CENTER for PRODUCE SAFETY

CPS 2016 RFP
FINAL PROJECT REPORT

Project Title
Control of *Listeria monocytogenes* on apple through spray manifold–applied antimicrobial intervention

Project Period
January 1, 2017 – December 31, 2018 (extended to January 31, 2019)

Principal Investigator
Meijun Zhu
Washington State University
School of Food Science
FSHN 232, 100 Dairy Road
Pullman, WA 99164
T: 509-335-4016
E: meijun.zhu@wsu.edu

Co-Principal Investigator
Trevor Suslow
University of California, Davis
Department of Plant Sciences
Davis, CA 95616
T: 530-754-8313
E: tvsuslow@ucdavis.edu

Objectives
1. *Validate the efficacy of selected sanitizers against Listeria monocytogenes on whole apple surfaces.*
2. *Verify the selected sanitizer interventions in the model/pilot packing line and representative commercial apple packing lines.*

Funding for this project provided by the Center for Produce Safety through:
CDFA SCBGP grant# SCB16071

FINAL REPORT

Abstract

Recent multistate outbreaks and recalls of fresh apples due to *Listeria monocytogenes* contamination have increased consumer concerns regarding fresh apple safety. The apple industry has an immediate need to begin the process of science-based improvements in *Listeria* control during packing and storage. The overall goal of this project was to comparatively assess and validate commercially practical and legally allowed sanitizer(s) against *L. monocytogenes,* and to further verify their efficacy on the pilot and multiple apple packing lines. Laboratory studies showed that chlorine-based sanitizers, either hypochlorite or (novel) mineral oxychlorides (JC9450) and neutral electrolyzed water (NEW), have limited efficacy against *L. monocytogenes* on apple surfaces, even at 100 ppm free available chlorine. Compared with chlorine-based sanitizers, peroxyacetic acid (PAA) at 80 ppm and practical contact time is more effective against *L. monocytogenes* on fresh apple surfaces. The anti-*Listeria* efficacy of PAA was not affected by the hardness of the wash water or the pH of the PAA solution, while efficacy improved dramatically when applied at elevated temperature. The 80 ppm PAA applied at 43–46 °C for 30-sec and 60-sec contact times reduced *L. monocytogenes* on apples by 2.2–2.4 and 2.3–2.6 Log_{10} CFU/apple, respectively. The anti-*Listeria* efficacy of PAA was further validated in the pilot spray-bar brush-bed washing line using *L. innocua* and *E. faecium* NRRL B-2354 as non-pathogenic surrogates, where 80 ppm PAA at 43–46 °C for 30-sec and 2-min exposure resulted in reductions of 1.5 and 1.6 Log_{10} CFU/apple, respectively. The efficacy of PAA at ambient temperature and elevated temperature was further validated at three commercial apple packing facilities by using *E. faecium.* PAA at ambient temperature for 30-sec contact time reduced *E. faecium* by 1.1–1.3 Log_{10} CFU/apple on inoculated Granny Smith (GS) or Fuji apples during commercial wash line validation. Elevating the temperature of PAA significantly improved the efficacy against *E. faecium* on inoculated apples. PAA at 43–45 °C for 30-sec contact time reduced *E. faecium* by 1.45, 1.94 and 2.19 Log_{10} CFU/apple on inoculated apples in facility A, B and C, respectively. Different reductions of *E. faecium* on inoculated apples might be related to the spray-bar brush-bed designs and dwell times in each facility. PAA spray treatment either at ambient temperature or elevated temperature could not prevent cross-contamination but reduced the cross-contamination rates. Dirty brush beds enhanced the cross-contamination rate. Additionally, the transfer rate could also be closely related to spray and brush parameters such as brush-bed speed, effective sanitation, nozzle and bar arrangements, and others. These data provide valuable information and reference points for the apple industry to further validate or verify process controls. The data also provide baseline parameters for an alternative intervention method for the apple industry to improve antimicrobial efficacy against foodborne pathogens.

Background

Listeria monocytogenes has been singled out due to its nature as a true environmental species, common prevalence in many produce-associated locales and operations, its ability to grow at refrigerated temperature (Chan & Wiedmann, 2009; Ells & Truelstrup Hansen, 2010; Golden, Brackett, & Beuchat, 1990), and high rate of mortality. Apples are an important global commodity, including in the Pacific Northwest. Recent *L. monocytogenes* multistate outbreaks associated with cantaloupes (CDC, 2012) and caramel apples (FDA, 2014), and an increasing number of recalls of possible *L. monocytogenes* contamination in fresh apples highlight the importance of controlling this pathogen in apples. Prevention and control of *L. monocytogenes*, in particular, has been identified as a priority research need by the tree fruit industry as a whole. Apples are routinely sorted, washed, and packed in packing facilities for further distribution and

marketing. Pathogenic microorganisms, including *L. monocytogenes*, can be introduced to apples at any stage, but it is generally held that the postharvest handling and packing environment is a significant source. Once contaminated, it is a challenge to reduce or eliminate pathogens on any produce item, including fresh apples. Various presumptive intervention steps have long been employed to reduce surface-borne pathogens on apples. One such intervention is the spray-bar sanitizer intervention. However, the specific practices and process designs are highly variable among industry operations. Scientifically controlled and collected data documenting the effectiveness of current spray-bar preventive controls in reducing human pathogens are sparse, especially for *L. monocytogenes*. Chlorine treatment has been utilized at various points during apple packing, but its effectiveness varies dramatically depending on the washing conditions such as pH and organic load (Beuchat et al., 1998; Francis et al., 2012). There is also a safety concern about the production of carcinogenic halogenated by-products resulted from chlorinated organic compounds (Parish et al., 2003). Peroxyacetic acid (PAA) is currently the most common single sanitizer used in spray-bar intervention. However, in laboratory studies on Golden Delicious apples, using 80 ppm PAA, ~80 sec of continuous contact was required to achieve a 1-Log reduction of *L. monocytogenes* (Rodgers et al., 2004). Based on the study design, it is unclear whether the spray-bar PAA intervention would achieve the desired microbial reduction under commercial practices. In addition, in recent years new GRAS (Generally Recognized as Safe) oxidizing formulation/wash process aids have been developed to control microorganisms. For example, JC9450, a mineral oxychloride ion, is a novel chlorine-based sanitizer that reacts in water to generate antimicrobial reactive oxygen species, such as peroxide and singlet oxygen, which exhibit strong oxidizing capacity and are potential bactericides for spray-bar intervention. Also, neutral electrolyzed water (NEW) is produced from the electrochemical reaction of water and salt, and on-site generation creates a sodium-free solution of hypochlorous acid with a high oxidation-reduction potential. Using NEW with 89 ppm free chlorine, researchers found a >4-Log reduction of *L. monocytogenes*, *Salmonella* and *E. coli* O157:H7 inoculated on tomato surfaces within 30 seconds (Deza, Araujo, & Garrido, 2003), showing a fast and broad spectrum of action against foodborne pathogenic microorganisms, which may be applicable for apples. However, there has been no study about the antimicrobial efficacy of NEW for apples. Furthermore, there is limited information available about the practical efficacy of registered and economical antimicrobial interventions under commercial packing conditions. Studies examining effective sanitizers against *L. monocytogenes*, and verification of laboratory-based pathogen inactivation outcome-data on packing lines would generate actionable information for the apple industry, apple packers and handlers. The overall goal of this project was to comparatively assess and validate commercially practical sanitizers against *L. monocytogenes,* and to further seek to verify this efficacy on multiple apple packing lines.

Research Methods and Results

Objective 1. Validate the efficacy of selected sanitizers against Listeria monocytogenes on whole apple surfaces.

Methods

Bacterial strains: *L. monocytogenes* NRRL B-57618, NRRL B-33053 and NRRL-33466 were used to prepare the 3-strain *L. monocytogenes* cocktail. *L. innocua* NRRL B-33197, TVS470 and TVS471 were used for the 3-strain *L. innocua* cocktail. *Enterococcus faecium* NRRL B-2354 was obtained from USDA-ARS.

Apple inoculation: Non-waxed apples of selected varieties, Granny Smith and Fuji, were individually and separately inoculated to establish 1×10^6 CFU/apple of the respective bacterial culture. .

Antimicrobial intervention: Apples at 24–48 h post-inoculation were immersed in respective antimicrobial solutions with agitation for 30 sec or 2 min; 10–12 apples were included per treatment. All antimicrobial solutions were used at room temperature (RT) unless otherwise specified. Each treatment combination was repeated independently at least three times. Chlorine, JC9450 and NEW concentrates were donated by Pace International (Wapato, WA), Jenfitch LLC (Walnut Creek, CA) and Aquaox (Loxahatchee, FL), respectively. The pH and oxidation/reduction potential (ORP) of chlorine-based wash solutions are listed in Table 1. Bioside HS (EnviroTech, Modesto, CA, USA) containing 15% of PAA was used to prepare PAA solutions (Table 2).

To evaluate the efficacy of antimicrobial water treatments in preventing cross-contamination of *L. monocytogenes* between apples in the lab setting, inoculated apples were loaded with non-inoculated apples at ratios of either 1:10 (Nou & Luo, 2010) or 6:6 (Pao et al.,2007), then subjected to the antimicrobial treatment as described above. Residual *L. monocytogenes* in the spent antimicrobial solutions was enumerated by filtration method.

Microbiological analysis: To detach bacteria from the apple surface, each apple was hand-rubbed for 1.5 min. Rub solutions were 10-fold serially diluted and plated in duplicate to respective plates per our established method (Sheng, Edwards, Tsai, Hanrahan, & Zhu, 2017; Sheng et al., 2018). For *L. monocytogenes or L. innocua*, plates of tryptic soy agar with yeast extract (TSAYE) overlaid with Modified Oxford agar (MOX) were used for enumeration; for *E. faecium*–inoculated apples, TSAYE plates overlaid with enterococcosel broth were used.

Statistical analysis: Data were analyzed by using GLM software (SAS Institute, Cary, NC). Mean values were compared by least significant difference (LSD) multiple-comparison test. *P* values of less than 0.05 were considered statistically significant. Results were reported as mean ± standard error mean (SEM).

Results

Antimicrobial efficacy of hypochlorite, JC9450, and NEW was first assessed against *L. monocytogenes* on Granny Smith (GS) and Fuji apples. Hypochlorite, 1:3 NEW, and 0.125% JC9450 exhibited a comparable but limited antimicrobial efficacy, and reduced *L. monocytogenes* on GS by ~1.0 Log_{10} CFU/apple (Fig. 1A & C). Increasing the JC9450 concentration improved its efficacy, as 0.5% of JC9450 reduced the number of viable *Listeria* by ~3.8 Log_{10} CFU/apple on GS apples (Fig. 1A & C). *L. monocytogenes* on Fuji apples exhibited similar residual survival in response to respective antimicrobial treatments as GS apples (Fig. 1C). In general, increasing contact time from 30 sec to 2 min (Fig. 2) or adjustment of the pH of the chlorine solution (Fig. 3) was not able to improve antimicrobial efficacy. Residual *L. monocytogenes* in spent sanitizer solutions was reduced to levels below detection limits, regardless of the contact time; however, a 2-min wash with water alone transferred ~6.6 Log_{10} CFU/100 ml *L. monocytogenes* from inoculated apples to water (Table 3). Furthermore, no *L. monocytogenes* was detected, either by direct plating or the enrichment method, on uninoculated apples loaded at low (1 inoculated: 10 non-inoculated) or high (6 inoculated: 6 non-inoculated) number of inoculated apples following any of the sanitizer treatments (Table 4). However, the non-inoculated apples from the 2-min water wash were contaminated with *L. monocytogenes* at ~3.8 and 4.2 Log_{10} CFU/apple, for the low and high contamination levels, respectively (Table 4). The results indicated that the three chlorine-based sanitizers at 100 ppm free available chlorine (FAC) have the potential to prevent cross-contamination.

PAA is approved to be used at 80 ppm as a wash water processing aide on fresh produce without further rinse requirement (FDA, 2017a), and is also the most commonly used antimicrobial in spray-bar rinse treatment during fresh apple packing and processing according to a survey conducted with the commercial apple packers in Washington State. Thus, the practical antimicrobial efficacy of PAA was further evaluated and optimized. At 24 h post inoculation, PAA at 40 ppm reduced *L. monocytogenes* on GS apples by 1.37 ± 0.12 Log_{10} CFU/apple at 2-min exposure, which was more effective than 100 ppm chlorine at pH 6.8 (Fig. 6A & B). Increasing PAA concentration significantly increased its bactericidal effects. PAA at 80 ppm and 2-min contact time reduced *L. monocytogenes* on GS apples by 2.17 ± 0.17 Log_{10} CFU/apple (Fig. 6). Extending the post-inoculation time from 24 to 48 h significantly reduced the efficacy of 80 ppm PAA, with a log reduction of 1.71 ± 0.11 Log_{10} CFU/apple at a 2-min treatment time, though it had a minor influence on PAA efficacy at 40 and 60 ppm (Fig. 6).

During the post-harvest process, foodborne pathogens can contaminate apples at any stage, thus, a bacterial attachment time of 48 h was used in the following study to mimic the harshest condition. PAA at 80 ppm was selected to mimic current industry practice and to assess the maximal expected reduction. In the commercial packing facility, the hardness and pH of wash water varies depending on the source of water. Therefore, impacts of water hardness or pH on PAA efficacy were further analyzed. PAA solutions made with water of different hardness had a similar efficacy against *L. monocytogenes* on GS apples, ranging from 1.8–2.0 Log_{10} CFU/apple reduction (Fig. 7A & B). PAA exerts a similar bactericidal effect at pH 2.5–6.3, which reduced *L. monocytogenes* on GS apples by ~1.7 Log_{10} CFU/apple (Fig. 7C & D).

However, increasing the temperature of PAA solution to 43 °C significantly improved PAA efficacy against *L. monocytogenes* (Fig. 8). PAA at 43 and 46 °C reduced *L. monocytogenes* on apples by 2.37 and 2.63 Log_{10} CFU/apple, respectively (Fig. 8A & B). However, further increasing PAA solution temperature to 49 and 52 °C was not able to further boost its effectiveness (Fig. 8A & B). Reducing contact time from 2 min to 30 s decreased bactericidal effects (Fig. 8 C & D). Furthermore, PAA at 46 °C also significantly improved its antimicrobial efficacy against apple background microflora compared with that at room temperature (data not shown). The concentration of PAA at all the tested temperatures remained stable during the wash treatment, while pH and ORP of PAA solutions gradually decreased with the elevation of temperature (Table 2). PAA at the elevated temperature slightly increased the surface temperature of apples depending on treatment temperature and contact time (Table 5).

To validate PAA anti-*Listeria* efficacy in commercial wash, its bactericidal effects against two commonly used surrogates, *L. innocua* and *E. faecium*, were further evaluated and compared. PAA intervention caused a similar reduction in both *L. innocua* and *E. faecium*. The 80 ppm PAA at 2-min RT intervention resulted in 1.81 ± 0.07, 1.96 ± 0.05, and 1.83 ± 0.20 log reductions for *L. monocytogenes, L. innocua,* and *E. faecium*, respectively, showing they are appropriate surrogates for the pilot model line and commercial packing line validation of PAA efficacy.

Objective 2. Verify the selected sanitizer interventions in model/pilot packing line and representative commercial apple packing lines.

Methods

Bacterial strains and apple inoculation: This was done as described for Objective 1. For the commercial packing facility validation, apples were inoculated with *E. faecium* with rifampicin resistance.

Pilot mini-spray bar intervention: The most effective sanitizer treatment identified from lab sanitizer intervention studies were further evaluated in a pilot spray washing line in the PI's lab

(Fig. 4) using *L. innocua and E. faecium* as non-pathogenic surrogates for *L. monocytogenes*. The mini spray-bar and brush-bed system is equipped with the both in-pipe PAA direct injection system and a 50-L sanitizer tank with a heating unit, two spray bars, and a flat brush bed with electronic control panel (Fig. 4). During each trial, ten apples of a single variety were inoculated with *L. innocua* or *E. faecium*, as described above, underwent spray bar intervention (i.e., sanitizer treatment at ambient temperature, heated water alone, or heated water with sanitizer treatments). The experiment was independently repeated three times.

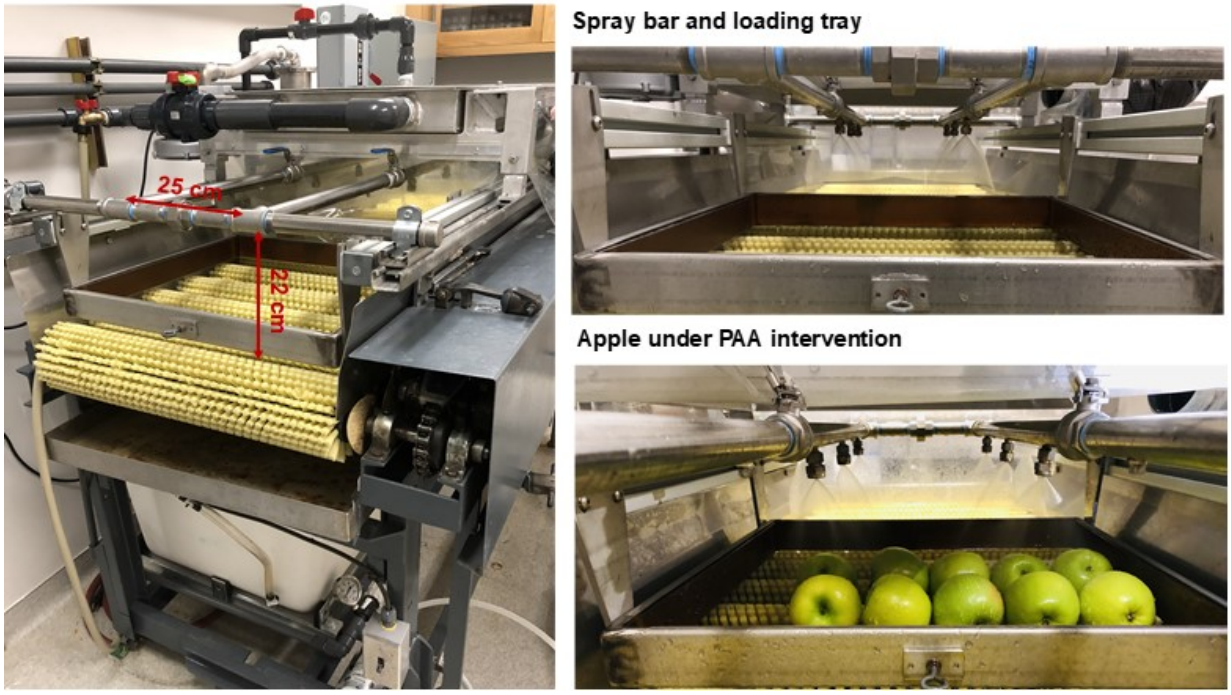


Figure 4. Schematic diagram of the pilot mini spray-bar brush-bed system in the PI's lab. Left: overview of spray-bar intervention facility; Right: apple loading tray and apples under PAA spray treatment. The flow rate of the spray bar is 0.26 gallon/min, and the brush bed rotating speed is 27 n/min (rpm).

Commercial packing facilities selected in the study: Three commercial apple packing facilities (Facility A, B, and C) with heated PAA spray-bar brush-bed systems were recruited for the PAA validation study. The spray-bar brush-bed system in the selected packing facilities includes a flat brush bed, three PAA spray bars and a water spray bar. However, each packing facility has its unique setting in terms of the distance between nozzles of the selected spray bar, the distance between the spray bars and the height from the spray bar to the brush bed. The specific parameters of the respective packing facility are outlined in Figure 5. The distance between spray bars ranged from 30–100 cm; the distance between nozzles is 20–30 cm. The heights from the spray bar nozzle to the brush bed were 20, 24 and 33 cm for Facility A, B and C, respectively. The flow rates of the spray bar were 0.3 gallon/min for Facility A and B, and 0.4 gallon/min for Facility C. The alternate brush rotating speeds were 45/120, 40/40 and 68/90 n/min (rpm) for Facility A, B and C, respectively (Table 6). Other parameters, including water pH, flow rate, contact time, and dwell time, are shown in Table 6.

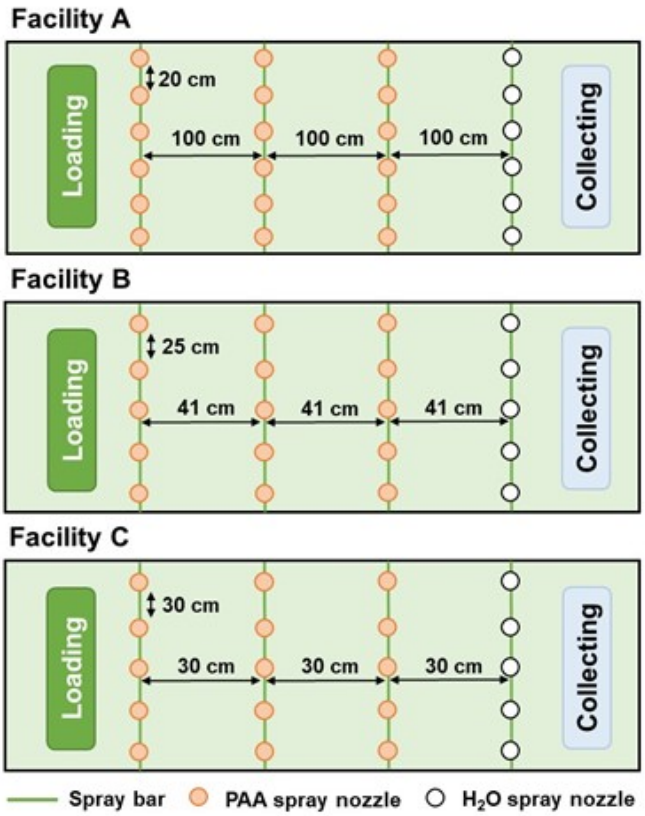


Figure 5. Schematic diagram of spray bar systems of 3 commercial apple packing facilities in Washington.

Apple processing in the commercial packing facility: In each packing facility, both GS and Fuji apples were tested. For each treatment with a different combination of sanitizer, temperature and contact time, 20–24 apples of a single variety inoculated with *E. faecium* underwent the spray bar interventions (heated water alone, heated 80 ppm PAA [(43–45 °C], and 80 ppm PAA at ambient temperature [17–22 °C]). During PAA spray-bar intervention, apples were exposed to a sanitizer treatment at the spray bar for the standard contact time (30 sec) and one additional duration (60 sec). Within each trial, 72–80 non-inoculated apples were introduced with inoculated fruit at a ratio of 1:3 (for Facility A) or 1:4 (for Facility B and C) to test for quantitative (enumeration) and qualitative (enrichment and qPCR confirmation) detection of cross-contamination. The PAA concentration was tested before and after each intervention. The temperatures of water and PAA solutions were measured before each treatment. The brush bed was sanitized with chlorine between spray-bar sanitizer treatments. Brush beds were swabbed before and after sanitizer disinfection.

Apples were sampled as follows: 1) right after to inoculation to document initial levels of inoculation; 2) 48 h post inoculation to examine the established *E. faecium*; and 3) after each stage of spray-bar treatment. The surface temperature was measured from 20 randomly selected apples by an infrared thermometer (Etekcity Corporation, Anaheim, CA). Immediately following spray-bar intervention, apples were transferred into stomach bags, one apple per bag, and then 10 ml of neutralizing buffer was added. Collected apple samples were immediately chilled to ~40 °F, stored in cooler and transported to the laboratory for analysis of quantitative and qualitative levels of the inoculated strain on both inoculated and non-inoculated apples. All apples were processed within 24 h after collection for microbiological analyses (see below).

Microbiological analysis: Survival on apple surfaces was analyzed as described previously. TSAYE plates overlaid with Modified Oxford agar were used for *L. innocua* enumeration, while TSAYE plates containing 40 μg/ml of rifampicin (TSAYE+Rif) were used for enumeration of *E. faecium,* which carries rifampicin resistance. For uninoculated apples, 1.0 ml of detached bacterial rub solution was plated onto three TSAYE+Rif plates to quantitatively enumerate the contaminated bacteria or was enriched in enterococcosel broth for 24 h to qualitatively detect cross-contamination. The enrichment-positive sample was streaked onto enterococcosel broth supplemented with 1.5% agar and TSAYE+Rif plates, and further confirmed with PCR by targeting *vanB* gene (Dutkamalen et al., 1995; Jayaratne & Rutherford, 1999).

Statistical analysis: Data were analyzed by GLM from Statistical Analysis Systems (SAS, Cary, NC). Mean values were compared by least significant difference (LSD) multiple-comparison test. *P* values of less than 0.05 were considered statistically significant. Results were reported as mean ± standard error mean (SEM).

Results

The antimicrobial efficacy of PAA at ambient and elevated temperature against *L. innocua* and *E. faecium* on GS apple surfaces was evaluated in a pilot-scale mini spray-bar and brush-bed system to compare fidelity as a surrogate for PAA spray-bar intervention (Fig. 1). Spray bar application of 80 ppm PAA at 22 °C for 30-sec or 2-min contact time resulted in ~1.0 Log_{10} CFU/apple reduction on GS apples (Table 7). A comparable or slightly smaller log reduction was observed for *E. faecium* on GS apples (Table 7). Similarly, 80 ppm PAA solution applied at ~46 °C for 30 sec and 2 min caused 1.5 and 1.6 Log_{10} CFU/apple reductions of *L. innocua* on GS apples, respectively, which were again comparable to the respective reduction of *E. faecium* on GS apples (Table 7). Consistently, PAA showed a similar antimicrobial efficacy against *L. innocua* and *E. faecium* on Fuji apples at different temperature and time combinations (Table 8).

The antimicrobial efficacy of PAA against *L. monocytogenes* on fresh apples at ambient and heated temperatures was further validated at three commercial apple packing facilities (Facility A, B and C) with heated PAA spray-bar brush-bed systems (Figs. 9–11). Hot water (43–45 °C) spray wash was included to show the bacterial reduction due to factors other than antimicrobial activities. PAA at ambient temperature for 30-sec contact time reduced *E. faecium* by 1.12, 1.28 and 1.23 Log_{10} CFU/apple on inoculated GS apples in Facility A, B and C, respectively (Figs. 9–11). Increasing the contact time to 60 sec slightly increased the reduction of *E. faecium* (Figs. 9–11). Similar results were also observed for *E. faecium*–inoculated Fuji apples. Elevating the temperature of PAA significantly improved the efficacy against *E. faecium* on inoculated apples (Figs. 9–11). PAA at 43–45 °C for 30-sec contact time reduced *E. faecium* by 1.45, 1.94 and 2.19 Log_{10} CFU/apple on inoculated apples in Facility A, B and C, respectively (Figs. 9–11). Different reductions of *E. faecium* on inoculated apples might be related to the spray-bar brush-bed designs or dwell times in each facility (Table 6). Compared with Facility B and C, Facility A had longer distance between their spray bar, which is correlated with a much lower reduction in heated PAA intervention. The surface temperature of apples post heated PAA treatment was 17–22 °C (Table 9), which is not expected to negatively impact fruit quality.

A 60-sec hot water spray-bar intervention caused transfer of ~3 Log_{10} CFU/apple of *E. faecium* to uninoculated apples at both 1:3 and 1:4 cross-contamination ratios (Fig. 12). PAA spray treatment either at ambient or elevated temperature could not prevent cross-contamination but reduced the cross-contamination rates (Fig. 12). Dirty brush beds enhanced the cross-contamination rate. Transfer rate or cross-contamination was much higher in Facility A, where the brush bed was not disinfected between treatments (Fig. 12). Additionally, the transfer rate could also be closely related to spray and brush parameters such as brush bed speed, effective sanitation, nozzle and bar arrangements, and others.

Outcomes and Accomplishments

Through extensive laboratory testing, pilot model system and commercial system spray bar validations, the project data collectively highlight that *L. monocytogenes*, once inoculated on the apple surface, is very difficult to eliminate. Laboratory studies conducted early in the project period showed that chlorine-based sanitizers, either hypochlorite or novel JC9450 and NEW, have limited efficacy against *L. monocytogenes* on apple surfaces even at 100 ppm free available chlorine level. PAA is relatively more effective against potential contamination of *L. monocytogenes* on apple surfaces compared with the chlorine-based sanitizers. In the later stage of the study, we focused on optimizing bactericidal activity of PAA and found that PAA at elevated temperature can achieve about 2-log reductions at practical concentration and contact time. The anti-*Listeria* efficacy of PAA was further validated in both the pilot wash line and multiple commercial packing facilities. The data provide valuable information and reference points for the apple industry to further validate or verify process controls. The data also provide baseline parameters for an alternative intervention method for the apple industry to improve antimicrobial efficacy against foodborne pathogens.

Summary of Findings and Recommendations

The chlorine-based sanitizers at 100 ppm free available chlorine level have limited efficacy against *L. monocytogenes* on apple surfaces. Compared with the chlorine-based sanitizers, PAA at practical concentration and contact time is more effective against *L. monocytogenes* on the surface of fresh apples. The anti-*Listeria* efficacy of PAA was not affected by the hardness of wash water or the pH of the PAA solution, and was improved dramatically when applied at elevated temperature. A 30-sec contact of 80 ppm PAA spray intervention at 43–45 °C can result in 1.4–2.3 log reduction of *L. monocytogenes* on fresh apples in commercial apple packing lines. It is important to point out that the tremendous variations in spray-bar brush-bed settings and nozzle alignments and parameters used will impact the practical efficacy of PAA. Therefore, the apple industry should take the unique settings of their packing lines into consideration before adopting the results of the current study. Data also indicate a need for further improvement in the efficacy of PAA or alternative sanitizers to control *L. monocytogenes* on apple surfaces.

APPENDICES

Publications and Presentations

Shen, X., L. Sheng, H. Gao, I. Hanrahan, T.V. Suslow, and M.J. Zhu. 2019. Enhanced efficacy of peroxyacetic acid against *Listeria monocytogenes* on fresh apples at elevated temperature. *Frontiers in Microbiology,* in revision.

Sheng, L., X. Shen, O. Ulloa, T.V. Suslow, I. Hanrahan, and M.J. Zhu. 2019. Antimicrobial efficacy of calcium oxychlorides and neutral electrolyzed water against *L. monocytogenes* on fresh apples. *Food Control,* submitted.

Shen, X., L. Sheng, H. Gao, I. Hanrahan, T.V. Suslow, and M.J. Zhu. Enhanced efficacy of peroxyacetic acid against *Listeria monocytogenes* on fresh apples. 119th General Meeting of the American Society for Microbiology, San Francisco, California, June 20–24, 2019.

Budget Summary

A total of $293,883 was awarded for this project. All grant funds awarded will be utilized by the end of the project period in the execution of the planned and modified objectives associated with this project.

Tables 1–9 and Figures 1–3, 6–12

Table 1. Physicochemical properties of antimicrobials used in this study.

Treatment	pH	ORP (mV)	FAC (ppm)
Deionized H_2O	6.65 ± 0.15	346.1 ± 12.7	0.0 ± 0.0
100 ppm hypochlorite	6.82 ± 0.01	882.5 ± 6.3	113.3 ± 1.0
0.01% JC9450	7.19 ± 0.03	672.4 ± 2.7	8.0 ± 0.0
0.125% JC9450	9.43 ± 0.15	643.3 ± 6.6	100.0 ± 0.0
	6.81 ± 0.00*	854.3 ± 4.7	100.0 ± 0.0
0.25% JC9450	9.84 ± 0.14	640.0 ± 11.8	200.0 ± 0.0
	6.81 ± 0.01*	887.5 ± 4.4	200.0 ± 0.0
0.50% JC9450	10.34 ± 0.08	612.7 ± 11.8	400.0 ± 0.0
	6.82 ± 0.00*	908.8 ± 1.8	400.0 ± 0.0
1:3 NEW	6.88 ± 0.15	883.7 ± 8.9	110 ± 0.0
1:7 NEW	6.74 ± 0.07	870.0 ± 8.0	55.0 ± 0.0
1:15 NEW	6.58 ± 0.02	823.2 ± 7.2	22.5 ± 0.0

Values are means ± SEM, n=3; NEW, neutral electrolyzed water; ORP, oxidation reduction potential; FAC, free available chlorine; *, pH of solution was adjusted to 6.8 with 6 N HCl.

Table 2. pH and oxygen reduction potential (ORP) of peroxyacetic acid (PAA) at different temperatures.

Temperature	PAA Conc. (ppm)	pH	ORP (RmV)
22 °C	80.0 ± 0.0	6.27 ± 0.01	375.0 ± 0.7
38 °C	80.0 ± 0.0	6.24 ± 0.01	367.0 ± 0.9
41 °C	80.0 ± 0.0	6.21 ± 0.01	363.2 ± 0.5
43 °C	80.0 ± 0.0	6.20 ± 0.02	359.4 ± 0.5
46 °C	80.0 ± 0.0	6.15 ± 0.03	359.4 ± 0.6
49 °C	80.0 ± 0.0	6.02 ± 0.03	351.0 ± 0.6
52 °C	80.0 ± 0.0	6.03 ± 0.01	350.5 ± 1.1

Data are presented as means ± SEM, n = 3.

Table 3. Enumeration of residual viable *Listeria monocytogenes* in spent wash solution following treatment of inoculated Granny Smith apples with chlorine-based sanitizers**.**

Treatment	Contact time	*L. monocytogenes* (Log_{10} CFU/100 ml)
Deionized H_2O	2 min	6.56 ± 0.01
100 ppm hypochlorite	30 s	< LOD
	2 min	< LOD
1:7 New	30 s	< LOD
	2 min	< LOD
1:3 NEW	30 s	< LOD
	2 min	< LOD
0.125% JC9450	30 s	< LOD
	2 min	< LOD
0.25% JC9450	30 s	< LOD
	2 min	< LOD
0.50% JC9450	30 s	< LOD
	2 min	< LOD

pH of each treatment was 6.6–6.8. NEW, neutral electrolyzed water; LOD, limit of detection, 1 CFU/100 ml; Mean ± SEM, n = 3.

Table 4. Efficacy of neutral electrolyzed water and commercial sanitizer JC9450 for the prevention of cross-contamination of Granny Smith apples with *Listeria monocytogenes* during treatments with different chlorine-based sanitizer washes.

Inoculated: uninoculated	Treatment	Inoculated apple (Log_{10} CFU/apple)	Non-inoculated apple (Log_{10} CFU/apple) (Positive*/total apple)
1:10	Deionized H_2O	6.40 ± 0.01^{a}	3.79 ± 0.07 (10/10)
	100 ppm hypochlorite	5.48 ± 0.07^{b}	ND (0/10)
	1:3 NEW	5.46 ± 0.09^{b}	ND (0/10)
	0.125% JC9450	5.42 ± 0.11^{b}	ND (0/10)
	0.25% JC9450	4.19 ± 0.11^{c}	ND (0/10)
	0.50% JC9450	2.76 ± 0.10^{d}	ND (0/10)
6:6	Deionized H_2O	6.47 ± 0.05^{a}	4.15 ± 0.11 (6/6)
	100 ppm hypochlorite	5.60 ± 0.03^{b}	ND (0/6)
	1:3 NEW	5.61 ± 0.04^{b}	ND (0/6)
	0.125% JC9450	5.58 ± 0.07^{b}	ND (0/6)
	0.25% JC9450	4.27 ± 0.02^{c}	ND (0/6)
	0.50% JC9450	2.86 ± 0.08^{d}	ND (0/6)

*, *L. monocytogenes*–positive apple refers to presence of *L. monocytogenes* on CHROMagar Listeria plates after enrichment of the surface-rub solution; NEW, neutral electrolyzed water; ND, not detected. Values are means ± SEM, n = 3; $^{a\text{-}d}$ Means with different lowercase letters differ significantly ($P < 0.05$). pH of each treatment was 6.6–6.8.

Table 5. Temperature of apple surface and peroxyacetic acid (PAA) solution at pre- and post-PAA intervention.

Treatment	Apple surface T (°C)		PAA solution T (°C)	
	Before	After	Before	After
PAA (43 °C, 30 s)	19.8 ± 0.0	34.8 ± 0.0	43.7 ± 0.2	42.8 ± 0.2
PAA (43 °C, 2 min)	19.8 ± 0.0	37.4 ± 0.3	43.8 ± 0.2	42.5 ± 0.3
PAA (46 °C, 30 s)	19.8 ± 0.0	36.3 ± 0.4	46.6 ± 0.1	45.3 ± 0.1
PAA (46 °C, 2 min)	19.8 ± 0.0	38.4 ± 0.4	46.5 ± 0.0	45.3 ± 0.2

Data are presented as means ± SEM, n = 3.

Table 6. Summary of PAA spray bar related parameters.

Facility	PAA (ppm)	pH	Flow rate (gallon/min)	Contact time (s)	Dwell Time (s)	Speed of brushes (n/min)
A	75-85	4	0.3 ± 0.0	30.2 ± 0.2	2.2 ± 0.2	Low: 45 ± 0
				60.0 ± 0.2	7.2 ± 0.2	High: 120 ± 0
B	75-85	4	0.3 ± 0.0	30.0 ± 0.0	6.7 ± 0.2	Low: 40 ± 1
				60.0 ± 0.0	12.3 ± 0.2	High: 40 ± 1
C	75-85	4	0.4 ± 0.0	30.0 ± 0.0	13.8 ± 0.3	Low: 68 ± 0
				60.0 ± 0.0	22.3 ± 0.7	High: 90 ± 0

[1.] Data are represented as means ± SEM, n = 5–6.

Table 7. The reduction of *L. innocua* and *E. faecium* on Granny Smith apples post PAA spray bar intervention at specified temperatures.

	Treatment	Time	Log reduction (Log_{10} CFU/apple)	
			L. innocua	***E. faecium***
22 °C	Water	30 s	0.23 ± 0.03^{aA}	0.27 ± 0.03^{aA}
		2 min	0.37 ± 0.05^{abA}	0.38 ± 0.04^{aA}
	PAA	30 s	0.97 ± 0.03^{cA}	0.83 ± 0.04^{bB}
		2 min	1.07 ± 0.06^{cA}	0.95 ± 0.04^{bA}
44 - 46°C	Water	30 s	0.42 ± 0.01^{bA}	0.33 ± 0.01^{aB}
		2 min	0.55 ± 0.05^{bA}	0.44 ± 0.01^{aA}
	PAA	30 s	1.48 ± 0.03^{dA}	1.54 ± 0.02^{cA}
		2 min	1.61 ± 0.07^{dA}	1.67 ± 0.03^{cA}

$^{a-d}$ Means within a column with different lowercase letters differ significantly ($P < 0.05$). $^{A-B}$ Means within a row with different uppercase letters differ significantly ($P < 0.05$). Mean ± SEM, n = 3.

Table 8. The reduction of *L. innocua* and *E. faecium* on Fuji apples post PAA spray bar intervention at specified temperatures.

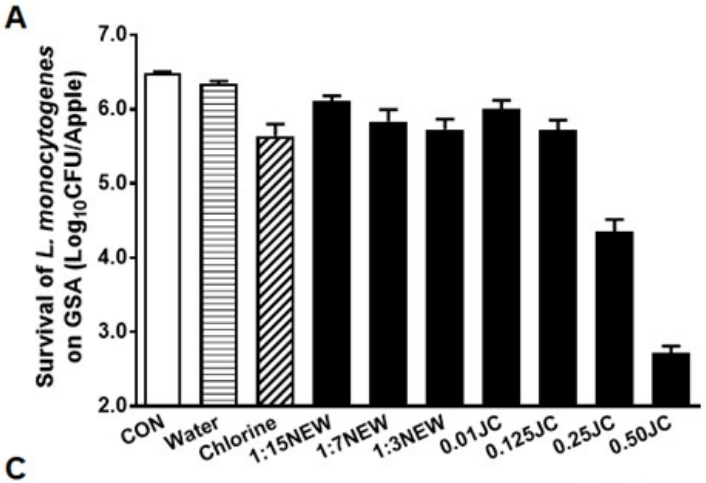
	Treatment	Time	Log reduction (Log_{10} CFU/apple)	
			L. innocua	***E. faecium***
22 °C	Water	30 s	0.19 ± 0.03^{aA}	0.24 ± 0.02^{aA}
		2 min	0.25 ± 0.04^{aA}	0.30 ± 0.01^{aA}
	PAA	30 s	0.88 ± 0.04^{bA}	0.95 ± 0.08^{bA}
		2 min	0.99 ± 0.02^{bA}	1.02 ± 0.10^{bA}
44 - 46°C	Water	30 s	0.37 ± 0.07^{aA}	0.27 ± 0.01^{aA}
		2 min	0.42 ± 0.07^{aA}	0.33 ± 0.03^{aA}
	PAA	30 s	1.62 ± 0.05^{cA}	1.61 ± 0.02^{cA}
		2 min	1.77 ± 0.03^{cA}	1.70 ± 0.02^{cA}

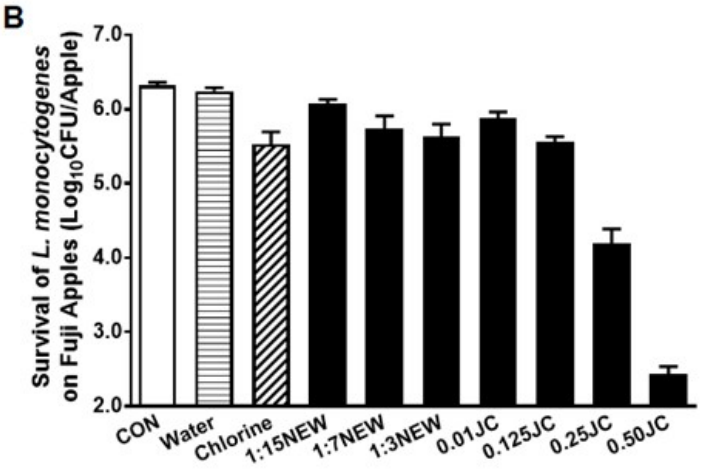
[a-d] Means within a column with different lowercase letters differ significantly ($P < 0.05$). [A-B] Means within a row with different uppercase letters differ significantly ($P < 0.05$). Mean ± SEM, n = 3.

Table 9. The surface temperature of Granny Smith (GSA) and Fuji apples subjected to PAA spray intervention in the packing facilities**.**

Treatment		Time	Facility A		Facility B		Facility C	
			GSA	Fuji	GSA	Fuji	GSA	Fuji
17-22 °C	PAA	30	14.4 ± 0.2	14.3 ± 0.1	16.2 ± 0.1	16.1 ± 0.0	13.5 ± 0.1	13.6 ± 0.2
		60	14.6 ± 0.1	14.9 ± 0.2	16.2 ± 0.2	15.9 ± 0.1	14.2 ± 0.1	13.7 ± 0.1
43– 45 °C	Water	60	17.3 ± 0.3	18.3 ± 0.1	18.3 ± 0.1	18.9 ± 0.1	/	22.1 ± 0.2
	PAA	30	17.2 ± 0.2	16.4 ± 0.0	18.9 ± 0.2	18.7 ± 0.1	21.5 ± 0.2	22.0 ± 0.2
		60	17.7 ± 0.1	17.1 ± 0.1	18.4 ± 0.2	18.4 ± 0.1	22.0 ± 0.2	21.9 ± 0.2

Data are presented as means ± SEM, n = 20.

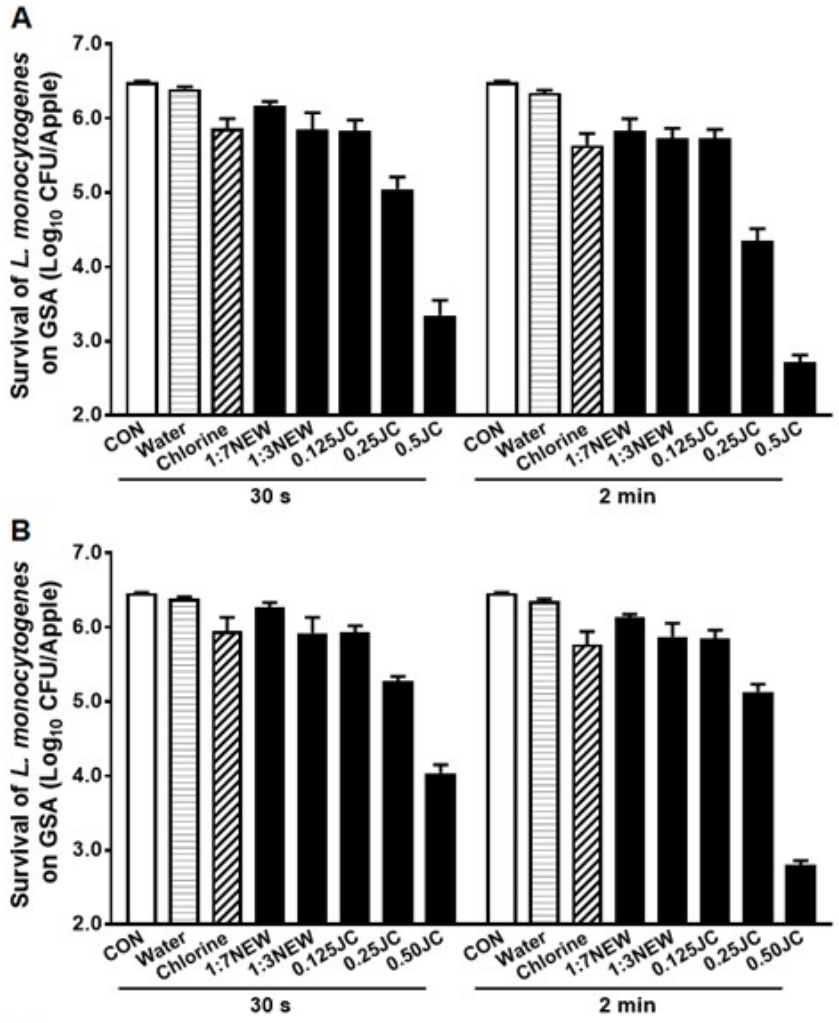




C

Variety	Water	Chlorine	NEW			JC			
			1:15	1:7	1:3	0.01%	0.125%	0.25%	0.50%
GSA	0.12 ± 0.02^{a}	1.15 ± 0.05^{b}	0.40 ± 0.01^{c}	0.68 ± 0.02^{d}	0.93 ± 0.07^{b}	0.45 ± 0.07^{c}	1.04 ± 0.08^{b}	2.06 ± 0.12^{e}	3.84 ± 0.10^{f}
Fuji	0.18 ± 0.03^{a}	1.14 ± 0.04^{b}	0.34 ± 0.05^{c}	0.73 ± 0.08^{d}	0.89 ± 0.12^{bd}	0.43 ± 0.08^{c}	1.15 ± 0.05^{b}	2.43 ± 0.15^{e}	3.87 ± 0.09^{f}

Figure 1. Antimicrobial efficacy of chlorine (in hypochlorite form), commercial sanitizer JC9450, and NEW (neutral electrolyzed water) against *Listeria monocytogenes* 24 h post-inoculation on Granny Smith apples (GSA) and Fuji apples with a contact time of 2 min. **A** and **B** show the numbers of surviving *L. monocytogenes* on Granny Smith and Fuji apples, respectively, following sanitizer washes. **C** shows Log_{10} reductions for all treatments. CON: untreated control; JC: JC9450, %; NEW: neutral electrolyzed water. $^{a\text{-}f}$ Mean values within rows with different lowercase letters are significantly different ($P < 0.05$). Error bars represent ± standard error of the means (SEM). Results displayed represent the mean values from 3 independent experiments (n = 3).

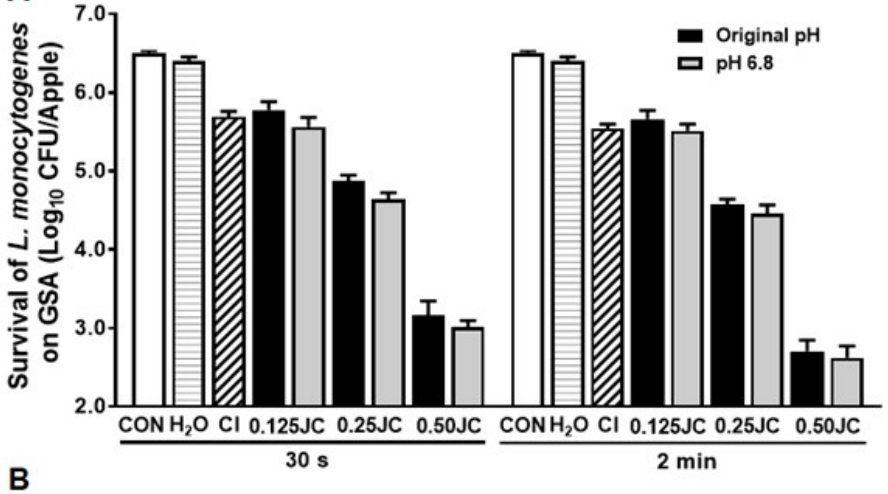


C

Treatment	24 h		48 h	
	30 s	2 min	30 s	2 min
Water	0.11 ± 0.01^{aA}	0.12 ± 0.02^{aA}	0.09 ± 0.03^{aA}	0.09 ± 0.02^{aA}
Chlorine	0.90 ± 0.04^{bA}	1.15 ± 0.01^{bB}	0.88 ± 0.07^{bA}	1.05 ± 0.10^{bAB}
1:7 NEW	0.50 ± 0.04^{cA}	0.66 ± 0.02^{cB}	0.50 ± 0.05^{cA}	0.58 ± 0.03^{cAB}
1:3 NEW	0.75 ± 0.09^{bA}	0.93 ± 0.09^{bA}	0.70 ± 0.10^{bcA}	0.88 ± 0.08^{bA}
0.125% JC	0.91 ± 0.04^{bA}	1.04 ± 0.08^{bAB}	0.82 ± 0.04^{bA}	0.88 ± 0.10^{bAB}
0.25% JC	1.58 ± 0.05^{dA}	2.06 ± 0.12^{dB}	1.56 ± 0.03^{dA}	1.84 ± 0.10^{dAB}
0.50% JC	3.27 ± 0.12^{eAB}	3.84 ± 0.10^{eC}	3.09 ± 0.14^{eB}	3.61 ± 0.05^{eC}

Figure 2. Influence of sanitizer contact time on the antimicrobial efficacy of chlorine-based sanitizers. Number of surviving *L. monocytogenes* on Granny Smith apples, 24 h (**A**) and 48 h (**B**) post-inoculation, following sanitizer washes at two different contact times. **C.** Log_{10} reductions for *L. monocytogenes* 24 and 48 h post-inoculation following sanitizer treatments with contact times of 30 s and 2 min. Chlorine was in hypochlorite form; CON: untreated control; JC: JC9450, %; NEW: neutral electrolyzed water. $^{a-e}$ Mean values within columns or $^{A-C}$ mean values across rows without common letters are significantly different ($P < 0.05$). Error bars represent ± standard error of the means (SEM), n = 3.

A

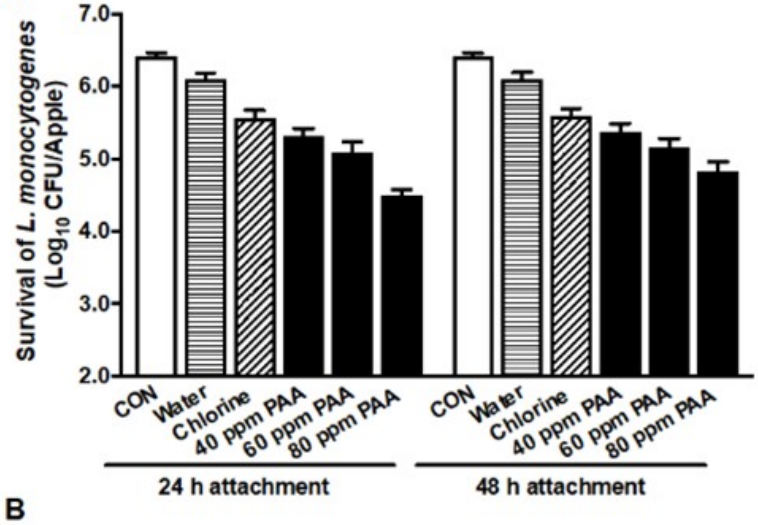


B

Treatment	Original pH		pH 6.8	
	30 s	2 min	30 s	2 min
0.125% JC	0.76 ± 0.03^{aA}	0.88 ± 0.10^{aAB}	0.96 ± 0.04^{aB}	1.02 ± 0.06^{aB}
0.25% JC	1.56 ± 0.03^{bA}	1.82 ± 0.10^{bB}	1.68 ± 0.04^{bAB}	1.84 ± 0.11^{bB}
0.50% JC	3.09 ± 0.14^{cA}	3.61 ± 0.05^{cB}	3.13 ± 0.08^{cA}	3.63 ± 0.08^{cB}

Figure 3. Influence of pH on antimicrobial efficacy of commercial sanitizer JC9450 against *Listeria monocytogenes* on Granny Smith apples (GSA). **A.** The number of surviving *L. monocytogenes* on GSA 48 h post-inoculation, following sanitizer treatments with contact times of 30 s or 2 min. **B.** Log_{10} reductions for *L. monocytogenes* following the treatments. CON: untreated control; Cl: Chlorine, in hypochlorite form; JC: JC9450, %. $^{a-c}$ Mean values within columns or $^{A-B}$ mean values within rows without common letters are significantly different ($P < 0.05$). Error bars represent ± standard error of the means (SEM). Results displayed represent the mean values from 3 independent experiments (n = 3).

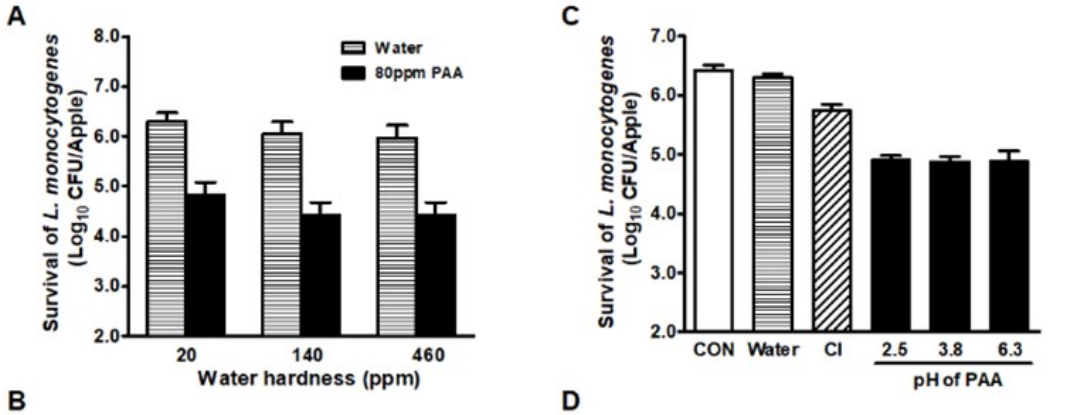
A



B

Treatment	24 h attachment (Log_{10} CFU/apple)	48 h attachment. (Log_{10} CFU/apple)
Water	0.15 ± 0.10^{aA}	0.10 ± 0.01^{aA}
Chlorine	0.91 ± 0.03^{bA}	0.71 ± 0.04^{bA}
40 ppm PAA	1.37 ± 0.12^{bcA}	1.34 ± 0.03^{cA}
60 ppm PAA	1.64 ± 0.15^{cA}	1.51 ± 0.08^{cA}
80 ppm PAA	2.17 ± 0.17^{dA}	1.71 ± 0.11^{dB}

Figure 6. Antimicrobial efficacy of peroxyacetic acid (PAA) against *L. monocytogenes* on Granny Smith apples (GSA) at a 2-min contact time at 22°C. **A.** Representative bar graph of survival of *L. monocytogenes* on GSA post PAA treatment. **B.** Log reduction of *L. monocytogenes* on apples, averaged from three independent experiments. $^{a-d}$ Means within a column with different lowercase letters differ significantly ($P < 0.05$), $^{A-B}$ Means within a row with different uppercase letters differ significantly ($P < 0.05$). Mean ± SEM, n = 3. 24 h-attachment: *L. monocytogenes* are allowed to attach to GSA for 24 h before antimicrobial treatment; 48 h-attachment: *L. monocytogenes* are allowed to attach to GSA for 48 h before antimicrobial treatment.



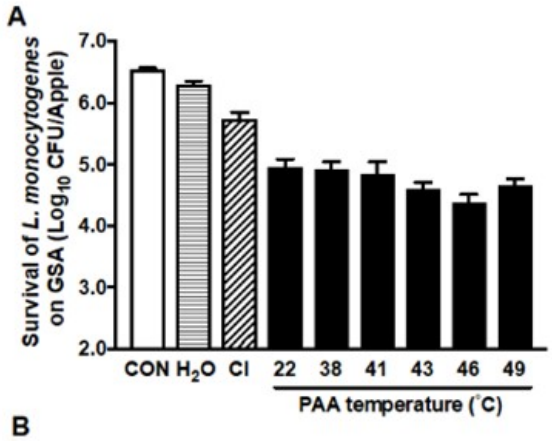
B

PAA (ppm)	Water hardness (ppm)	Log reduction (Log_{10} CFU/apple)
80	20.0 ± 0.0	1.77 ± 0.09[a]
80	140.0 ± 0.0	1.98 ± 0.23[a]
80	460.0 ± 0.0	1.93 ± 0.20[a]

D

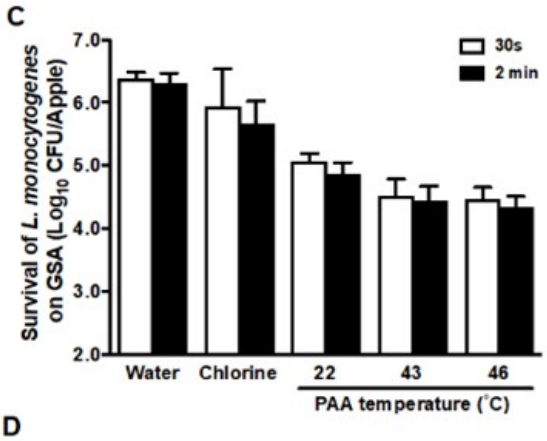
PAA (ppm)	pH	Log reduction (Log_{10} CFU/apple)
80	2.50 ± 0.00	1.73 ± 0.16[a]
80	3.81 ± 0.00	1.78 ± 0.10[a]
80	6.30 ± 0.01	1.73 ± 0.18[a]

Figure 7. Antimicrobial efficacy of peroxyacetic acid (PAA) against *L. monocytogenes* on Granny Smith apples (GSA) under different water hardness and pH at 22°C. *L. monocytogenes* were allowed to attach to GSA for 48 h before antimicrobial treatment. **A** and **C**. Representative bar graphs of *L. monocytogenes* survival on GSA; **B** and **D**. Log reduction of *L. monocytogenes* on apples, averaged from three independent experiments. [a] Means within a column with different lowercase letters differ significantly ($P < 0.05$). Mean ± SEM, n = 3.



B

Temperature	Log reduction (Log_{10} CFU/apple)
22 °C	1.78 ± 0.05[a]
38 °C	1.89 ± 0.15[ab]
41 °C	2.00 ± 0.15[ab]
43 °C	2.37 ± 0.06[bc]
46 °C	2.63 ± 0.04[c]
49 °C	2.35 ± 0.18[bc]



D

Temperature	Contact time	Log reduction (Log_{10} CFU/apple)
22 °C	30 s	1.33 ± 0.08[a]
	2 min	1.75 ± 0.08[b]
43 °C	30 s	2.22 ± 0.07[b]
	2 min	2.34 ± 0.05[b]
46 °C	30 s	2.35 ± 0.02[b]
	2 min	2.64 ± 0.03[c]

Figure 8. Influence of temperature and contact time on antimicrobial efficacy of peroxyacetic acid (PAA) against *L. monocytogenes* on Granny Smith apples (GSA). **A** and **C**. Representative bar graphs of *L. monocytogenes* survival on GSA. **B** and **D**. Log reduction of *L. monocytogenes* on apples, averaged from three independent experiments. [a-c] Means within a column or a temperature with different lowercase letters differ significantly ($P < 0.05$). Mean ± SEM, n = 3.

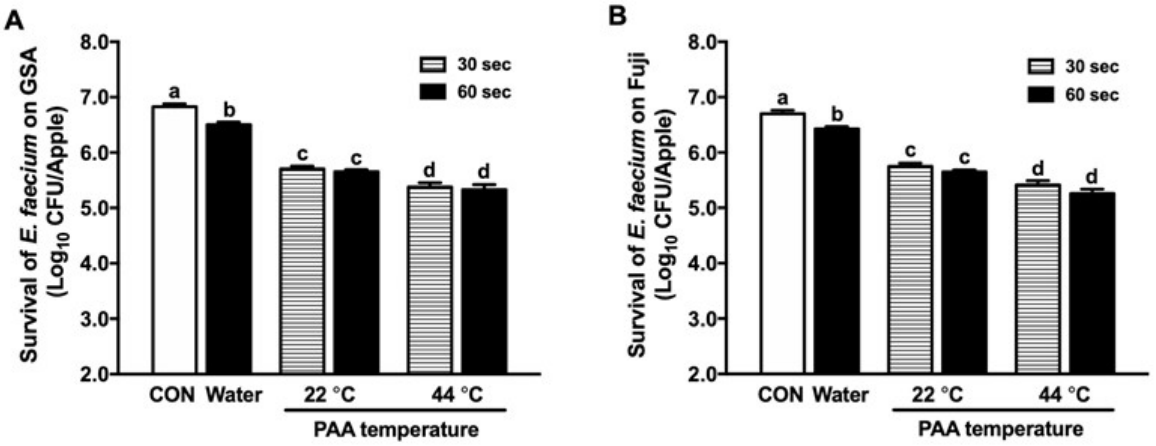


Figure 9. The reduction of *E. faecium* on inoculated fresh apples post PAA spray bar intervention at the packing facility A. **A.** Survival of *E. faecium* on inoculated Granny Smith apple (GSA). **B.** Survival of *E. faecium* on inoculated Fuji apples. [a-d] Bars with different lowercase letters differ significantly ($P < 0.05$). Mean ± SEM, n = 20–24. Temperature is averaged from 6 measurements during in-plant testing. CON: The *E. faecium* population level of inoculated apples before spray bar wash; Water: Inoculated apples were subjected to hot water rinse (43–45°C) for 60 sec, which was used as a negative control.

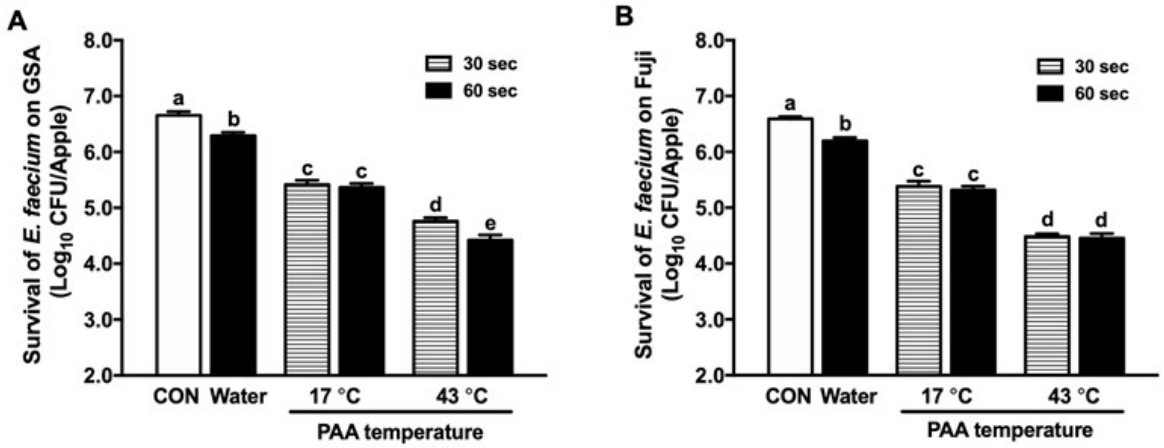


Figure 10. The reduction of *E. faecium* on inoculated fresh apples post PAA spray bar intervention at the packing facility B. **A.** Survival of *E. faecium* on inoculated Granny Smith apples (GSA). **B.** Survival of *E. faecium* on inoculated Fuji apples. [a-d] Bars with different lowercase letters differ significantly ($P < 0.05$). Mean ± SEM, n = 20. Temperature is averaged from 6 measurements during in-plant testing. CON: The *E. faecium* population level of inoculated apples before spray bar wash; Water: Inoculated apples were subjected to hot water rinse (43–45°C) for 60 sec, which was used as a negative control.

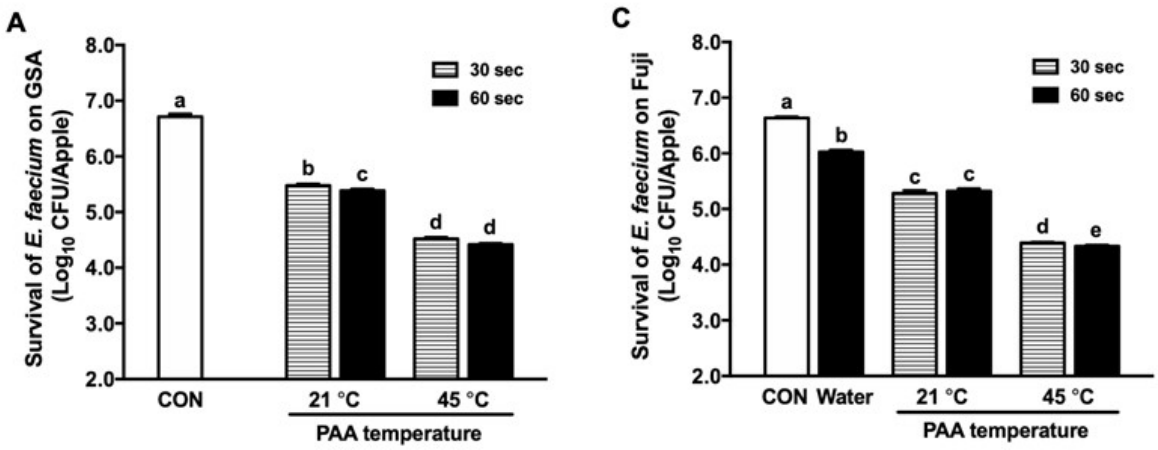
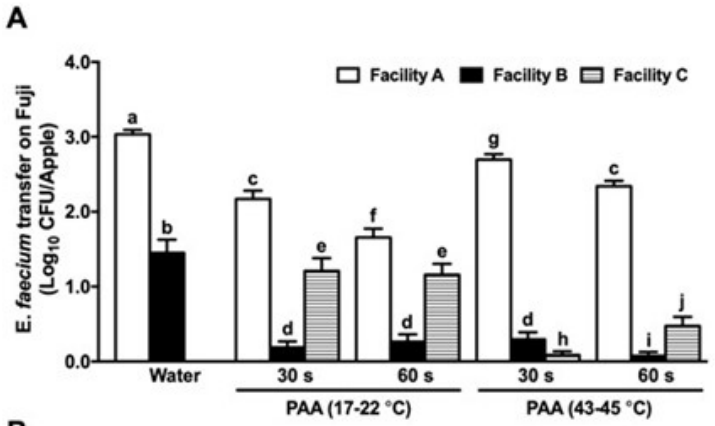
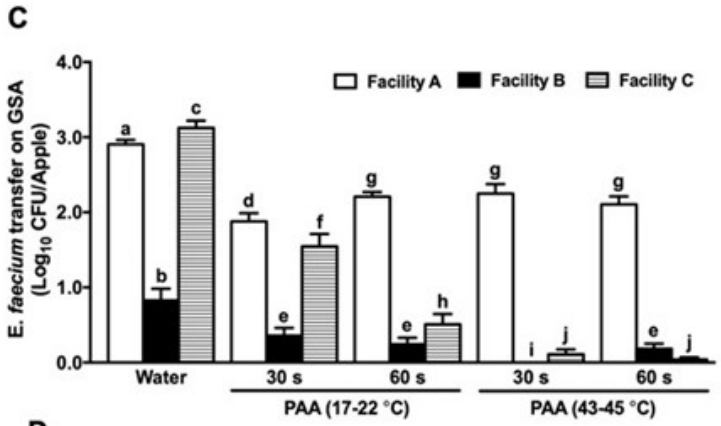


Figure 11. The reduction of *E. faecium* on inoculated fresh apples post PAA spray bar intervention at the packing facility C. **A.** Survival of *E. faecium* on inoculated Granny Smith apple (GSA). **C.** Survival of *E. faecium* on inoculated Fuji apples. [a-d] Bars with different lowercase letters differ significantly ($P < 0.05$). Mean ± SEM, n = 20–24. Temperature is averaged from 6 measurements during in-plant testing. CON: The *E. faecium* population level of inoculated apples before spray bar wash; Water: Inoculated apples were subjected to hot water rinse (43–45°C) for 60 sec, which was used as a negative control.





B

	Treatment	Time	Facility A	Facility B	Facility C
17 - 22 °C	PAA	30 sec	65/72#	5/70	59/70
		60 sec	56/72	7/70	39/70
43 – 45 °C	Water	60 sec	70/70	35/70	/
	PAA	30 sec	71/72	2/70	5/70
		60 sec	71/72	5/70	17/70

D

	Treatment	Time	Facility A	Facility B	Facility C
17 - 22 °C	PAA	30 sec	63/72	13/70	41/70
		60 sec	72/72	8/70	13/70
43 – 45 °C	Water	60 sec	72/72	22/70	63/65
	PAA	30 sec	61/72	0/70	3/70
		60 sec	65/72	7/70	8/70

Figure 12. *E. faecium on un*-inoculated apples transferred from inoculated fresh apples during the spray bar interventions at the cross-contamination ratio of 1:3 (facility A) and 1:4 (facility B and C). **A.** *E. faecium* counts on uninoculated Fuji apples. **B.** Enrichment positive rate of *E. faecium* on uninoculated Fuji apples, presented as enrichment positive/total apples tested. **C.** *E. faecium* counts on uninoculated Granny Smith apples (GSA). D. Enrichment positive rate of *E. faecium* on uninoculated Granny Smith apples, presented as enrichment positive/total apples tested. [a-j] Bars with different lowercase letters differ significantly ($P < 0.05$). Mean ± SEM, n = 65–72. In facility A, the brush bed was not sanitized between treatments, which contributed to the cross-contamination.

References cited

Beuchat, L. R., Nail, B. V., Adler, B. B., & Clavero, M. R. (1998). Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. *J Food Prot, 61*(10), 1305-1311.

CDC. (2012). Multistate outbreak of Listeriosis linked to whole cantaloupes from Jensen Farms, Colorado. *CDC website: http://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/082712/*.

Chan, Y. C., & Wiedmann, M. (2009). Physiology and genetics of *Listeria monocytogenes* survival and growth at cold temperatures. *Crit Rev Food Sci Nutr, 49*(3), 237-253.

Deza, M. A., Araujo, M., & Garrido, M. J. (2003). Inactivation of *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes* on the surface of tomatoes by neutral electrolyzed water. *Lett Appl Microbiol, 37*(6), 482-487.

Dutkamalen, S., Evers, S., & Courvalin, P. (1995). Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant *Enterococci* by PCR *J Clin Microbiol, 33*(5), 1434-1434.

Ells, T. C., & Truelstrup Hansen, L. (2010). Growth of *Listeria* spp. in shredded cabbage is enhanced by a mild heat treatment. *J Food Prot, 73*(3), 425-433.

FDA. (2014). FDA investigates *Listeria monocytogenes* illnesses linked to caramel apples. *http://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm427573.htm*.

FDA. (2017). BAM: detection and enumeration of *Listeria monocytogenes.* https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071400.htm.

Francis, G. A., Gallone, A., Nychas, G. J., Sofos, J. N., Colelli, G., Amodio, M. L., & Spano, G. (2012). Factors affecting quality and safety of fresh-cut produce. *Crit Rev Food Sci Nutr, 52*(7), 595-610.

Golden, D. A., Brackett, R. E., & Beuchat, L. R. (1990). Efficacy of direct plating media for recovering *Listeria monocytogenes* from foods. *Int J Food Microbiol, 10*(2), 143-155.

Jayaratne, P., & Rutherford, C. (1999). Detection of clinically relevant genotypes of vancomycin-resistant *enterococci* in nosocomial surveillance specimens by PCR. *J Clin Microbiol, 37*(6), 2090-2092.

Nou, X., & Luo, Y. (2010). Whole-leaf wash improves chlorine efficacy for microbial reduction and prevents pathogen cross-contamination during fresh-cut lettuce processing. *Journal of Food Science, 75*(5), M283-290.

Pao, S., Kelsey, D. F., Khalid, M. F., & Ettinger, M. R. (2007). Using aqueous chlorine dioxide to prevent contamination of tomatoes with *Salmonella enterica* and *Erwinia carotovora* during fruit washing. *Journal of Food Protection, 70*(3), 629-634.

Parish, M. E., Beuchat, L. R., Suslow, T. V., Harris, L. J., Garrett, E. H., Farber, J. N., & Busta, F. F. (2003). Methods to reduce/eliminate pathogens from fresh and fresh-cut produce. *Comp. Rev. Food Sci. Food Safety, 2*(Supplement s1), 161-173.

Rodgers, S. L., Cash, J. N., Siddiq, M., & Ryser, E. T. (2004). A comparison of different chemical sanitizers for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes* in solution and on apples, lettuce, strawberries, and cantaloupe. *J Food Prot, 67*, 721-731.

Sheng, L., Edwards, K., Tsai, H., Hanrahan, I., & Zhu, M. J. (2017). Fate of *Listeria monocytogenes* on fresh apples under different storage temperatures. *Front Microbiol, 8*, 1396.

Sheng, L., Hanrahan, I., Sun, X., Taylor, M. H., Mendoza, M., & Zhu, M. J. (2018). Survival of *Listeria innocua* on Fuji apples under commercial cold storage with or without low dose continuous ozone gaseous. *Food Microbiol, 76*, 21-28.