Efficacy of Peroxyacetic Acid and Lactic Acid Against *E. Coli 0157:H7* On Beef Surfaces

Background

The purpose of this study was to perform a side-by-side comparison on the efficacy of peroxyacetic acid and lactic acid against the hazardous bacteria, *E. coli 0157:H7* that had been inoculated onto beef surfaces. Mixtures of peroxyacetic acid and lactic acid were also compared to determine whether there were any synergistic or antagonistic effects. The efficacy vs. unit time of peroxyacetic acid is very quick, and those of lactic acid are relatively more long-term, so the combination would have a more over-all beneficial effect.

Methods

A stock solution of *E. coli 0157:H7* (ATCC # 43888) was incubated at 35 degrees C for four days in Sigma Nutrient Broth for Microbial Culture. The broth and bacteria mixture was then centrifuged leaving the *E. coli* to be re-suspended in 300mL Butterfield's Buffer.

1) One small chuck roast was cut into twelve equal two-inch cubes. The cubes of meat were marinated in the 300mL *E. coli* Butterfield's Buffer solution for one hour, turning every 15 minutes.
2) Then the twelve cubes of meat were removed from the *E. coli*. Butterfield's Buffer bath, and two cubes were shaken dry using sterile gloves and immersed in each of the following test solutions:
    a) 200mL hard water (city water) to serve as the control.
    b) 200mL 0.5% lactic acid and hard water solution.
    c) 200mL 1.0% lactic acid and hard water solution.
    d) 200mL of 190ppm PAA prepared from a solution of Perasan MP-2 and hard water. .
    e) 200mL of 190ppm PAA (from MP-2) and 0.5% lactic acid in one solution using hard water.
    f) 200mL of 190ppm PAA (from MP-2) and 1.0% lactic acid in one solution using hard water.

The MP-2 used to prepare the test solutions containing PAA were made from MP-2 that tested at 5.36% Hydrogen Peroxide and 15.55% PAA. Perasan MP-2 is a product that contains 15.5% peroxyacetic acid, 5.5% hydrogen peroxide, 35% acetic acid, and 1.1% HEDP and if used less than 200 ppm as peroxyacetic acid complies in all respects to 21 CFR 173.370 for use on meat, poultry and seafood (FCN #699).

3) After soaking in the test solution for one and two minutes, the cubes of meat were removed for swabbing using 3M Quick Swabs. One Quick Swab was used for each cube. The end of the swab was wetted with the 1 mL vial of nutrient media that came with the swab, then one entire side of the cube was swabbed using a

rolling motion over surface. The swab was then returned to the 1mL buffer, vortexed, removed and then swabbed again on the opposite side of the beef cube. The same swab was re-vortexed into the 1 mL of nutrient media which was serially diluted (10^1, 10^3, and 10^5) and then plated onto 3M E.coli / Coliform petrifilms that were incubated at 35 degrees C for 48 hours.

Results and Discussion

The whole process was performed in duplicate (i.e. two pieces of meat in each test solution), to be swabbed at one minute and at two minutes. Figure 1 compares the $\log_{10}$ CFU's/swab on the six test solutions at one minute and two minute intervals. It can be seen that there is little difference between the $\log_{10}$ CFU's/swab values for the control and both 0.5% and 1.0% lactic acid solutions. The decrease in the 190ppm PAA solution (from MP-2) and combination 190ppm PAA (from MP-2) plus lactic acid solutions are also shown in Figure 1.

Figure 1

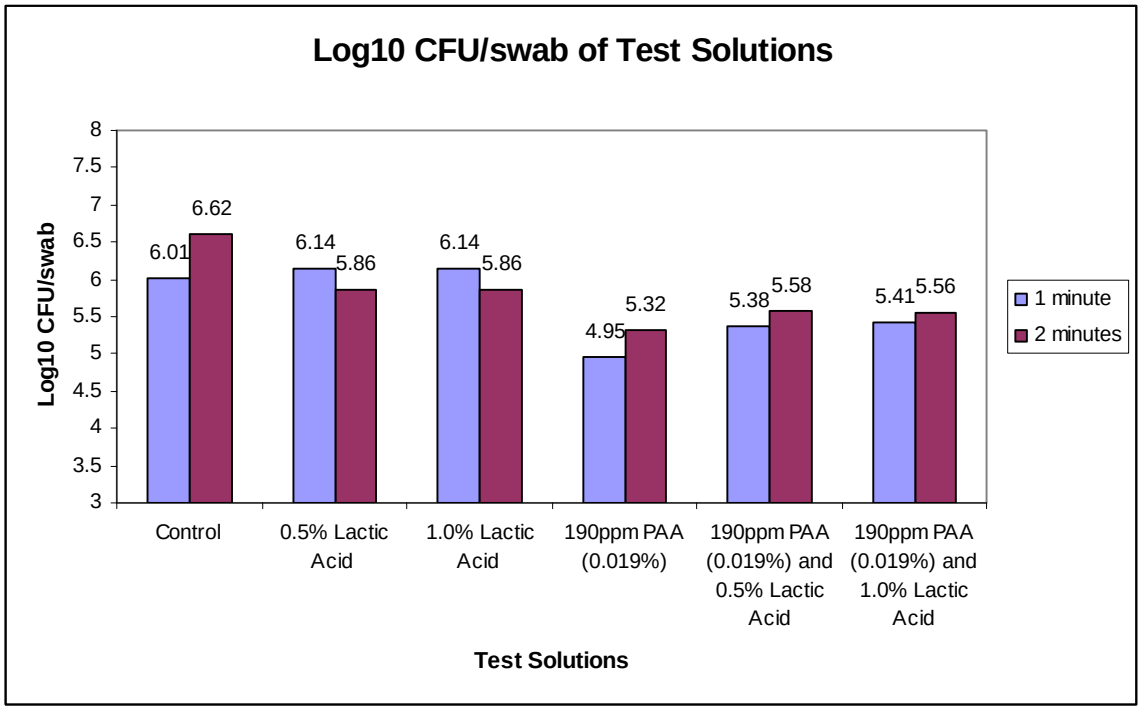

Figure 2 demonstrates the $\log_{10}$ reduction for the 0.5% lactic acid solution, 1.0% lactic acid solution, the 190ppm PAA (from MP-2) solution, and both 0.5% lactic acid/190ppm PAA and 1.0% lactic acid/190ppm PAA solutions compared to the control. As expected, the data indicates that there was a somewhat higher $\log_{10}$ reduction when the cubes of beef were treated for two minutes compared to being treated for one minute. Compared

to the untreated (control) solution, within the statistical variability of the experiment, there was no $\log_{10}$ reduction in bacteria for the 0.5% lactic acid and 1.0% lactic acid solutions when treated for one minute. There was a $\log_{10}$ reduction of 1.06 CFU/mL when treated at one minute and a $\log_{10}$ reduction of 1.30 CFU/mL when treated at two minutes for the solution containing 190 ppm PAA (from MP-2). There was a $\log_{10}$ reduction of 0.63 CFU/mL when treated for one minute and a $\log_{10}$ reduction of 1.04 CFU/mL when treated for two minutes for the solution containing 190ppm PAA (from MP-2) and 0.5% lactic acid. There was a $\log_{10}$ reduction of 0.60 CFU/mL for the solution containing 190ppm PAA (from MP-2) and 1.0% lactic acid when treated for one minute, and a $\log_{10}$ reduction of 1.06 CFU/mL when treated for two minutes.

Figure 2

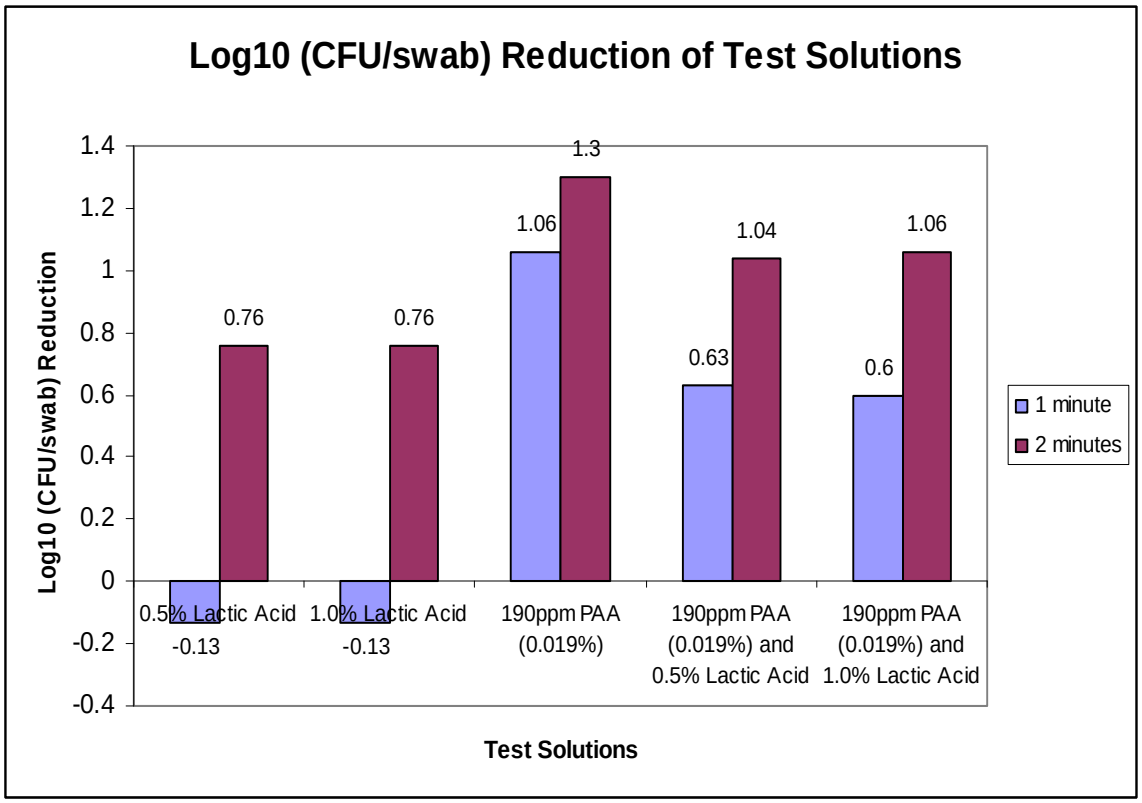

Figure 3 demonstrates the $\log_{10}$ percent reduction of the test solutions when compared to the control at one minute and at two minutes. There was no percent reduction for both lactic acid solutions at one minute. At two minutes there was an 82.6% $\log_{10}$ reduction for both of the lactic acid solutions. The 190ppm PAA test solution (from MP-2) had the highest percent $\log_{10}$ reduction for both one minute and two minutes. It can be seen in Figure 3 that the efficacy of the combination PAA and lactic acid test solutions are far greater than the lactic acid test solutions alone. Figure 3 also shows that the 190ppm

PAA solution (from MP-2) has the highest $\log_{10}$ reduction of surface-associated bacteria, which was 95.0% treated two minutes.

Figure 3

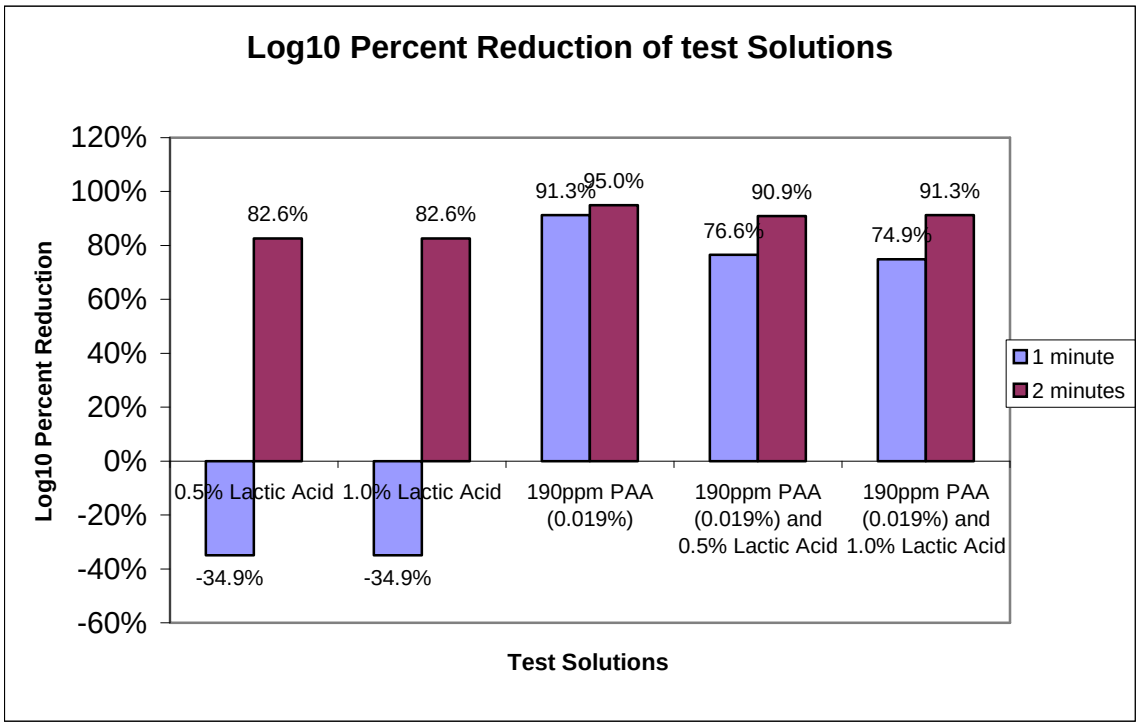

Table 1 reports the pH of all test solutions used in this study. The control (untreated) solution had a pH of 7.56. All solutions containing lactic acid solutions had far lower pH's than the PAA solution alone. This indicates that the antimicrobial effect of the lactic acid solutions may be due only to the lower pH conditions. *E.coli 0157:H7* is known to be a pH-sensitive organism. On the other hand, although 190 ppm PAA from MP-2 also causes a pH depression, the results indicate that the efficacy of PAA cannot be solely attributed to low pH conditions.

Table 1

Test Solution	**pH**
Control (Hard Water)	7.56
0.5% Lactic Acid	2.57
1.0% Lactic Acid	2.38
190ppm PAA (from MP-2)	3.00
190ppm 0.5% Lactic Acid Solution	2.65
190ppm 1.0% Lactic Acid Solution	2.48

Conclusions

- The results of this experiment show that the use of a 0.5% lactic acid solution and a 1.0% lactic acid solution had negligible effect on *E.coli 0157:H7* inoculated beef when treated for one minute. There was a somewhat higher $\log_{10}$ reduction when the cubes of beef were treated for two minutes compared to those treated for one minute.

- There was a $\log_{10}$ reduction of 1.06 CFU/mL when treated at one minute and a $\log_{10}$ reduction of 1.30 CFU/mL when treated at two minutes for the solution containing 190 ppm PAA (from MP-2). There was a $\log_{10}$ reduction of 0.63 CFU/mL when treated for one minute and a $\log_{10}$ reduction of 1.04 CFU/mL when treated for two minutes for the solution containing 190ppm PAA (from MP-2) and 0.5% lactic acid. There was a $\log_{10}$ reduction of 0.60 CFU/mL for the solution containing 190ppm PAA (from MP-2) and 1.0% lactic acid when treated for one minute, and a $\log_{10}$ reduction of 1.06 CFU/mL when treated for two minutes.

- The 190ppm PAA test solution (from MP-2) had the highest percent $\log_{10}$ reduction for both one minute and two minutes. It can be seen in Figure 3 that the efficacy of the combination PAA and lactic acid test solutions are far greater than the lactic acid test solutions alone. Figure 3 also shows that the 190ppm PAA solution (from MP-2) itself has the highest $\log_{10}$ reduction of surface- associated bacteria corresponding to 95.0% when treated for two minutes.

- PAA and lactic acid do not exhibit synergistic behavior. In fact, combinations of 0.5% and 1% lactic acid in the presence of 190 ppm PAA actually display slightly reduced efficacy compared to 190 ppm PAA alone. The effect of lactic acid on suppressing the performance of PAA is not statistically significant.

- The results indicate that the antimicrobial effect of lactic acid may be due only to the lower pH conditions. *E.coli 0157:H7* is known to be a pH-sensitive organism. On the other hand, although 190 ppm PAA from MP-2 also causes a pH depression, the results indicate that the efficacy of PAA cannot be solely attributed to low pH conditions.

- Utilizing a two-part antimicrobial solution where each is recognized as an independently acceptable antimicrobial treatment will increase the confidence, efficacy and margin of safety in these applications should one of the treatment systems cease, due to mechanical or operator error.

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