Effectiveness of Lactic Acid and Peroxyacetic Acid Treatments on Reducing Generic and Pathogenic *E. coli* on Fresh Apples

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Abstract

Identifying effective antimicrobial interventions to reduce foodborne pathogen risk are important for the fresh apple packing industry. Apple packing antimicrobial interventions are commonly applied for short application times using spray bars and longer application times in flumes. The objective of this study was to investigate the potential for using lactic acid for spray bar applications and peroxyacetic acid (PAA) for flume applications based on reduction of generic and pathogenic *Escherichia coli* (*E. coli*) on fresh apples. For each replication, fresh apples (n=220) were randomly assigned to treatments. Uninoculated controls (5 apples) were examined for background microbial levels (total coliforms, generic *E. coli* and aerobic plate counts) and the remainder were inoculated with pathogenic (*E. coli* O157:H7) or generic *E. coli*. Lactic acid (1% and 2%) and water treatments were examined at application times of 5, 15 and 30 seconds to mimic spray bar applications. PAA (60 and 80 ppm) and water treatments were examined at application times of 2, 3.5 and 5 minutes to mimic flume applications. Apples were sampled using appropriate serial dilutions for microbial levels and plated on Violet Red Bile Agar (VRBA) for generic *E. coli* and Sorbitol-MacConkey Agar supplemented with Cefixime and Potassium Tellurite (CT-SMAC) for *E. coli* O157:H7. For examination of lactic acid as a potential spray bar application, the main treatment effect was significant (p<0.01). Inoculated control samples averaged 5.3 log cfu/ml and were significantly (P<0.05) higher (about 1 log cfu/ml) than all other treatments. However, all lactic acid treatments were similar to water treatments. For lactic acid, application time did not influence microbial reductions. Microbial reductions were also observed for the treatments reflecting flume applications with a significant (p<0.01) treatment main effect for water and PAA treatments. Treatments with 60 ppm PAA at 3.5 and 5 minutes and 80 ppm PAA for all application times were significantly (p<0.05) lower than water treatments for all application times (average 0.5 log cfu/ml reduction) and the inoculated control treatment (average 1.5 log cfu/ml reduction). Lactic acid (1 and 2%) treatments with short application times do not appear to reduce microbial levels sufficiently for consideration as a spray-bar application for fresh apple packing. PAA treatments of 80 ppm for 2-5 minutes and 60 ppm for 3.5-5 minutes reduced microbial levels sufficiently for consideration as a flume application for fresh apple packing.

Introduction

- Annually, about 48 million people acquire foodborne illnesses in the United States. Several foodborne pathogens contribute to this statistic, and *E. coli* O157:H7 is among the top five pathogens causing hospitalization due to foodborne illness (CDC, 2011).
- Due to increasing foodborne illnesses associated with fresh produce consumption, identifying effective antimicrobial interventions to reduce foodborne pathogen risk is important for the produce industry (Sapers, 2002). The tree fruit industry is important to the state of Washington, and fresh apple packing industry is interested in validating current food safety interventions.
- The most common apple packing antimicrobial interventions involve spray bar applications with contact times of 5-30 seconds using peroxyacetic acid or chlorine dioxide, and flume applications with contact times of 2-5 minutes using chlorine or an acid treatment.
- Lactic acid and acetic acid are two organic acids traditionally used as antimicrobial carcass sprays in the meat industry (Jasass. 2008). Lactic acid is being explored for use in fresh produce to reduce microbial levels (Park et al., 2011).

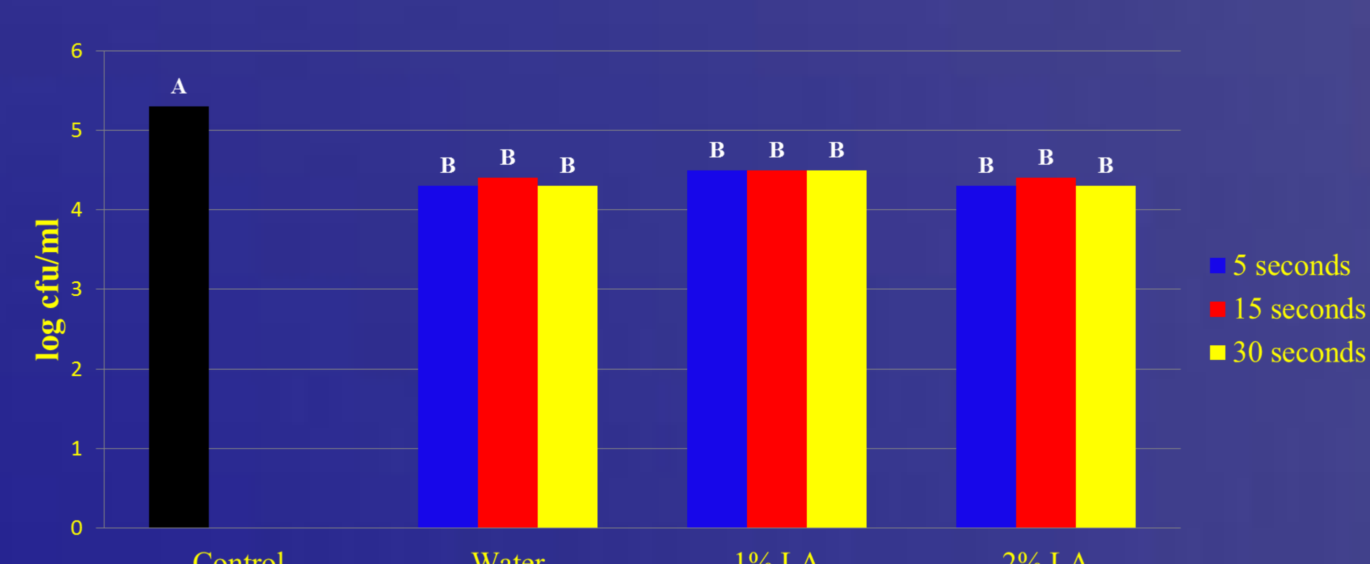
Objective

The objective of this study was to investigate the potential for using lactic acid for spray bar applications and peroxyacetic acid (PAA) for flume applications through examination of reduction of generic and pathogenic *E. coli* on fresh apples.

Materials and Methods

- For each replication, fresh, Gala apples (n=220) were randomly assigned to treatments.
- Uninoculated controls (10 apples) were examined using 3M Petrifilm™ for aerobic plate counts, total coliform counts and generic *E. coli* levels Violet Red Bile Agar (VRBA) was also utilized to examine total coliform counts and Sorbitol-MacConkey Agar supplemented with Cefixime and Potassium Tellurite (CT-SMAC) was utilized to examine for the presence of pathogenic *E. coli*.
- Cocktails of generic *E. coli* (ATCC 25922, 11755, 23716) and pathogenic *E. coli* (*E. coli* O157:H7 43890, 43895, SEA13B88) were prepared. For apple inoculation with each bacterial type, 2.5L of 0.1% peptone water, 300ml of the appropriate cocktail and 147ml of sterile horse serum were mixed in sterile, biowaste bags lining a 5 gallon bucket. Due to the size of the experiment, each replication was completed over two days. On each day, data was collected for either generic or *E. coli* O157:H7.
- Ten apples were placed in the inoculum, and apples were massaged for 10 minutes. Apples were placed under forced air in a biosafety cabinet to dry for one hour to allow for bacterial attachment.
- For all treatments, spring water (Super Chill ™) was purchased and used to ensure initial water pH values were near 7.0. (Tables 1 and 2).
- Lactic acid (1 and 2%) and water treatments were examined at application times of 5, 15 and 30 seconds to mimic spray bar applications. PAA (60 and 80 ppm) and water treatments were examined at application times of 2, 3.5 and 5 minutes to mimic flume applications.
- After water and sanitizer treatments, apples were allowed to dry briefly for 10 seconds. Dey-Engley neutralizing (DE) broth (99 mL) was placed in a sterile stomacher bag with each apple and massaged for 2 minutes. The purpose of the (DE) broth was to neutralize the acidic effect of the sanitizers and recover microbes from apple surfaces.
- Apples were sampled using appropriate serial dilutions for microbial levels and plated on VRBA for enumeration of generic *E. coli* and CT-SMAC for *E. coli* O157:H7. Inoculated plates were incubated at 37°C for 24±2 hours.
- Microbial counts were logarithmically transformed. Statistical analysis for examination of lactic acid and peroxyacetic acid were performed separately. For each compound, the study design was a randomized complete block with a factorial arrangement of treatments. Fixed effects included bacteria and treatment (combinations of sanitizer concentration and application time) and replication as the block was considered a random effect.

Figure 1: Average generic and *E. coli* O157:H7 levels on apples after inoculation, direct application lactic acid (concentrations 1 and 2%) for 5, 15 and 30 seconds

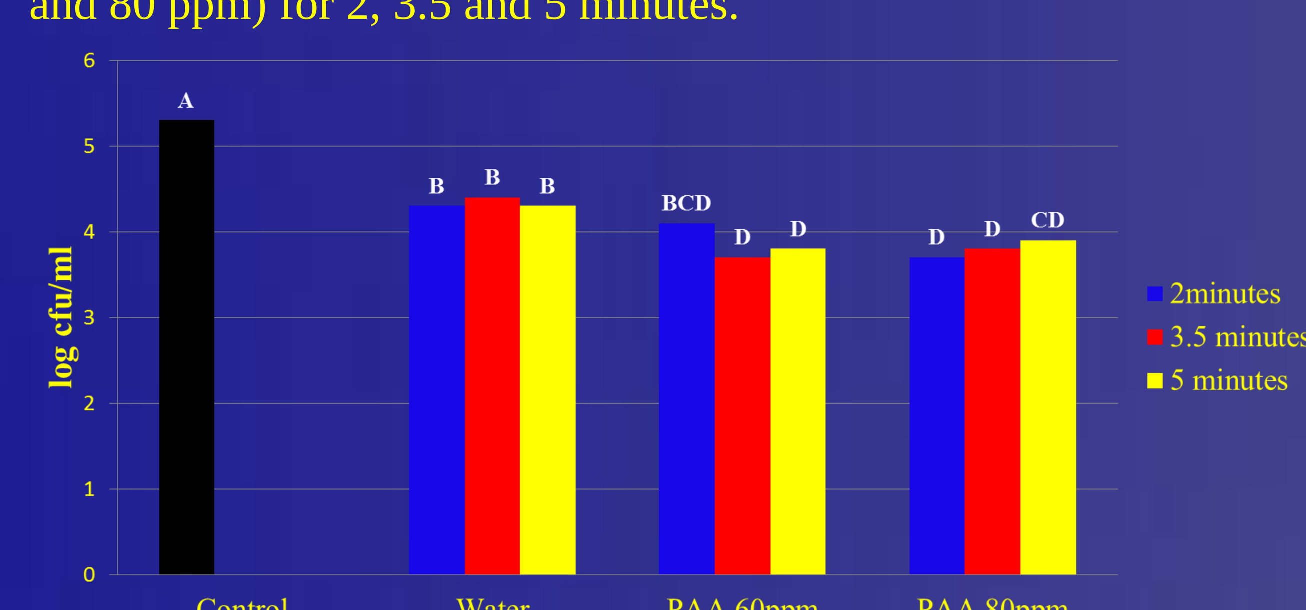


A-B Values without a common superscript differ significantly (p<0.05)

Table 1: Solution pH and temperature for water and lactic acid treatments.

Treatment	pH	Solution Temp °C
Water	6.6	21.3
1% LA	2.3	21.5
2% LA	2.1	21.6

Figure 2: Average generic and *E. coli* O157:H7 levels on apples after inoculation, direct application peroxyacetic acid (concentrations 60 and 80 ppm) for 2, 3.5 and 5 minutes.



A-D Values that do not share a common superscript differ significantly (p<0.05).

Table 2: Solution pH and temperature for water and peroxyacetic acid treatments.

Treatment	pH	Solution Temp °C
Water	6.6	21.3
PAA 60ppm	4.3	21.5
PAA 80ppm	4.1	21.5

Results and Discussion

- Uninoculated controls averaged 4.1 log/ml for aerobic plate counts (APC), 0.15 log/ml for total coliforms and 0.1 log/ml for generic *E. coli* (EC) on Petrifilm™. On VRBA, total coliform levels averaged 0.3 log/ml. Pathogenic *E. coli* O157:H7 were not observed on CT-SMAC plates.
- For apple inoculation, the inoculum level averaged 8.04 log/ml for *E. coli* O157:H7 and 7.94 log/ml for generic *E. coli*.
- Inoculated control apples averaged 5.3 log cfu/ml.

Lactic Acid

- The main treatment effect was significant (p<0.01).
- Application time did not influence microbial reductions for water, 1% or 2% lactic acid treatments (Figure 1).
- The water treatments averaged 4.3 log cfu/ml (averaged over all application times) and a significant (p<0.05) reduction (about 1 log cfu/ml) was observed compared to the inoculated control.
- For 1% lactic acid, an average of 4.5 log cfu/ml was observed; representing about a significant (P<0.05) 1 log cfu/ml reduction compared to the inoculated control.
- For 2% lactic acid, an average of 4.3 log cfu/ml was observed and which was a significant (p<0.05) reduction (about 1 log cfu/ml) compared to the inoculated control.
- Average bacterial levels observed for 1 and 2% lactic acid treatments were not significantly (p>0.05) different than levels observed on the water treatments.

Peroxyacetic Acid

- The main treatment effect was significant (p<0.01).
- For water and PAA treatments reflecting flume applications, significant reductions were observed (p<0.01). (Figure 2).
- Water treatments averaged 4.4 log cfu/ml produced a significant (P<0.05)1 log cfu/ml reduction compared to the inoculated control.
- Peroxyacetic acid at 60 ppm averaged 3.9 log cfu/ml (or a 1.4 log cfu/ml reduction compared to the inoculated control);
- For the peroxyacetic acid 80 ppm treatment averaged 3.8 log cfu/ml.
- Treatments with 60 ppm PAA at 3.5 and 5 minutes and 80 ppm PAA for all application times were significantly (p<0.05) lower than water treatments for all application times (average 0.5 log cfu/ml reduction).

Conclusion

- Lactic acid (1 and 2%) treatments with short application times do not appear to reduce microbial levels sufficiently for consideration as a spray-bar application for fresh apple packing.
- PAA treatments of 80 ppm for 2-5 minutes and 60 ppm for 3.5-5 minutes reduced microbial levels sufficiently for consideration as a flume application for fresh apple packing.

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