Comparative Efficacy of Peroxyacetic Acid and Sodium Hypochlorite Bleach against Enterobacteria, *E. coli* and Yeasts Molds on Cherries

Purpose

The primary purpose of this study was to determine whether the current disinfection process using peracetic acid (from Perasan®) on line 2 at a large cherry packing company in California's central valley is effective against Enterobacteriaceae bacteria (Enterobacteria), *E. coli* as well as yeast and mold spoilage microorganisms. Enterobacteriaceae is a large family of bacteria, including many of the more familiar pathogens, such as *Salmonella* and *Escherichia coli.* Many members of this family are a normal part of the gut flora found in the intestines of humans and other animals, but can cause serious illness when ingested, and in some cases at very small doses, which is demonstrated below in Table 1.

Table 1: Estimated infectious dose of bacteria species

Bacteria Species	**Estimated infectious dose (bacteria cell number)**	**Disease**
E. coli O157:H7	10 to 100	Hemorrhagic colitis
E. coli	1,000,000 to 100,000,000	Traveler's diarrhea
Salmonella	100 to 1,000,000,000	Salmonellosis
Principal source: Foodborne Pathogens: Risks and Consequences, Report No. 122, CAST-Council for Agricultural Science and Technology, September 1994.		

The secondary purpose of this study was to compare the efficacy of peracetic acid and sodium hypochlorite bleach against the same bacteria in addition to and yeasts and molds. At the start of this study, the cherry packing house used two different antimicrobials during the processing of freshly picked cherries. Peracetic acid (PAA) was used on Line 2 and sodium hypochlorite bleach (NaOCl) was used on Line 3. Microbiological testing was performed on untreated and treated cherries picked from the respective lines.

Methods

Zip-lock bags of cherries were received at Envirotech Chemical Services on the afternoon of May 15, 2009 and stored in the refrigerator for microbiological testing later that same day. The zip-lock bags that contained the cherries had the following information on them:

1) Line 2: Pre-treat – no chemicals used

2) Line 2: Post-Hydrocooler – 50ppm PAA used

3) Line 3: Primary Dump Tank – no chemicals used

4) Line 3: Post-hydrocooler – 40ppm NaOCl

One at a time, 600g of cherries from each of the four chosen zip-lock bag samples and 500g of Modesto city water were placed in a new, sterile zip-lock bag, sealed and gently tumbled for one minute.

After being tumbled for one minute, the rinse water was plated at 10^0 and 10^2 dilutions on 3M Enterobacteriaceae Plates, 3M E.coli Plates and 3M Yeast and Mold Plates. The 3M Enterobacteriaceae Plates, 3M E.coli Plates were incubated at 35 degrees C for 24 hours and the 3M Yeast and Mold Plates were kept at room temperature for five days, after which the plates were enumerated. The 3M Enterobacteriaceae plates contain a special indicator that differentiates acid-producing bacteria from other bacteria present on the cherries. All *Salmonella* species are examples of acid-producing bacteria. The 3M E. coli plates also contain a special indicator which allows the microbiologist to differentiate *E.coli* species from other bacteria present on the cherry samples.

Results and Discussion

Table 2 shows the $\log_{10}$ CFU/mL of Enterobacteria, *E. coli*, yeasts and molds on the untreated cherries from Line 2 and Line 3. The $\log_{10}$ CFU/mL and $\log_{10}$ reduction on the cherries treated with 50ppm PAA and 40ppm NaOCl is also demonstrated in Table 2.

The data in Table 2 show that treating the cherries with 50ppm PAA produces a log10 reduction in Enterobacteria of >1.41 CFU/mL corresponding to a 100% reduction, meaning that there were no Enterobacteria present after PAA treatment. On the other hand, the NaOCl treated cherries from Line 3 only had a log10 reduction in Enterobacteria of 0.36 CFU/mL corresponding to 56.34%. The results were similar when the cherries were subjected to microbiological analysis to determine the presence of *E. coli* species. The cherries that were treated with PAA had no detectable *E. coli* present, but the use of NaOCl on cherries only reduced the number of *E. coli* by 77.10% (log10 reduction of 0.64 CFU/mL). This data shows that PAA at 50ppm is very effective in eliminating Enterobacteria and *E.coli* contaminated cherries.

Table 2 also shows the efficacy of PAA and NaOCl against yeasts and molds present on cherries. Both PAA and NaOCl are very effective against yeast and mold. The use of PAA yielded a 98.3% reduction in yeast present on the surface of the cherries, and a 100% reduction in surface associated mold. The cherries treated with NaOCl also showed significant reduction in surface associated yeast and mold with reductions of 99.8% and 100%, respectively.

Table 2:

Enterobacteria Plate Results

Description	Line 2 Control: (Untreated)	Line 2: 50ppm PAA	Line 3: Control (Untreated)	Line 3: 40ppm NaOCl
Log10 (CFU/mL)	1.41	1.41	1.76	1.40
Log10 Reduction (%)	N/A	>1.41 (100%)	N/A	> 0.36 (56.34%)

E. coli Plate Results

Description	Line 2 Control: (Untreated)	Line 2: 50ppm PAA	Line 3: Control (Untreated)	Line 3: 40ppm NaOCl
Log10 (CFU/mL)	1.34	1.34	1.68	1.04
Log10 Reduction (%)	N/A	>1.34 (100%)	N/A	0.64 (77.10%)

Yeast Plate Results

Description	Line 2 Control: (Untreated)	Line 2: 50ppm PAA	Line 3: Control (Untreated)	Line 3: 40ppm NaOCl
Log10 (CFU/mL)	4.12	2.33	5.15	2.23
Log10 Reduction (%)	N/A	1.79 (98.38%)	N/A	2.92 (99.88%)

Mold Plate Results

Description	Line 2 Control: (Untreated)	Line 2: 50ppm PAA	Line 3: Control (Untreated)	Line 3: 40ppm NaOCl
Log10 (CFU/mL)	2.60	2.60	2.90	2.90
Log10 Reduction (%)	N/A	>2.60 (100%)	N/A	>2.90 (100%)

Figures 1 and 2, seen below, chart the number of Enterobacteria, *E. coli*, yeasts and molds present on the surface of cherries before washing and chemical treatment post hydrocooler. Figure 1 shows the above mentioned microorganisms present on the cherries sampled from Line 2, in which PAA was used to treat the cherries. The amount of these microorganisms present on the cherries sampled from Line 3, in which NaOCl was used during processing, is charted in Figure 2. When comparing these Figures, it can be seen that there is a tremendous difference in the number of Enterobacteria and *E.coli* present on the cherries after being treated with PAA and NaOCl. PAA eliminated all potentially harmful bacteria on the surface of the cherries from Line 2, but NaOCl provided minimal reduction in these potentially harmful bacteria.

Important note for readers interested in the absolute number of bacteria present in the rinse waters: for example let's look at the PAA yeast plate results. The untreated control had a $\log_{10}$ 4.12 CFU/ml, antilogging this number on a calculator by entering 4.12 into the display and pressing 2nd LOG raises 10 to the power 4.12 ($10^{4.12}$) which is 13182 CFU/ml. Hardly surprising then that microbiologists prefer to report their results using the $\log_{10}$ scale.

Figure 1:

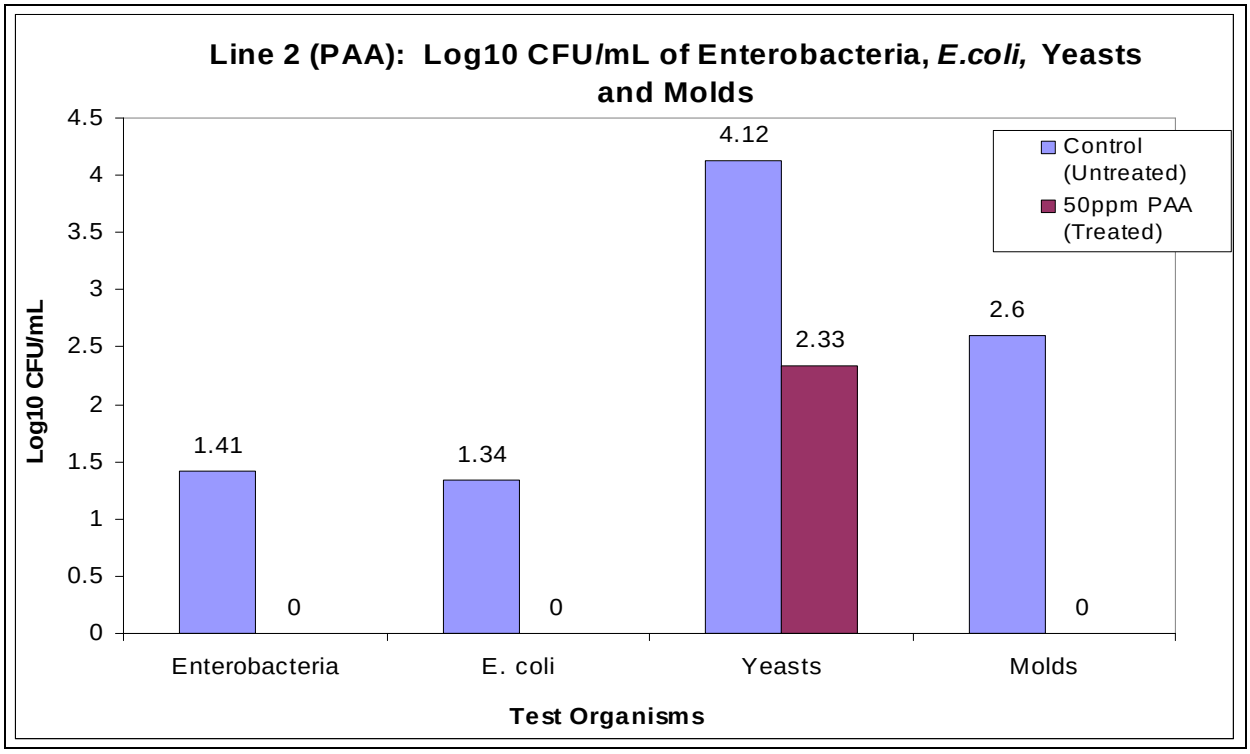
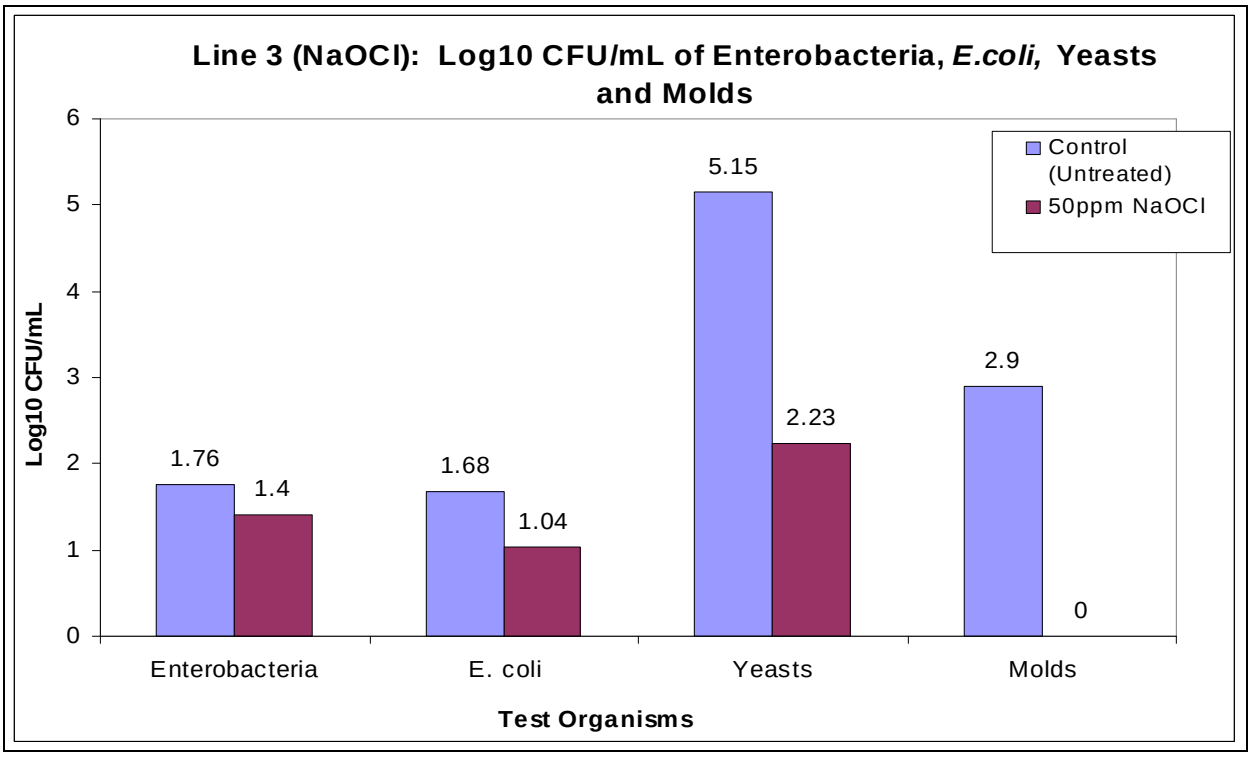


Figure 2:



Conclusions

- Figure 1 and Table 2 show a dramatic decrease in Enterobacteria, *E. coli*, mold cells and yeast cells when cherries are treated with 50ppm PAA from Perasan®. The use of PAA provided a 100% reduction in Enterobacteria and *E. coli* contaminated cherries washed from the cherries and into the aqueous phase. PAA also reduced the number of yeasts and molds washed from surface of the cherries by 98.38% and 100%, respectively.

- Figure 2 and Table 2 demonstrate that the use of NaOCl reduced the number of yeast cells washed from the cherries into the aqueous phase by 99.88% and mold cells by 100%. Although this study shows that there was slightly great efficacy of NaOCl against yeast and mold on cherries, there was only a 56.34 % reduction in Enterobacteria and 77.10% reduction in *E. coli* present on the cherries.

- This study was designed only to compare the relative performance of PAA and NaOCl microorganism washed from the cherries and into the rinse water against that for a simple non chemical treatment water wash. The data reported herein does not represent the amount of surface associated bacteria that may still be resident on the fruit.

- The data collected and reported from this study show that PAA proved to be not only an effective antimicrobial for post harvest cherries, but also shows the significantly higher efficacy against both Enterobacteriaceae and *E. coli* compared to the use of NaOCl as an antimicrobial. It is somewhat concerning that the chemically untreated cherry samples contained a potentially infective dose of Enterobacteria and *E. Coli*, depending on the strain of *E. coli*. Past *E. coli* outbreaks have been linked to contaminated irrigation water, uncomposted manure used as fertilizer, the presence of wildlife, and the personal hygiene habits of field workers handling the crops. The usage of PAA at 50ppm as an antimicrobial during the processing of cherries at the packing house will alleviate concerns of a potential human health problem as well as extend the shelf life and freshness of the cherries.

- The data from this study on cherries is entirely consistent with other studies performed in these laboratories which indicate 50ppm PAA offers meaningful performance against fruit and vegetables contaminated Enterobacteria, *E. coli*, yeast and molds.

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