FINAL PROJECT REPORT **YEAR**: No-Cost Extension

Project Title: Increasing the efficacy of antimicrobial chemicals with surfactants

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Cooperators: Various apple packing houses in Yakima and Wenatchee (Borton Fruit, Washington Fruit, Double Diamond), Wesmar, Inc., Decco, CleanLogix, Inc., ZEP, and others

Total Project Request: **Year 1:** $47,148 **Year 2:** 53,575 **Year 3: $0**

Other funding sources: **None**
Amount:
Agency Name:
Notes: Donation of apples, sanitizer, and surfactants will be requested to decrease the cost of this research.

WTFRC Budget: NONE

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Item	2019	2020	2021
Salaries	$17,098	$17,782	
Benefits[1]	$2,172	$2,270	
Wages[1]	$14,880	$15,475	
Benefits[1]	$1,488	$1,548	
RCA Room Rental	0	0	
Shipping	0	0	
Supplies[2]	$10,000	$15,000	
Travel[3]	$1,510	$1,500	
Plot Fees	0	0	
Miscellaneous	0	0	
Total	$47,148	$53,575	0

Footnotes:
[1] Salaries, Wages and Benefits for technical and student support
[2] Supplies and analysis fees, including for microbial testing
[3] Travel costs of trips to the packing facilities in Wenatchee and Yakima.

JUSTIFICATION

This proposal addresses the priority "*Holistic approach to food safety and sanitation"* for the 2019 RFP.
The food safety risks in fresh apples can be minimized my reducing the cross-contamination through excellent sanitation practices and washing of the apples. Washing the apples with water and with different sanitation chemicals can help reduce the microbial loads on the apples. Various studies emphasized the difficulty of reducing microbial load on apples (Jo et al., 2014; Kim et al., 2018). From our previous work we have shown that besides possessing difficult to clean, irregular shapes (presence of calyx and stem cavities), apple surface is covered with numerous microstructures, such as microcracks or lenticels, which harbor bacteria and protect it from the cleaning interventions (Pietrysiak & Ganjyal, 2018). Apples are also covered with natural layer of wax which makes the apple surface hydrophobic. Hydrophobic characteristic of the apple surface prevents water and aqueous solutions of commonly used sanitizers from spreading on the surface uniformly and thus may decrease their effectiveness.

To address this issue, we are proposing the use of washing treatments that combine sanitizers with surfactants. Surfactant is a chemical compound with ability to reduce surface tension between the liquid and solid. This greatly facilitates spreading of the washing solution over the surface of the produce and improves surface decontamination. Surfactants should be combined with sanitizer to ensure inactivation of the detached bacteria. Based on its dissociation in water, surfactant can be classified into cationic, anionic, and nonionic (Salager, 2002). Chemical characteristics of the surfactants determines their functional properties and ability to react with other components, thus it is important to evaluate different types of surfactants to indicate the most suitable ones for cleaning the fresh apples.
In this project we are proposing to evaluate the efficiency of surfactants (with different chemical properties), combined with sanitizers, for the removal of *Listeria* from fresh apples. Efforts will be made to select appropriate surfactants to fit the surface properties of the fresh apples. Cleaning treatments will be designed to suit the conditions and equipment of typical apple packing line. Furthermore, the impact of the treatments on the quality of apples during post packing storage will be examined.

OBJECTIVES

In this project, we proposed to evaluate the efficiency of surfactants (with different chemical properties), combined with sanitizers, for the removal of *Listeria* from fresh apples.

1. Examine the efficacy of different types of surfactants combined with a standard sanitizer, for the removal of *Listeria* from fresh apples during the packing process.
2. Assess the impact of optimum treatment on quality of the most significant apple varieties, such as Gala, Fuji, Granny Smith, Honeycrisp, and Cosmic Crisp.

SIGNIFICANT FINDINGS

1st year of study

- Combining three selected surfactants, including Tween20 (T-20), sodium dodecyl sulfate (SDS), and lauric arginate (LAE) with peracetic acid (PAA), decreased the population of *L. innocua* on apples.
- Treating apples with PAA-T20 reduced the load of *L. innocua* by 2.2 log.
- Stem bowl and calyx cavity are difficult to reach areas during the cleaning operation.

- Cleaning treatments were not completely effective in removing all *L. innocua* from apples.
- Additional commercially available surfactants will be examined on their cleaning efficacy.

2nd year of study

- Significant differences in bacterial reduction were observed between Gala and Granny Smith apples when subjected to the same cleaning treatments.
- Additional five commercially available surfactants combined with PAA were examined.
- The addition of new surfactants to PAA increased the bacterial reduction in Granny Smith apples, but it did not have the same effect on Gala apples.
- The new type of PAA was used in the 2nd year of study, showing greater antimicrobial potential than PAA used during the 1st year of study.
- The formulations of commercial PAA solutions can have a significant impact on their effectiveness.
- The cleaning solutions resulting in the greatest *L. innocua* reductions were: PAA in Gala apples (2.77 log) and 0.2% Norfox 90 with PAA in Granny Smith apples (2.76 log).
- Extending the dipping time from 1 min to 4 and 7 minutes did not significantly increase the bacteria reduction on apples.

3rd year of study

- Overall, the surfactants did not negatively impact the quality and texture over the storage time studied outside of normal aging and ripening.
- Except, when an acidic surfactant was used (Tsunami 200), it led to burnt skin for Granny Smith apples only. Thus, it is important to consider the pH of the surfactant and its impact on the apple variety before making the final selections.

METHODS

Objective 1. Examine the efficacy of different types of surfactants combined with a standard sanitizer, for the removal of *Listeria* from fresh apples during the packing process.

<u>Apples</u>

Apple varieties most significant to the state of WA (Gala, Fuji, Granny Smith, Honeycrisp, and Cosmic Crisp) at commercial maturity and without wax treatment will be obtained from the cooperators. Apple varieties differ based on their morphological characteristics, such as shape, amount of natural wax and presence of surface microstructures. This difference may influence the number of bacteria attached to the apple and the process of bacterial detachment. Therefore, several varieties will be selected to evaluate the attachment of *Listeria* to apple surface in aqueous conditions (such as dump tank and flumes) and effectiveness of the cleaning treatments.
Apples will be transported to the Washington State University (Pullman, WA) and stored in a walk-in cooler (~40°F) until further analysis. Efforts will be made to have all selected apples of similar size and free of visible surface disorders, and mechanical injuries.

Inoculum preparation. *L. innocua* 51742 (ATCC) isolate was used as a non-pathogenic surrogate for *L. monocytogenes*. The inoculum was later diluted with room temperature water to reach the proper concentration.

Apple inoculation. Fifteen apples were placed into 5L of 10^7 CFU/mL *L. innocua* solution and gently agitated for 10 minutes. Apples were then removed and allowed to dry at room temperature until visibly dry under a chemical hood.

Preparation of cleaning solutions. Surfactant solutions used included DP081901 (Decco US Postharvest, Inc., Monrovia, CA), Barlox 12 (Wesmar Company, Inc., Lynnwood, WA), Barlox 10S (Wesmar Company, Inc.), Stepanol EHS (Wesmar Company, Inc.), and Norfox 90 (Wesmar Company, Inc.). The surfactants were used at high and low concentrations delineated in Table 1, and all were combined with 80ppm peracetic acid (PAA) (Tsunami ™ 200, EcoLab Saint Paul, MN, U.S.A). PAA concentration was measured using a titration kit (LaMotte, Chestertown, MD, U.S.).

Table 1. Surfactants used for surfactant concentration treatments in conjunction with 80 ppm peracetic acid (PAA).

Name	Company	Composition	Final Concentration	Disassociation in H_2O
DP081901 (DP)	Decco	D-glucopyranose, oligomeric, C10-16, alkyl glycosides (% by weight: 20-25%); D-glucopyranose, oligomers, decyl octyl glycosides (% by weight: 25-50)	0.05% [DP-L] 0.2% [DP-H]	nonionic
Barlox 12 (B12)	Wesmar	Amines, cocoalkyldimethyl, n-oxides 30%	0.05% [B12-L] 0.2% [B12-H]	nonionic
Barlox 10S (B10)	Wesmar	N,N-dimethyldecylamine N-oxide 30%	0.05% [B10-L] 0.2% [B10-H]	nonionic
Stepanol EHS (ST)	Wesmar	Sodium 2-ethylhexyl sulfate 40-50%	0.02% [ST-L] 0.2% [ST-H]	anionic
Norfox 90 (N90)	Wesmar	Sodium dodecylbenzene sulfonate 90%	0.05% [N90-L] 0.2% [N90-H]	anionic

Cleaning procedure. The Cleaning procedure is summarized in Figures 1 and 2. Briefly, each apple was dipped into 250 mL of cleaning solution and kept submerged for 1, 4, and 7 minutes. Once removed from the cleaning solution, the apple was gently rubbed with gloved hands for one minute to replicate the brush bed during the apple packing process. Then, the apple was sprayed with approximately 4.2 mL of an 80 ppm PAA solution. The apples were allowed kept under a chemical hood until visibly dry. Nine apples were used for each treatment. Inoculated, untreated apples were subjected to microbial enumeration as a control.

Enumeration of *L. innocua* after cleaning. Dry apples were peeled using a sterile knife. The apple peels from each apple were placed into separate sterile filtered stomacher bags, weighed, and diluted with 25 mL D/E N broth (Dey-Engley Neutralizing Broth) (Hardy Diagnostics, Lacey, WA). Samples were homogenized and serially diluted in 9 mL PBS and plated on TSAYE. Plates were then inverted and incubated for three to four hours at 35°C (95˚F) before being overlaid with approximately 10 mL of Modified oxford agar (MOX) (Criterion, Hardy Diagnostics, Santa Maris, CA) at 40˚C (104˚F). The overlay solidified after 30 minutes, then inverted and incubated at 35˚C (95˚F) for 48 hours before being counted.

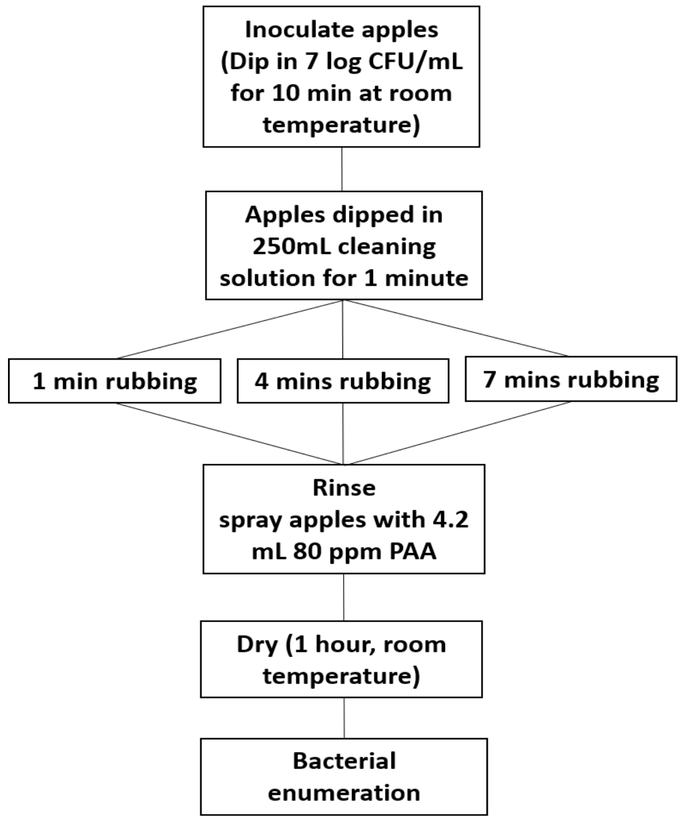

Figure 1. Cleaning treatment procedures.

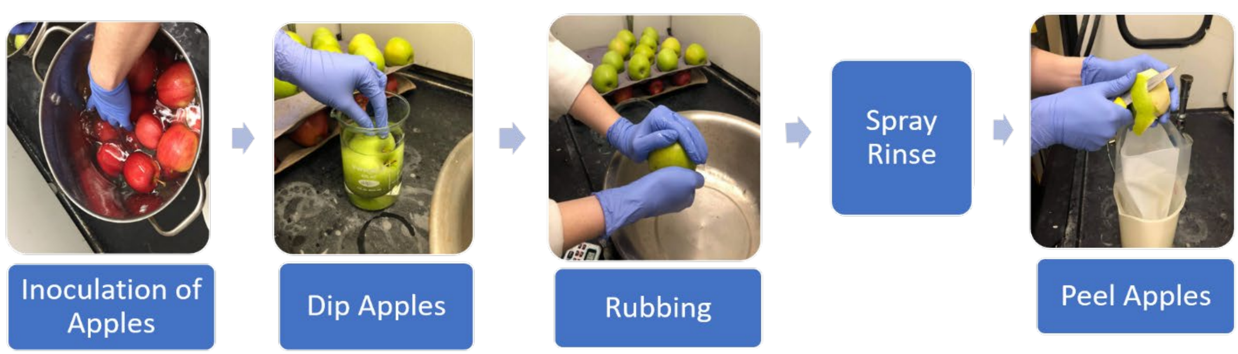

Figure 2. Cleaning treatment process.

Statistical analysis. Results were presented as means with standard deviations. Data were analyzed using a one-way analysis of variance (ANOVA). The least significant difference test, LSD Fisher, was completed with Minitab 19 (Minitab Inc., State College, PA, U.S.).

P.T.O.

Objective 2. Assess the impact of optimum treatment on quality of the most significant apple varieties, such as Gala, Fuji, Granny Smith, Honeycrisp, and Cosmic Crisp.

Fruit quality analysis

To evaluate the impact of applied treatments on the quality of fruits, quality measurements including appearance and firmness will be assessed. Apples will be evaluated soon after receiving and during post-treatment storage (day 0, 3, 7, and 14). Apples treated with cleaning solutions will be stored in refrigerated temperature (40°F) and room temperature (71°F). These conditions are selected to simulate conditions of retail storage and household storage, respectively.

Fruit firmness will be assessed with a fruit texture analyzer using a 1 cm diameter probe. Measurement will be repeated three times independently with a sample size of 5 apples per replication per storage condition and time point. Changes in the appearance will recorded by taking pictures (in similar lighting conditions) before treatment and during the storage to detect visible changes in the apple quality such as color, shriveling, and presence of other disorders. Ten apples of each of the selected varieties and treatments will be evaluated. For fruit quality analysis apples will not be inoculated with bacteria.

RESULTS & DISCUSSION

Objective 1:

Enumeration of *L. innocua* after cleaning. Preliminary studies on Gala and Granny Smith varieties determined that treatments only using a surfactant yielded lower log reductions than treatments using both a surfactant and PAA. Following this conclusion, all experiments with data shown in Figures 3, 4, and 5 were performed using EcoLab Tsunami 200 PAA at 80 ppm.

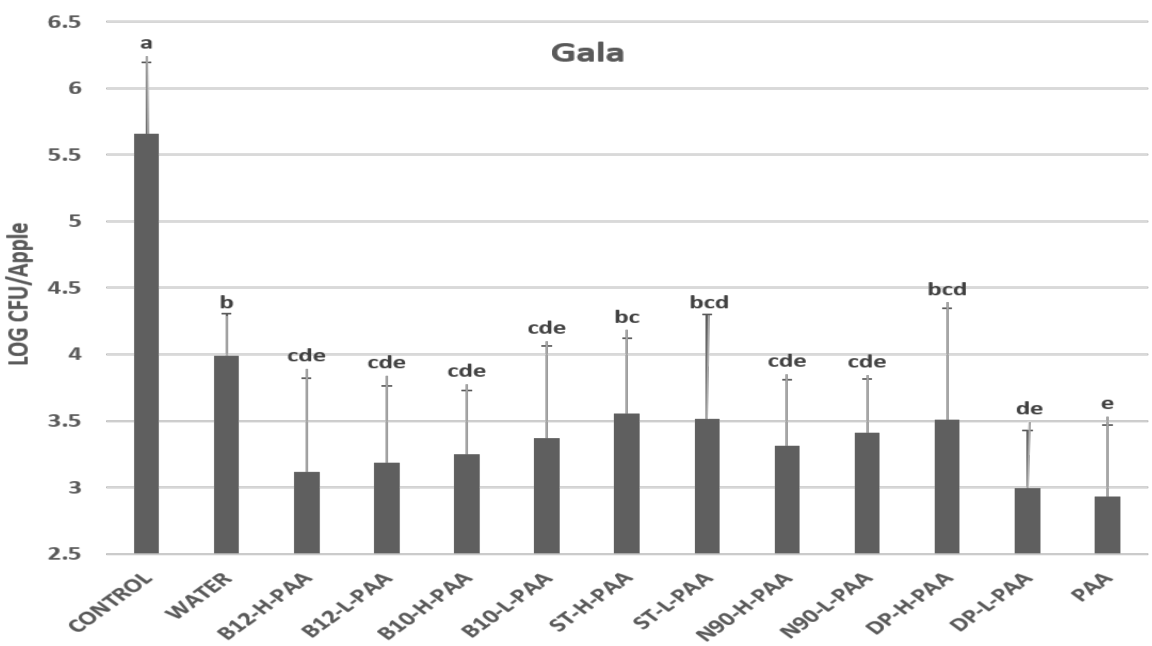

Figure 3. L. innocua Log 10 CFU/apple counts on Gala's using surfactant and PAA (Tsunami 200, EcoLab) treatments. Mean ± standard deviation; n = 9. Bars labelled with different letters indicate a significant difference (P<0.05).

The PAA and DP-L-PAA treatments on Gala's yielded the greatest log reductions, as shown by the lowest log 10 counts in Figure 3. Using only PAA resulted in the highest log reduction of 2.72. These results are not consistent with previous studies (Pietrysiak et al., 2019), where the addition of surfactants consistently performed better than PAA alone. Several surfactant treatments, ST-H & L, and DP-H, did not significantly reduce *Listeria* compared to treatment of only water. Overall, the results from Figure 3 indicate that *Listeria* on Gala apples can be removed and deactivated effectively without the addition of a surfactant to the cleaning treatment.

All surfactant/PAA combination treatments on Granny Smith apples were significantly different than the water-only treatment (Figure 4), which had a log 10 reduction of 1.02. *Listeria* on Granny Smith apples appeared to be more securely attached compared to that on Galas, as a water-only treatment on Galas had a reduction of 1.67 logs. The best treatment on Granny Smith apples was N90-H, an anionic surfactant, with a reduction of 2.76 log CFU/apple.

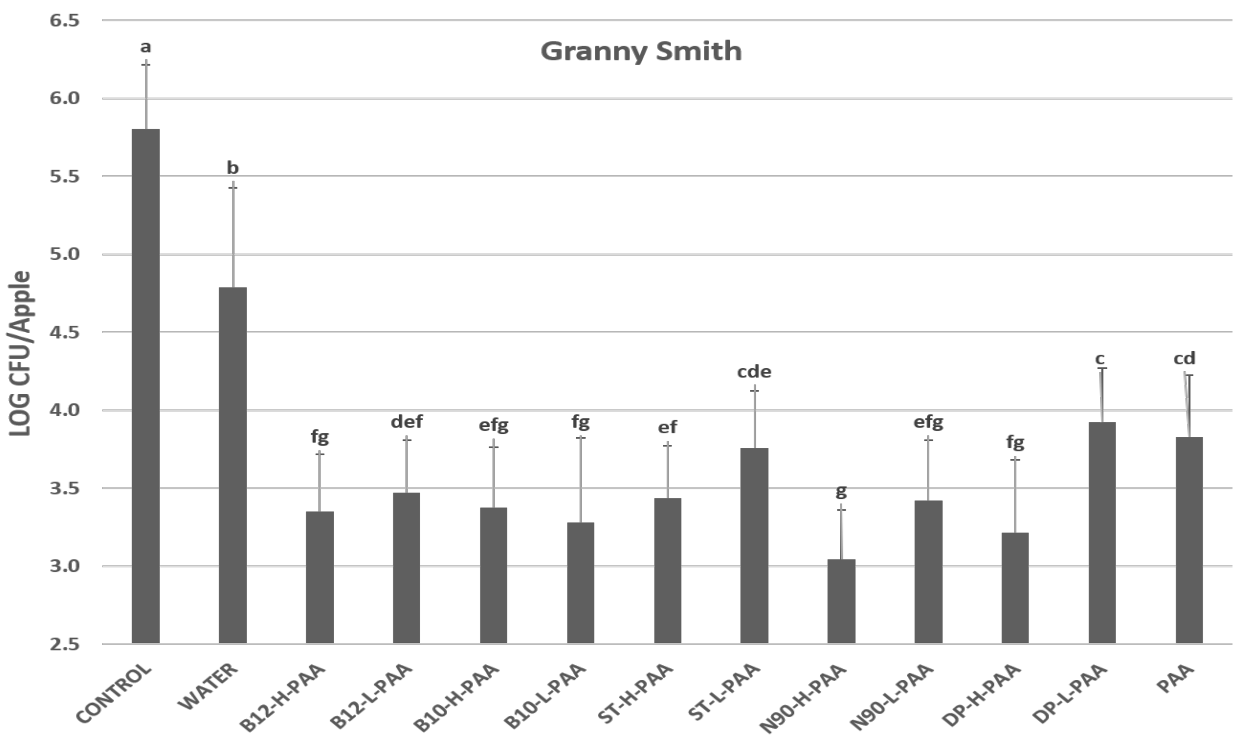

Figure 4. L. innocua Log 10 CFU/apple counts on Granny Smith's using surfactant and PAA treatments. Mean ± standard deviation; n = 9. Bars labelled with different letters indicate a significant difference (P<0.05).

The PAA treatment with Granny Smiths was also less effective, resulting in a reduction of 1.97 logs compared to 2.72 on Galas. This could indicate that the addition of surfactants is more important in cleaning Granny Smiths, the opposite of what was concluded from the results on Galas in Figure 3. When developing cleaning treatments for apple packing lines, it can be concluded that the variety of the apples being treated is an important factor to consider and can change which type of treatment will be most effective at enhancing food safety.

When the dipping time was increased from 1 minute to 4 and 7 minutes, shown in Figure 5, the log reductions for both N90-H-PAA and ST-H-PAA increased. With N90-H-PAA yielding log reductions of 3.00 and 3.26 at 4 minutes and 7 minutes, respectively. Using the ST-H-PAA treatment log reductions of 2.52 and 2.63 were recorded at 4 and 7 minutes, respectively. While this is an increase in log reduction, the amount of time needed for an incremental log reduction is impractical for application purposes. Approximately 3 logs of bacteria have remained stubbornly attached to the apple surface, regardless of changes in the dipping time.

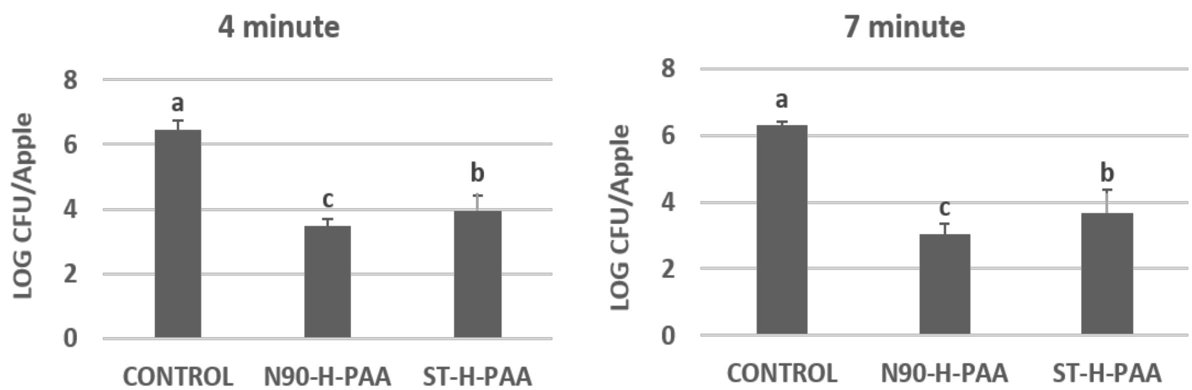

Figure 5. L. innocua Log 10 CFU/apple counts on Granny Smith's using surfactant and PAA treatments for 4 and 7 minute dipping times. Mean ± standard deviation; n = 9. Bars labelled with different letters indicate a significant difference (P<0.05).

In summary, the experiments indicate the potential for PAA used with or without surfactants in the apple cleaning process and how the activity of certain surfactants are more effective than others. The results further highlight the challenges that different apple varieties present in developing the most effective cleaning treatments, as well as the difficulty of removing bacteria that are sheltered by the apple structure. Further research is necessary to assess the quality impact the cleaning treatments have on several apple varieties, as well as explore how different PAA formulations can influence the efficacy of the cleaning treatments.

<u>Objective #2:</u>

Four varieties of apples (Granny Smith, Gala, Fuji, and Honey Crisp) were used for the storage studies. The apples were subjected to various treatments (combinations of the two PAA solutions and three surfactants) and were stored in two different temperature conditions (room temperature and refrigeration) for a total of four weeks. The apple quality (firmness using a texture analyzer and visual observations) was analyzed on a weekly basis throughout the four weeks of storage. A total of five apples were tested for every treatment and for every week. The average of the five data points along with standard deviations is presented in Figure 6. The data for only the Fuji apples are sown in Figure 6 due to the limited space. (Note: All data will be provided to WTFRC and its members upon request).

Texture analysis using force (in Nm) showed the average firmness of each treatment throughout the storage time of four weeks. The main observation throughout the storage time shows an overall decrease in firmness of apples with each week, with apples being exposed to room temperature having a greater difference in values as opposed to apples stored in cold storage.

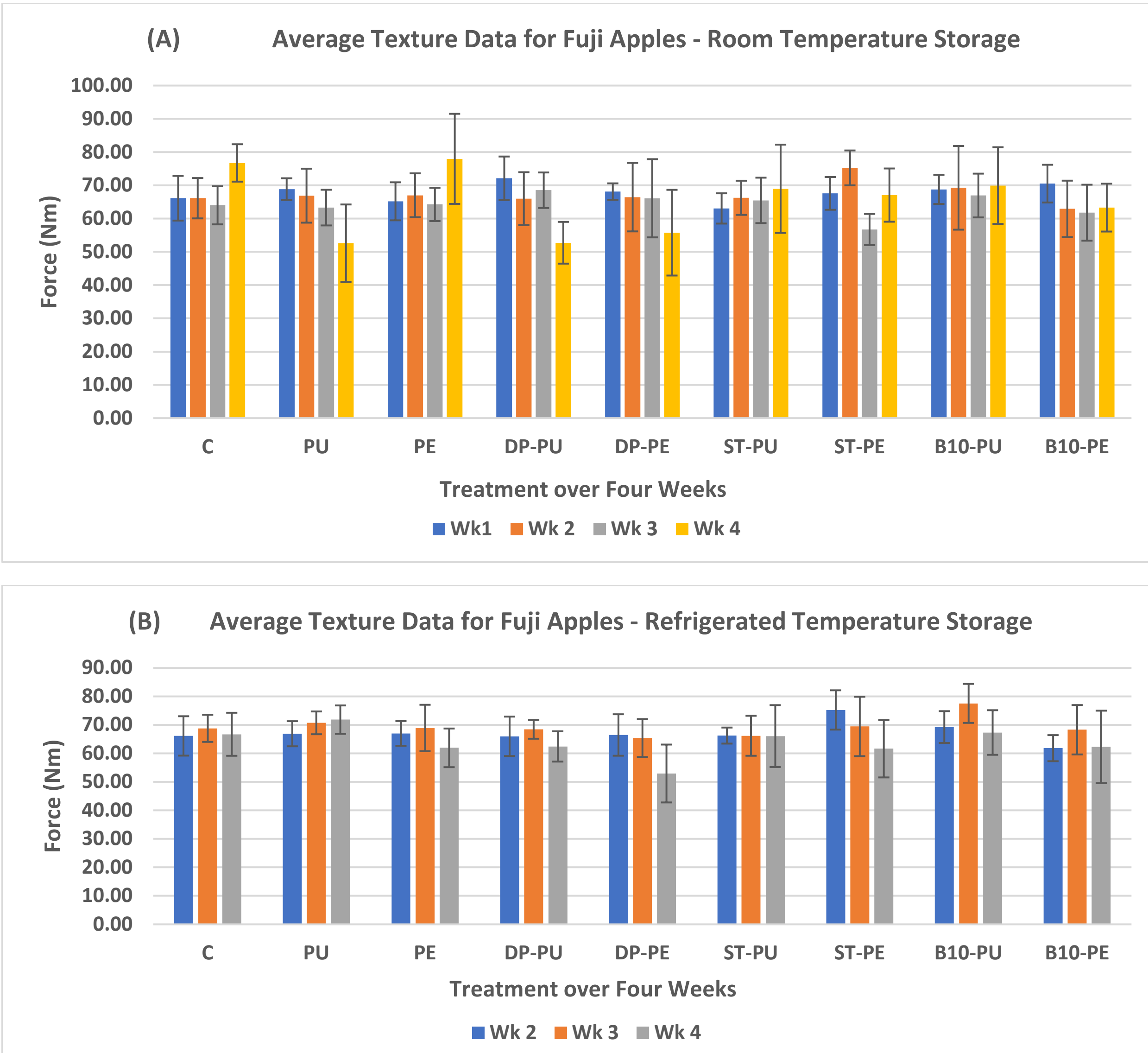

Figure 6. Firmness of Fuji apples measured with a Texture Analyzer (T.A.) for two different storage setting over four weeks (A) – room temperature storage and (B) refrigerated temperature storage. (C – control, no surfactant used.; PU – Purisan Paracitic acid (80 ppm); PE – Perox Paracitic acid (80 ppm); DP-PU – DP081901 with Purisan; DP-PE – Surfactant DP081901 with Perox; ST-PU – Stepanol with Purisan; ST-PE – Stepanol with Perox; B10-PU – Barlox 10s with Purisan; B10-PE – Barlox 10s with Perlox.)

In summary, the experiments indicate there were not significant negative impacts with the use of the surfactant treatments. The only major negative impact observed was in the case of the surfactant with a very low pH (Tsunami 200) when used on the Granny Smith variety. The acidic nature of the surfactant led to burnt spots on the skin of the Granny Smith apples. This shows that it is critical to assess the acidity of the surfactant before utilizing it on fresh apples. We recommend the apple industry conduct short preliminary studies to assess the impacts of the surfactant on the skin of the apples before utilizing them in the packing processes.

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Executive Summary

Project title: Increasing the efficacy of antimicrobial chemicals with surfactants

Key words: Surfactants, Antimicrobial Efficacy, Apple Quality, Storage.

Abstract:

This project evaluated the impacts of the various types of surfactants in enhancing the antimicrobial efficacy of the peracetic acid (PAA) on select apple varieties. Further, the impacts of the treatments on the quality of the apples were also studied over a storage period of fours weeks in two different storage conditions.

The experiments indicated the potential for PAA used with or without surfactants in the apple cleaning process and how the activity of certain surfactants is more effective than others. The results further highlight the challenges that different apple varieties present in developing the most effective cleaning treatments, as well as the difficulty of removing bacteria that are sheltered specifically in the hard-to-reach areas such as the stem bowl and the calyx.

The storage experiments indicated, in general, there were no significant negative impacts with the use of the surfactant treatments. The only major negative impact observed was in the case of the surfactant with a very low pH when used on the Granny Smith variety. The acidic nature of the surfactant led to burnt spots on the skin of the Granny Smith apples. This shows that it is critical to assess the acidity of the surfactant before utilizing it on fresh apples. We recommend conducting short preliminary studies to assess the impacts of the surfactant on the skin of the apples before utilizing them in the packing processes.