complexes. PAA is much less reactive to organic nitrogen, and the efficacy of PAA is much less compromised by the presence of soils and other organic material.

- The data strongly highlights the need for PAA to be used for food safety purposes. At 60-100 ppm PAA, 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).