CPS 2023 RFP – FINAL PROJECT REPORT

Project Title:
Survival of pathogens on work-in-process fresh-cut produce ingredients

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Objectives:

1. Evaluate the survival of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* on the surface of work-in-process (WIP) ingredients during holding time as affected by time, temperature, pre- and post-sanitization, pathogen populations, microbiota, and natural antimicrobials.
2. Study the transfer of pathogens from WIP ingredients to holding containers and the reusability of holding containers.
3. Model factors contributing to the survival and growth of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* on WIP ingredients and the transfer between pathogens and holding containers.

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Summary of Findings and Recommendations

- Washing with water, chlorine, and peracetic acid (PAA) reduced (0.6-1.0 log averages) initial pathogen populations when compared to no-wash control.
- In most cases, there were no statistical differences in the efficacy of the three washes for reducing initial pathogen populations among water, chlorine or PAA.
- PAA was more effective than chlorine in reducing initial *Listeria monocytogenes* populations on some WIP ingredients.
- *Salmonella* and *E. coli* O157:H7 populations on WIP ingredients often declined during 7 days of storage at 4 and 8°C, but increased at 12°C.
- *Listeria* populations on WIP ingredients did not grow at 4°C, sometimes increased at 8°C, and always increased at 12°C.
- Natural anti-Listerial activities were present in carrots and Brussels sprouts.
- Bacterial mesophile, psychrotroph, and yeast and mold populations increased much faster than foodborne pathogens on WIP ingredients, even grew at 4°C.
- *Listeria* and microbiota populations on PAA-washed samples sometimes grew more rapidly than on other samples at 8 and 12°C.
- Mathematical models were developed to predict pathogen growth, as a function of time, temperature, and sanitizer wash, for each WIP ingredient.
- Pathogens were readily transferred between WIP ingredients and holding containers, and survived well on holding containers at low temperatures.
- Quick rinses of holding containers in either 100 ppm chlorine or 80 ppm PAA solutions could not ensure that all pathogens on the containers were inactivated or removed.
- Temperature is the most critical factor affecting pathogen growth. Maintaining low temperature (4°C) is essential to preventing pathogen growth during WIP ingredient holding, especially for PAA-washed samples.

Abstract

The objective of the study was to evaluate *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella* survival on selected work-in-process (WIP) ingredients during holding time, as well as pathogen transfer potential onto storage containers. WIP ingredients (carrot, red cabbage, yellow onion, kale, Brussels sprout, and broccoli stalk), inoculated with 5-strain cocktails of cold-adapted *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella,* were washed in water, chlorine (minimum 10 ppm, pH 6.5) and 80 ppm peracetic acid (PAA) for 1 min before storage at 4, 8, and 12°C for up to 7 days. Populations of inoculated pathogens, along with native microbiota (mesophiles, psychrotrophs, and yeast and mold) were determined during storage. Pathogen transfer potential onto holding containers was determined by placing inoculated WIP ingredients into containers, followed by tracking pathogen populations on container surfaces. The contaminated WIP ingredients containers were reused for holding WIP ingredients to study the pathogen transfer from the containers to WIP ingredients. In addition, the efficacy of washing contaminated containers in chlorine and PAA solutions to eliminate pathogens was evaluated. Results revealed that washing with sanitizers had mostly similar efficacy in reducing initial pathogen populations, when compared to water washing. Temperature is critical to prevent the growth of foodborne pathogens. Washing with PAA sometimes led to higher *Listeria* and microbiota growth rates during storage at elevated temperatures (8 and 12°C), when compared to

water and PAA washes. Pathogens were readily transferred between WIP ingredients and holding containers. Washing containers with 100 ppm chlorine for 1 min, followed by a water rinse, often eliminated pathogens on holding containers. Mathematical models were developed to predict the survival of pathogens as a function of time and temperature. The study identifies conditions that prevent pathogen growth and transfer and will help the industry establish a standardized science-based approach to manage WIP fresh-cut produce ingredients.

Background

Work-in-process or work-in-progress (WIP) ingredients are fresh-cut fresh produce items (such as shredded carrot and cabbage) that are held temporarily before being mixed into finished products (e.g., salads). WIP fresh-cut ingredients, often minor components of final salad products, still have processing to undergo, including mixing and packing. WIP ingredients are often held in variously sized containers for hours or days before being utilized. WIP ingredients may be prepared in the same processing facility where the final products will be assembled. In some cases, WIP ingredients are supplied by different processing plants to reduce costs by eliminating the capital investment needed to prepare many types of WIP ingredients in the same plant. WIP ingredients may potentially be contaminated with human pathogens, such as *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella,* prior to and following harvest, as well as during processing and handling. These three pathogens have been recovered from prepackaged salads as well as from several fresh vegetables, including cabbage and radish. It is known that *L. monocytogenes*, a psychrotrophic bacterium, can multiply under refrigeration, while *Salmonella* and *E. coli* cannot grow but can survive at low temperatures. The U.S. Food and Drug Administration (FDA) requires that WIP ingredients must be handled in a manner that protects against contamination and growth of undesirable microorganisms. WIP fresh-cut produce represents a critical yet underexplored stage in the production chain, where contamination can be introduced and multiply before final packaging. There is a need to evaluate the growth and survival of bacterial pathogens on WIP ingredients during this holding period. Therefore, we studied the survival of *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella* on select WIP ingredients during holding time, as well as pathogen transfer between WIP ingredients and holding containers. The WIP ingredients were selected because they are either commonly used by the industry or are those which are seldomly evaluated.

Research Methods

Objective 1. Evaluate the survival of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* on the surface of work-in-process (WIP) ingredients.

Preparation of WIP Ingredients

Whole red cabbage, carrots, yellow onions, Brussels sprouts, and kale were purchased from a local supermarket via special pre-order. Broccoli stalks were provided by a local processing plant. Outer layers of whole red cabbage, yellow onions, and Brussels sprouts were removed. Whole fresh produce items were washed with 100 ppm chlorine (pH 6.5, applied as sodium hypochlorite [NaOCl]) followed by a rinse in deionized (DI) water. Whole red cabbage was sliced into quarters, while onions, broccoli stalks, and Brussels sprouts were halved. Most produce items were then sliced into 3–5 mm wide strips using an electric vegetable slicer (Newtry, Jiangsu, China). Carrots were cut into strips (3–5 mm x 4–5 cm) using an electric cheese grinder. Kale was manually cut into strips (ca. 1 cm wide, 3–4 cm long) with knives. The cut WIP ingredients were then separately mixed in a large pan to ensure sample consistency.

Bacterial Strains and Preparation of Bacteria

Five strains each of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella enterica* (applied as individual 5-strain composites) were used in this study (**Table 1**) (n= 15 pathogen strains). To prepare the bacterial cultures, frozen stocks of the strains of three pathogens, stored at -80°C, were revived by transferring them into Tryptic Soy Broth (TSB) (BD Difco, Sparks, MD) and incubating at 37°C for 24–48 h, depending on bacterial strain. Before use, the resuscitated cultures were grown in 10 ml of TSB at 37°C and transferred twice at 24-hr intervals. To facilitate cold adaptation, isolates grown at 37°C were stored in TSB at 8°C for 5 days. The cultures were then centrifuged, and each bacterial pellet was resuspended in phosphate-buffered saline (PBS) (Fisher Scientific, Fair Lawn, NJ). Each representative pathogen strain was aseptically combined to create an 8-log CFU/mL cocktail of the five selected strains.

Table 1. List of bacterial isolates used in this study

Bacterial name	ID	Serotype	Source
E. coli	TW00975	O157:H7	First case of EHEC, 1975
E. coli	TW00118	O157:H7	Human, 1987 USA outbreak Tarr
E. coli	TW07492	O157:H7	Food (meat), Asia
E. coli	TW07496	O157:H7	Cow, France
E. coli	TW1290 DEC4E	O157:H7	Denmark, diarrhea
Salmonella	02-515-1	St. Paul	Cantaloupe outbreak
Salmonella	H0558	Stanley	Stool sample, 1995 sprout outbreak
Salmonella	G4639	Montevideo	1993 raw tomato outbreak strain
Salmonella	H1275	Newport	Sprouts
Salmonella	CFSAN038692	Poona	Cucumber
L. monocytogenes	J1-0108	4b	1981 Canadian cole slaw/cabbage outbreak
L. monocytogenes	LS320	1/2a	Broccoli
L. monocytogenes	LS327	1/2a	Carrot
L. monocytogenes	LS808	1/2a	Celery
L. monocytogenes	LS1857	2a	Enoki mushroom

Inoculation, Treatment, and Storage

Briefly, each 600 g of cut WIP ingredients were inoculated with 6 ml of an 8-log CFU/ml inoculum of a 5-strain cocktail, each, of *E. coli* O157:H7, *L. monocytogenes*, or *Salmonella*, respectively. The inoculum was applied in multiple spots across the produce surface using a 10 ml serological pipette, followed by 3 min of continual bag inversion/rotation to ensure uniform distribution. Samples were then either left untreated (control) or subjected to a 1-min wash with either ca. 100 ppm free chlorine (pH 6.5), PAA (ca. 80 ppm), or sterile DI water. All treated samples received a subsequent 1-min DI water rinse in a large pan to remove residual sanitizers. Inoculated and washed (as well as unwashed control) samples (25 g each) were stored at 4, 8, or 12°C for 7 days in 24 oz. (710 ml) Whirl-Pak bags (Pleasant Prairie, WI, USA), with microbial analyses performed on days 0, 1, 4, and 7. Each experiment was conducted in two separate biological replicates with duplicate technical replicates for each, and independently repeated with different produce batches.

Recovery and Enumeration

Sterile 0.1% peptone water (100 ml) (BD Difco) was added to the 25-g samples in individual bags and pummeled with a stomacher (Interscience, Woburn, MA) for 1 min at 260 rpm. The homogenized samples (1 ml) were serially diluted into 0.1% peptone water and spread plated onto Sorbitol McConkey Agar (SMAC) (BD Difco) + 80 ppm rifampicin, Xylose-Lysine-Tergitol 4 Agar (XLT-4) (BD Difco) + 80 ppm rifampicin, or Polymyxin Acriflavin Lithium-chloride Ceftazidime Esculin Mannitol Agar (PALCAM) (BD

Difco) plates for *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes*, respectively. *E. coli* O157:H7 and *Salmonella* plates were incubated at 37°C for 18–24 h, while the *L. monocytogenes* plates were incubated for 48 h before colonies were counted. In addition, samples were evaluated for total mesophilic bacteria, aerobic psychrotrophic bacteria, and yeasts and molds. Total mesophilic bacteria and aerobic psychrotrophic populations were assessed by plating on TSA, and Plate Count Agar (PCA) (BD Difco), respectively, followed by incubating at 37°C for 24 h (mesophiles) and at 8°C for 9 days (psychrotrophs). Yeast and mold populations were assessed by surface plating samples on Dichloran Rose-Bengal Chloramphenicol Agar (DRBC) (BD Difco), and colonies that formed on plates, after 25°C, five-day incubation, were counted.

Statistical Analysis

Pairwise means comparisons among washes at each temperature and among temperatures for each wash were conducted using SAS PROC MIXED (SAS version 9.4). To examine pathogen growth potential, 7-day slopes were calculated (slope = [(log CFU Day 7) – (log CFU Day 0)] / 7) within each of the inoculation trials by pairing the day 7 replicates #1 and 2 with each of the day 0 replicates #1 and 2, due to the independence of day 0 and day 7 experimental units. For the separate wash treatments for each pathogen, the slope was specified as the dependent variable in a two-way ANOVA model, incorporating heterogeneous within-wash and temperature variances, using variance grouping, when variance magnitudes were >4x, using SAS PROC MIXED. Significance level was set at $P = 0.05$.

Objective 2. Study the transfer of pathogens from WIP ingredients to holding containers and the reusability of holding containers.

Experiment #1 - Pathogen transfer from WIP ingredients to containers: Work-in-process (WIP) red cabbage, carrot, or onion samples were inoculated with an 8-log CFU cocktail of either *E. coli* O157:H7, *Salmonella,* or *L. monocytogenes*. Sixty grams of inoculated WIP produce were placed into 8-oz. rigid plastic containers (filled to the top) or in thin-film bags and held for either 30 min or 24 h at 4 or 8°C. After holding, the WIP ingredients were discarded and bacterial pathogens were recovered from the containers by swabbing with a cotton ball, followed by adding 50 mL of 1X PBS into the container and shaking it at 250 rpm for 1 min. Bacteria attached to the swabbed cotton ball were recovered separately using 25 mL of 1X PBS and stomaching for 1 min. Aliquots (0.1 and 1.0 mL) were plated onto selective media and incubated at 37°C for 24–48 h.

Experiment #2 - Pathogen survival on container surfaces: WIP red cabbage, carrot, or onion samples were inoculated with an 8-log CFU cocktail of either *E. coli* O157:H7, *Salmonella* or *L. monocytogenes*. Sixty grams of inoculated WIP produce were filled to the top in rigid plastic containers and held for 30 min, after which the contents were discarded. After removing WIP ingredients, pathogen survival on the contaminated (emptied) container surfaces was monitored at day 0 (30 min) and after 24 h at 4 and 8°C.

Experiment #3 - Pathogen transfer from reused containers to WIP ingredients: WIP red cabbage, carrot, or onion samples were inoculated with an 8-log CFU cocktail of either *E. coli* O157:H7, *Salmonella* or *L. monocytogenes*. Sixty grams of inoculated WIP produce were filled to the top in rigid plastic containers and held for 30 min, after which the contents were discarded. Subsequently, 60 g of uninoculated WIP ingredients were placed into the same containers and held for either 30 min or 24 h (at 4 or 8°C for 24 h). WIP ingredients were then transferred into stomacher bags, and the pathogens were recovered and enumerated from WIP ingredients using 100 mL of 1X PBS.

Experiment #4 - Washing contaminated containers: WIP red cabbage, or onion samples were inoculated with an 8-log CFU cocktail of either *E. coli* O157:H7, *Salmonella* or *L. monocytogenes*. Sixty grams of inoculated WIP produce were filled to the top in rigid plastic containers, held for 30 min, and then discarded. Containers were washed with either 100 ppm chlorine or 80 ppm peracetic acid for either 10 s or 1 min, followed by rinsing with DI water for the same exposure time to determine requisite washing conditions to inactivate (and/or remove) the pathogens from the containers. Bacteria were recovered from the containers by swabbing with a cotton ball, followed by adding 50 mL of TSB to the sanitized containers and shaking at 250 rpm for 1 min to remove bacteria from the container surfaces. One milliliter of TSB was then plated onto selective media prior to enrichment, after which the remaining TSB was enriched at 37°C for 24 h, and 100 µL plated onto selective media, followed by incubating at 37°C for 24h.

Objective 3. Model factors contributing to the survival and growth of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* on WIP ingredients and the transfer between pathogens and holding containers.

Data obtained from Objective 1 were used to develop statistical models to predict *L. monocytogenes, E. coli* and *Salmonella* population data as a function of storage time, storage temperature, type of sanitizer, microbiota, and type of WIP ingredient for each of the WIP vegetables. The models were used to identify the conditions (temperature, time, etc.) at which the observed *L. monocytogenes*, *E. coli* and *Salmonella* growth was <0.5 log with a 95% confidence interval.
For each pathogen/microbiota under each wash treatment, a quadratic response surface model was fitted to the microbial population values across the grid of observed design points, defined by temperature (4, 8, 12°C) and day (0, 1, 4, 7), using SAS PROC RSREG (SAS version 9.4). The risk (probability) of cross-contamination between WIP ingredients and containers was estimated for each observed laboratory condition as the proportion of observed containers in which contamination was transferred to uninoculated WIP ingredients, to identify low-risk transfer conditions.

Research Results

All three objectives proposed in the project have been completed, even though we faced challenges and delays in recruiting qualified researchers to work on the project, because of changes in USDA hiring policies. We managed to recruit two capable and diligent research fellows. Detailed results are as follows.

Objective 1. Evaluate the survival of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* on the surface of work-in-process (WIP) ingredients.

Efficacy of sanitizer washes in reducing initial pathogen populations

Red cabbage: WIP red cabbage, treated with chlorine and PAA, reduced ca. 0.7 log of inoculated *E. coli* O157:H7, whereas DI water inactivated ca. 0.4 log, and there was no statistical difference among the three washes. The three washes reduced *Salmonella* by 0.7–0.9 log, when compared with the unwashed control; however, there was no significant difference ($P > 0.05$) among the washes. When compared with the unwashed control, chlorine and PAA washing significantly reduced ($P < 0.05$) initial *L. monocytogenes* populations by 0.8 and 1.3 log, respectively, with PAA resulting in greater reductions than chlorine.

Carrot: When compared with the unwashed control, water washing significantly reduced initial *E. coli* O157:H7 populations (0.8 log CFU/g) on carrots at day 0. Chlorine and PAA further reduced populations from 5.27 to 3.44 and 3.59 log, respectively, achieving >1.5 log reductions, when compared

with the unwashed control. The initial *Salmonella* populations were reduced by all three wash treatments, when compared with the unwashed control. Water-wash reductions (0.7 log) were not significant, when compared with the unwashed control, whereas chlorine and PAA treatments effected significant reductions of ca. 1.0 log. When compared with the initially-inoculated *E. coli* populations, initially-inoculated *L. monocytogenes* populations were 3-4 log lower on day 0, likely due to the anti-Listerial effects of inherent, antimicrobial carrot chemical constituents.

Yellow onion: When compared with the unwashed control, the initial *E. coli* O157:H7 population was reduced by 0.51, 0.39, and 0.84 log, following water, chlorine, and PAA washes, respectively; however, these reductions were not statistically significant. Initial *Salmonella* populations were not significantly affected by any washes, when compared with the unwashed control. The initial *L. monocytogenes* population was significantly reduced by all three treatments, with water and chlorine treatments lowering populations by ca. 0.7 log and PAA by ca. 1.0 log, when compared with the unwashed control.

Broccoli stalk: When compared with the unwashed broccoli stalk samples, washing with water significantly reduced initial *E. coli* O157:H7 populations by 0.38 log, similar to chlorine wash (0.41 log). PAA wash achieved an additional 0.41 log reduction of *E. coli* O157:H7 (ca. 0.82 total reduction). When compared with the unwashed control, only chlorine significantly reduced initial populations of *Salmonella* (by 1.10 log) on WIP broccoli stalk, although not significantly different from the PAA wash. Washing with water, chlorine or PAA significantly reduced initial *L. monocytogenes* populations on WIP broccoli stalk, with PAA being the most effective (1.21 log reduction).

Brussels sprout: When compared with the unwashed control, chlorine and PAA significantly reduced initial *E. coli* O157:H7 populations inoculated onto WIP Brussels sprout by 0.44 and 0.59 log, respectively, although the reductions were not significantly different between chlorine- and water-washed samples. Although chlorine and PAA significantly reduced initial *Salmonella* populations, reductions were only 0.41 and 0.36 log, respectively. Washing WIP Brussels sprout with water, chlorine and PAA significantly reduced initial *L. monocytogenes* populations by 0.71, 0.85 and 1.0 log, respectively.

Kale: Washing with water, chlorine and PAA reduced *E. coli* O157:H7 populations on WIP kale by 0.79, 0.87 and 1.21 log, respectively, and there was no significant difference among the three washes. Water, chlorine or PAA washing did not significantly reduce *Salmonella* populations. Washing WIP kale with water, chlorine and PAA reduced *L. monocytogenes* populations by 1.37, 1.80 and 1.54 log, respectively.

Changes in pathogen and microbiota populations during storage

Red cabbage: At 4°C, all treatments resulted in a gradual decline of ca. 1 log of *E. coli* O157:H7 populations during 7 days, while the trends differed significantly among treatments at 8 and 12°C. At 8°C, *E. coli* O157:H7 populations in chlorine and water-treated samples declined steadily (by ca. 1.0-1.5 log) during the 7 days, while *E. coli* O157:H7 populations in PAA-treated samples fluctuated, with an overall declining trend. At 12°C, *E. coli* O157:H7 populations generally increased over time, particularly in PAA-treated samples, where an initial increase of 1.5-2.0 log occurred on day 4 before stabilizing. The significant variations in bacterial populations at 12°C suggest that temperature influences pathogen survival in both treatments, with chlorine washing resulting in more consistent *E. coli* O157:H7 reductions, whereas PAA resulted in population variability during post-sanitization storage.

At 4°C, *Salmonella* populations declined (1.2-1.5 log) in red cabbage samples of all treatments during the first 4 days, although there were no significant differences among the three washes. Over

time, bacterial population stabilized across all treatments, suggesting limited regrowth under cold storage conditions. However, chlorine washing resulted in greater variability, as indicated by the larger error bars on day 4. At 8°C, *Salmonella* populations in all samples declined, with no significant population differences among the washes. In contrast, at 12°C, a different trend was observed, with *Salmonella* populations increasing over time. Notably, samples treated with PAA resulted in a significantly higher *Salmonella* population log increase (ca. 2.3 log), when compared to chlorine (1.6 log) and water (1.7 log), during the 7-day, 12°C storage. The overall trend highlights that, while lower temperatures help suppress *Salmonella* population growth, regardless of treatment, elevated temperatures (i.e., 12°C) promote *Salmonella* regrowth.

At 4°C, *L. monocytogenes* populations in all red cabbage samples declined during the 7-day storage period, with PAA samples maintaining the lowest populations throughout the holding period. At 8°C, *L. monocytogenes* populations increased across all treatments, with populations in PAA-treated samples increasing from ca. 5.0 to ca. 6.6 log, while chlorine- and water-treated samples increased more slowly, reaching similar populations by day 7. At 12°C, significant. *L. monocytogenes* population growth occurred, with water- and chlorine-treated samples reaching ca. 7.0 log, while *L. monocytogenes* populations in PAA-treated samples resulted in more rapid growth, exceeding 7.0 log by day 7.

Carrot: *E. coli* O157:H7 populations on carrot declined on all samples over 7 days at 4°C. The greatest reductions at this condition were achieved by chlorine, which reduced populations below the limit of detection (LOD = 0.8 log) by day 7. Throughout the 7-day storage period at 4°C, carrot samples washed with chlorine and PAA maintained significantly lower *E. coli* O157:H7 populations than water-washed samples. Similar to the trend at 4°C, *E. coli* O157:H7 declined in all samples at 8°C, with chlorine effecting the most rapid decline to <1.0 log. Once again, chlorine- and PAA-washed samples had lower *E. coli* O157:H7 populations than water-washed samples through 7-day, 8°C storage. At 12°C, *E. coli* O157:H7 increased on unwashed samples, and on carrots washed with DI water and PAA, while chlorine, conversely, inhibited growth. At the end of 7 days at 12°C, *E. coli* O157:H7 populations on chlorine-washed samples were significantly lower than on all other samples. At the three tested temperatures, chlorine-washed samples had significantly lower *E. coli* O157:H7 populations than PAA-washed samples on days 1, 4 and 7.

At both 4 and 8°C, *Salmonella*, in washed carrot samples, followed a similar pattern, declining up to day 4, followed by a relatively stable population through day 7, which was significantly lower than the unwashed control. After 7 days at 4°C, populations decreased to 1.52 and 1.68 log in chlorine- and water- washed samples, respectively, representing a >1.3 log reduction, when compared with the unwashed control, while the populations on PAA-washed samples were below the limit of detection. After 7 days at 8°C, *Salmonella* populations decreased to 2.88 and 2.24 log on water- and chlorine-washed samples, respectively, whereas PAA declined to 1.23 log, significantly lower than the unwashed and water-washed treatments. At 12°C, *Salmonella* increased until day 1, before stabilizing through the rest of the 7-day storage period in water-, chlorine-, and PAA-washed samples. Significant reductions (>1.0 log) were maintained up to day 4, when compared with unwashed samples. By day 7, populations increased to 5.07, 4.93, and 4.92 log in water-, chlorine-, and PAA- treated samples, respectively, which were not significantly different from the unwashed control.

L. monocytogenes populations did not change significantly during the 7-day storage period, even at 12°C, suggesting that the anti-Listerial effects were present during the entire 7 days. There were no significant differences among any treatments on any of sampling days, regardless of temperature, except that PAA-treated samples had significantly higher *L. monocytogenes* populations than other treatments at day 7 and 12°C.

Yellow onion: At 4°C, *E. coli* O157:H7 populations remained relatively stable throughout the 7-day storage period, with only the unwashed treatment declining by >1.0 log, when compared with the

initial population. At 8°C, populations remained stable until day 4, after which the populations in the water-treated samples declined the greatest (down to 2.36 log at day 7), followed by PAA (down to 3.31 log) and chlorine (down to 3.38 log), all by day 7. By day 7 no significant differences occurred among all treatments. Greater reductions in the water-washed samples may be a function of reduced background microbiota in the chemically treated samples, which would affect pathogen survival. At 12°C, *E. coli* O157:H7 populations increased across all treatments, reaching 5.44 log in the unwashed control and 5.05 log in the water treatment after 7 days, both of which were significantly higher than populations in the chlorine- (4.28 log) and PAA-treated samples (3.95 log). Notably, at 12°C, PAA washing maintained the lowest populations, (ca.1.5 log reduction) when compared with the unwashed control by day 7.

During 4°C storage, *Salmonella* populations remained relatively stable in all onion samples, with PAA samples consistently being the lowest. After 7 days at 4°C, chlorine- and water-washed samples had significantly lower populations than the unwashed control, and populations in PAA-treated samples were significantly lower than in all other treatments. At 8°C, all treatments reached peak populations by day 4, followed by a decline through day 7. By the end of storage, unwashed samples at 8°C decreased to 2.89 log, while water and PAA maintained lower populations of 1.78 and 1.60 log, respectively, both significantly lower than the unwashed samples. At 12°C, *Salmonella* grew rapidly and stabilized by day 4, reaching 6.93 log in the unwashed control by day 7, although differences were not statistically significant.

During 4°C storage, *Listeria* populations remained relatively stable in all onion samples. The *L. monocytogenes* population in all three washed samples were significantly lower than in the unwashed control at all sampling days. At 8°C, however, populations increased in all samples during the first 4 days, which were greater in the water- and PAA-washed samples. No significant differences were detected among treatments at day 7 and 8°C. At 12°C, *L. monocytogenes* grew more rapidly, especially during the first 4 days, with unwashed and water-washed samples exceeding 7.1 log by day 7, whereas chlorine and PAA treatments increased to 6.41 and 6.38 log, respectively. There were no significant differences among any treatments at days 4 and 7 at 12°C. Across all temperatures, PAA induced the greatest reductions at days 0 and 1, relative to the unwashed controls.

Broccoli stalk: During storage, washed samples maintained significantly lower *E. coli* O157:H7 populations on most sampling days, regardless of storage temperature. At 4 and 8°C, all sample populations decreased by ≥0.51 log during the 7 days, while PAA-washed samples consistently had the lowest *E. coli* O157:H7 populations. During 12°C storage, *E. coli* O157:H7 populations on all samples increased by ≥0.80 log, especially between days 1 and 4. There was no significant difference in *E. coli* O157:H7 populations between chlorine and PAA samples on days 4 and 7, even though PAA-treated samples had lower initial *E. coli* O157:H7 populations at days 0 and 1.

During 4°C storage, *Salmonella* populations tended to decline, but there was no difference in *Salmonella* populations among water, chlorine and PAA samples. There was also no difference between chlorine and PAA samples on days 4 and 7 during 8°C storage. During storage at 12°C, *Salmonella* populations in all samples increased by ≥2.43 log; however, there was no difference among water, chlorine and PAA samples.

During 4°C storage, washed broccoli stalk samples maintained lower *Listeria* populations than the unwashed controls, with PAA-washed samples being the lowest, except on day 7 where there was no significant difference among the four samples. On day 7, PAA-washed sample populations reached levels comparable to those on unwashed samples, with populations significantly higher than those on water- and chlorine-washed samples. During 8°C storage, *L. monocytogenes* populations increased on all samples, while PAA-washed samples increased the most. On day 7, PAA-washed samples had significantly higher *L. monocytogenes* populations than water- and chlorine-washed samples. Similar to the results at 8°C, *L. monocytogenes* populations on all samples increased during 12°C storage. The population on PAA-washed samples increased more rapidly (2.88 log) than those of water and chlorine

samples (1.72-1.81 log), resulting in significantly higher populations on PAA samples than on water and chlorine samples on days 4 and 7. Our results indicate that although PAA washes are more effective in reducing initial *L. monocytogenes* populations than chlorine, populations in PAA-washed sample became higher than on chlorine-washed samples during the latter storage periods at 8 and 12°C, as a result of rapid population growth in PAA-washed samples. Storage temperature amplified these differences.

Brussels sprout: *E. coli* populations declined at 4 and 8°C storage, and increased at 12°C. After 4 days storage at all temperatures, PAA-washed samples had lower *E. coli* O157:H7 populations than other samples. After 7 days storage at 4 and 12°C, there was no difference among the four treatments, while washed samples maintained lower populations than unwashed samples at 8°C.

Salmonella populations declined at 4 and 8°C storage and increased at 12°C. The PAA-washed samples had the lowest *Salmonella* populations during the entire storage periods at all temperatures, which was often significantly lower than on the unwashed samples.

During 4°C storage, the all-washed Brussels sprout samples maintained lower *L. monocytogenes* populations, with PAA washed samples having lower *L. monocytogenes* populations than chlorine- and water-washed samples on days 1, 4 and 7. During 8°C storage, *L. monocytogenes* populations declined on unwashed samples but increased on washed samples, eliminating the initial differences among all the treatments on days 4 and 7. During 12°C storage, *L. monocytogenes* populations in washed samples increased, while unwashed samples changed little. After 7 days, populations on unwashed samples were significantly lower than those on chlorine and PAA-washed samples.

Kale: During 4 and 8°C storage, chlorine and PAA maintained significantly lower *E. coli* O157:H7 populations than the unwashed control. During 12°C storage, *E. coli* O157:H7 populations increased by 0.69-1.28 log on all samples, with washed samples consistently having populations 1.06-1.74 log lower than the unwashed control. During storage at either 4, 8 or 12°C, there were no significant differences between chlorine and PAA washed samples.

Salmonella populations increased by 1.65-2.68 log on all samples at 12°C, but not at 4 or 8°C.

During 4 and 8°C storage, the washed kale samples had lower *L. monocytogenes* populations than the unwashed samples on all sampling days, with chlorine-washed samples often being the lowest. During 12°C storage, *L. monocytogenes* populations increased on all samples, while unwashed samples had the least growth, resulting in <0.5 log differences among all samples by day 7.

Growth of microbiota during storage

Red cabbage: Mesophilic bacteria populations declined at 4 and 8°C, but increased at 12°C. Yeast and mold, and psychrotrophic bacteria populations increased during the 7 days at all temperatures. Samples washed with PAA had the highest populations of yeast and mold, and psychrotrophic bacteria during the 7 days, regardless of temperature, while chlorine-washed samples often had the lowest psychrotrophic bacteria populations.

Carrot: Mesophile, yeast and mold, and psychrotroph populations, increased during holding time, regardless of temperature. Chlorine or PAA washing did not prevent native microbiota growth, when compared with plain water washing. PAA-washed samples had the highest populations of all three types of microbiota sampled at 8 and 12°C.

Yellow onion: Mesophile, yeast and mold, and psychrotroph populations increased at all temperatures. Growth was greater at higher temperatures (viz., greater at 8 and 12°C than at 4°C). Within 4 days at 12°C, all three types of microbiota populations reached maximal 8 to >9 log. At 8°C, maximum microbiota populations occurred by day 7.

Broccoli stalk: Mesophile, yeast and mold, and psychrotroph populations increased during the holding time, regardless of temperature. Mesophile population growth was less pronounced, when compared to yeast and mold, and psychrotrophs. PAA-washed samples had higher growth potentials for yeast and mold, and psychrotrophs than other samples at all temperatures.

Brussels sprout: Yeast and mold, and psychrotroph populations increased during the holding time, regardless of temperature. Mesophile population growth was less pronounced, when compared to yeast and mold, and psychrotrophs. Washed samples often had higher growth potentials than unwashed samples.

Kale: Mesophile, yeast and mold, and psychrotroph populations increased during the holding time, regardless of temperature. Washing with chlorine and PAA had limited effects on the growth of native microorganisms, when compared to water washing.

Objective 2. Pathogen transfer from WIP ingredients to holding containers and the reusability of holding containers.

Pathogen transfer between WIP items and holding containers

Results revealed that all three foodborne pathogens inoculated on WIP fresh-cut ingredients (red cabbage, carrot, or onion) were easily transferred to holding containers. After WIP ingredients with ca. 6 log/g of pathogens were placed into containers, 1-3 log CFU/cm^2 of the pathogens were found on container surfaces. The pathogens on the containers survived well during 4 and 8°C storage. There was no significant change in pathogen populations on containers after 1 day at 4°C. Results also demonstrated that the pathogens on containers were readily transferred to "clean" WIP ingredients. After "clean" WIP ingredients were placed into contaminated containers with pathogen populations of 1-3 log/cm^2, 1-3 log CFU/g pathogens were found on the WIP ingredients. Under the conditions in the experiment, ca. 0.02% of pathogen cells were transferred from initial inoculated WIP to clean WIP via containers. Therefore, if the initial contamination level on WIP were 1,000/g, then ca. 2 bacteria (i.e., 2 CFU) would be transferred to clean WIP, if the contaminated holding containers were not sanitized.

To evaluate whether the contaminated containers could be sanitized effectively, we immersed the containers in either 100 ppm or 80 ppm PAA solutions for either 10 seconds or 1 min. Results revealed that immersed containers, contaminated from red cabbage into the sanitizer solutions for 10 sec, rendered pathogens non-detectable (detection limit = 0.2 log CFU/cm^2) when the recovered bacteria were not enriched. However, after enrichment, *Salmonella* cells were detectable on PAA-washed containers, while *E. coli* were detectable on both chlorine and PAA washed samples. *Listeria* were undetectable on either chlorine, or PAA-washed containers. After 1 min washing with chlorine and PAA, all three pathogens were undetectable, even after enrichment.

For containers contaminated with yellow onions, washing with chlorine and PAA for 10 sec did not eliminate any of the three pathogens, after enrichment. After the 1 min wash, there were no detectable pathogens on the containers washed with chlorine, while all three pathogens were found on the PAA-washed containers. Overall, our results from this limited study (4 containers used) suggest that longer wash times (1 min) were better than shorter wash times (10 sec) in reducing pathogen populations on containers. Chlorine washing for 1 min eliminated all three pathogens on containers contaminated with both red cabbage and yellow onions. PAA washing for 1 min eliminated the populations of the three pathogens on containers contaminated with red cabbage, but did not eliminate them on containers contaminated with yellow onions, suggesting that the nature of fresh-cut produce influences sanitization protocols for washing containers. Namely, washing with 80 ppm PAA for up to 1 min cannot guarantee complete pathogen inactivation on containers contaminated by yellow onions.

Objective 3. Modeling factors contributing to the survival and growth of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* on WIP ingredients and the transfer between pathogens and holding containers.

Red cabbage:

E. coli O157:H7. For each type of wash and pathogen, a response surface model was fit to the Log CFU values as a quadratic function of temperature and day. The quadratic models are: Log CFU = 6.63 – 0.54 x **Temp** – 0.42 x **Day** + 0.036 x **Temp^2** + 0.035 x **Day x Temp** + 0.0076 x Day^2 for water-washed red cabbage samples, and Log CFU = 6.77 – 0.64 x **Temp** – 0.31 x **Day** + 0.041 x **Temp^2** + 0.042 x **Day x Temp** - 0.012 x Day^2 for chlorine-washed red cabbage samples. The only term in the models with a coefficient that is not statistically different from zero is for the Day^2 term. The model indicated that populations of *E. coli* O157:H7 declined with increased temperature and storage time but declined with increasing Temp^2 and Day x Temperature. The model for PAA-washed samples was: Log CFU = 6.02 – 0.51 x **Temp** + 0.011 x Day + 0.034 x **Temp^2** + 0.040 x **Day x Temp** - 0.046 x **Day^2**. Considering the significant effects of Temp, Temp^2, Day x Temp, and Day^2, the model indicated that *E. coli* O157:H7 populations declined with increasing temperature and Day^2 but declined with increasing Temp^2 and Day x Temperature.

Salmonella. Quadratic models were Log CFU = 6.73 - 0.66 x **Temp** - 0.56 x **Day** + 0.044 x **Temp^2** + 0.054 x **Day x Temp** + 0.012 x Day^2 for water-washed samples, Log CFU = 6.07 - 0.55 x **Temp** - 0.59 x **Day** + 0.040 x **Temp^2** + 0.049 x **Day x Temp** + 0.020 x Day^2 for chlorine-washed samples, and Log CFU = 7.12 - 0.88 x **Temp** - 0.49 x **Day** + 0.059 x **Temp^2** + 0.061 x **Day x Temp** + 0.0040 x Day^2. The terms in the models with coefficient that are statistically different from zero are storage Temp, Day, Temp^2 and Day x Temp, indicating that *Salmonella* populations declined with increasing storage temperature and time, but increased with Temp^2 and Day x Temp.

L. monocytogenes: Quadratic models were Log CFU = 5.46 + 0.049 x Temp - 0.19 x **Day** - 0.0014 x Temp^2 + 0.030 x **Day x Temp** + 0.0013 x Day^2 for water-washed samples, Log CFU = 5.22 + 0.27 x Temp - 0.14 x **Day** - 0.00074 x Temp^2 + 0.034 x **Day x Temp** - 0.083 x Day^2 for chlorine-washed samples, and Log CFU = 4.43 + 0.13 x Temp - 0.16 x **Day** - 0.0077 x Temp^2 + 0.054 x **Day x Temp** - 0.014 x Day^2 for PAA-washed samples. The terms in the models with coefficient that are statistically different from zero are Day and Day x Temp, suggesting that *L. monocytogenes* populations declined with storage time (day). However, there were significant ($P < 0.001$) interactions between storage time and temperature (i.e., at high temperature [8 and 12°C], *L. monocytogenes* populations increased).

Note: Models for the other WIP ingredients have also been developed. At the time of this report, these models are available *upon request* by contacting principal investigator Xuetong Fan. Some of the other models will be presented at the 2026 CPS Research Symposium. The models for red cabbage, as provided above, have been published (see Amarasekara et al., 2026, International Journal of Food Microbiology 444:111451). In addition, the research team intends to publish the models for the other five WIP ingredients.

Outcomes and Accomplishments

- WIP fresh-cut red cabbage, carrot, yellow onion, broccoli stalk, Brussels sprout, and kale were studied.
- The efficacies of chlorine and PAA in reducing initial pathogen populations, when compared with water wash and unwashed samples, were evaluated. Results revealed that chlorine and PAA washing had limited efficacy in reducing initial inoculated pathogen populations on WIP fresh-cut ingredients.
- Variation existed in chlorine and PAA demands (i.e., concentration stability) in wash solutions among WIP items evaluated. PAA was more stable than chlorine in the wash solution during the 1 min wash.
- The effect of washes on the survival of three pathogens and microbiota populations (viz., total mesophilic bacteria, aerobic psychrotrophic bacteria, and yeast and mold) was studied during post-wash storage at various temperatures. Results demonstrated that temperature was the most critical factor in controlling these populations. Maintaining temperature is essential to minimizing the prefoliation of foodborne pathogens and microbiota during storage.
- *Listeria* and microbiota may grow faster in PAA-washed WIP ingredients than on other washed samples, especially at 8 and 12°C.
- Foodborne pathogen transfer between WIP items and containers, and pathogen survival on holding containers, were investigated. The efficacy of sanitizing holding containers with chlorine and PAA solutions in removing/reducing foodborne pathogens on containers was evaluated. Results demonstrated that long washes (≥1min) were needed to inactivate pathogens on containers.
- Mathematical models were developed to predict the fate of foodborne pathogens and spoilage microorganisms on WIP ingredients.
- The variation in growth rates among various *L. monocytogenes* strains was also evaluated at different temperatures.

APPENDICES

Publications and Presentations

Peer-reviewed publications:
Amarasekara, Nirosha Ruwani, Deepak Subedi, Joshua B. Gurtler, Bryan T. Vinyard, and Xuetong Fan. 2026. Survival of *Escherichia coli* O157: H7, *Listeria monocytogenes* and *Salmonella enterica* on work-in-process (WIP) fresh-cut red cabbage. International Journal of Food Microbiology 444:111451. https://doi.org/10.1016/j.ijfoodmicro.2025.111451

Counihan, Katrina L., Nirosha Ruwani Amarasekara, Joshua B. Gurtler, and Xuetong Fan. 2026. Genetic variations and accelerated growth at low temperatures in a *Listeria monocytogenes* isolate. Microbiology Spectrum 14:e03786-25. https://doi.org/10.1128/spectrum.03786-25 [open access]

Presentations:
Amarasekara, Nirosha Ruwani, Deepak Subedi, Joshua B. Gurtler, Bryan T. Vinyard, and Xuetong Fan. 2025. Survival of *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella enterica* on Work-In-Process (WIP) Fresh-Cut Red Cabbage. IAFP Annual Meeting, July 27–30, 2025, Cleveland, OH.

Amarasekara, Nirosha Ruwani, Deepak Subedi, Joshua B. Gurtler, Bryan T. Vinyard, and Xuetong Fan. 2026. Survival of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* and Growth of Native Microbiota on Work-in-process Fresh-cut Carrot and Onion During Storage After Chlorine and Peracetic Acid Washes. IAFP Annual Meeting, July 26–29, 2026, New Orleans, LO.

Budget Summary
This project was awarded a total of $300,872 in research funds. As of 30 April 2026, the budget has a surplus of funds for salary due to the delay in hiring researchers for the project, based on USDA-prohibitive hiring practices, and recent changes to the federal hiring structure. Some surplus funds were used for supplies due to increased prices for lab materials. Due to federal restrictions on travel, Co-PIs could not attend the 2024 or 2025 CPS Research Symposiums. So, there currently is a surplus of funds for travel, although this will be used for traveling to the 2026 CPS Research Symposium in Nashville, TN.