CPS CENTER for PRODUCE SAFETY

CPS 2015 RFP
PROJECT REPORT

Project Title
Factors that influence the introduction, fate and mitigation of foodborne pathogens on mangos throughout the production chain

Project Period
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Objectives
1. Evaluate the fate of foodborne pathogens on the surfaces of whole and fresh-cut mangoes.
2. Assess the risks of contamination with foodborne pathogens from handling practices that are common in mango packinghouses.
3. Evaluate the ability of Salmonella spp. to infiltrate into mangoes and to determine the fate of internalized cells after infiltration.

FINAL REPORT

Abstract

Mangoes have previously been linked to outbreaks of salmonellosis in North America and limited information about their persistence or control on mangoes exists in the published literature. The objectives of this study are designed to generate a scientific basis for minimizing and controlling pathogen presence on fresh mangoes during postharvest packing, distribution, and retail. Three objectives are included in this proposal: 1) Evaluate the fate of foodborne pathogens on the surfaces of whole and fresh-cut mangoes; 2) Assess the risk of contamination with foodborne pathogens from practices that are common in mango packinghouses; and 3) evaluating the ability of *Salmonella* to infiltrate into mangoes and to determine the fate of the internalized cells. The majority of the work focused on *Salmonella* and 'Tommy Atkins' mangoes, but experiments also evaluated *Listeria monocytogenes,* as well as 'Ataulfo' and 'Kent' mangoes. No meaningful reductions of *Salmonella* or *L. monocytogenes* were observed on the surface of whole mangoes during storage at temperature typical of those used during mango holding and handling. These results imply that postharvest microbial reduction rates should not be relied on as a Corrective Measure to meet the produce safety rule requirements of mangoes. Though *L. monocytogenes* did not grow substantially on fresh cut mangoes under the typical storage temperatures tested, it has the potential to survive for the shelf life of fresh-cut mangoes. When compared to previously published work on *Salmonella* and *E. coli* O157:H7, these organisms grew significantly more ($p \leq 0.05$) than *Listeria monocytogenes.* If control of bacterial hazards is identified during the cold chain as a Process Preventive Control in a Food Safety Plan under the Preventive Controls for Human Foods Rule of FSMA, *Salmonella*, not *L. monocytogenes* should be identified as the bacterial hazard, as it has the fastest growth rate. The use of sanitizer sprays (free chlorine, 100 ppm, pH 6.5-7.5; (chlorine dioxide, 5 ppm; ozone, 2 ppm; and PAA, 80 ppm) can significantly reduce *Salmonella* populations on the surface of mangoes on both PVC and brush rollers. Significant reductions are apparent after 5 s for most sanitizers, and continue for up to 60 s. Following 60 s, reductions exceed 3.5 log CFU/mango with all sanitizers except ozone, and exceed 4 log CFU/mango with 100 ppm free chlorine and 80 ppm PAA. *Salmonella* internalizes into whole mangoes with some mango varieties and regions on the mango surface having greater susceptibility to internalization. The addition of the rest period between heat treatment and hydro cooling significantly reduces *Salmonella* infiltration. Once internalized, *Salmonella* populations may increase at higher storage temperatures, and persist at all storage temperatures evaluated.

Background

In 2011, the Food Safety Modernization Act (FSMA) was signed into law and two rules will impact mango growers, packers, and those who import mangoes into the US. The Produce Safety Rule (PSR; US FDA, 2015a), will impact growers and some packers. One of the most controversial aspects of the PSR is its requirements on the microbiological quality of water that contacts the harvested portion of the fruit. Alternatives have been offered to growers who need to use water that does not meet the proposed microbiological quality standards, called Corrective Measures. These include (but are not limited to) applying either a postharvest intervention, such as commercial washing, where microbial removal rates have been documented; or a time interval (in days) between harvest and the product reaching a retail location, where microbial reductions over time are known. In both cases, microbial reduction needed during the postharvest intervention or the postharvest storage times are dictated by the initial quality of the water used. Currently, no scientific data exists to support a postharvest intervention or a postharvest storage time for mangoes.

Some mango packers, importers, and anyone involved in fresh cut or value added mango products will fall under the Preventive Controls (PC) for Human Foods Rule (US FDA, 2015b), under FSMA. The PC rule requires all food handling companies have a Food Safety Plan, including a hazard analysis. A cornerstone of these plans is the validation of risk-based preventive controls. Previously, no scientific data existed for industry use in complying with the Produce Safety Rule, specifically related to identification of the appropriate pathogen of concern, and validated reduction strategies.

Five documented *Salmonella* outbreaks in North America have been associated with mangoes, occurring in 1998, 1999, 2001, 2003, and 2012 from *Salmonella* Oranienburg, Newport, Saintpaul, and Braenderup and Worthington, respectively (Beatty et al., 2004; CDC, 2003; PHAC, 1998; Sivaapalasingam et al., 2003; CDC, 2012). In all cases, imported fresh mangoes were the source of the outbreak. While *Salmonella* is the only organism currently linked to any North American outbreaks, concerns over *L. monocytogenes* exist due to recent outbreaks and recalls associated with fresh fruits.

Research has shown that *Salmonella* can internalize into intact 'Tommy Atkins' mango flesh during postharvest heating and cooling treatments, to which all imported fresh mangoes linked to salmonellosis outbreaks had undergone (Penteado et al., 2004; Bordini et al., 2007). Growth of *Salmonella* that has internalized into mango pulp over 6 days at 22°C has also been reported (Bordini et al., 2007). Internalization into other mango varieties, and the fate of internalized pathogens during typical distribution temperatures has not previously been reported.

Little research exists detailing the growth and survival of *Salmonella* or other pathogens on the surface of mangoes. On fresh-cut mangoes, *Salmonella* grew at 23 and 12°C and survived long term at 4 and -20°C; *Escherichia coli* O157:H7 grew at 23°C, and survived at 12, 4 and -20°C (Strawn and Danyluk, 2010). In fresh mango pulp, *E. coli* O157:H7 survived for up to 13 days when stored at either 6 or -10°C (Leite et al., 2002); *Salmonella* Enteritidis grew at 25°C and survived at 10, 4, and -20°C, while *Listeria monocytogenes* grew at both 25 and 10°C, and survived at 4 and -20°C (Penteado, et al., 2014). Acid adapted and non-acid adapted *E. coli* O157:H7 survived in mango juice held at ambient (25°C) and refrigeration (7°C) temperatures for 6 and 8 days, respectively, with better survival under refrigeration; regardless of strain or acid adaptation, *E. coli* O157:H7 population counts quickly declined at ambient temperature (Hsin-Yi and Chou, 2001). No published studies previously evaluate the survival of pathogens on whole mangoes.

Limited published information previously evaluated methods to control pathogen populations that may be present on the surface of mangoes. Penteado et al. (2014) describe the potential for *Salmonella* and *L. monocytogene*s cross-contamination from contaminated knives during processing. While Fernandes et al. (2014) have described the adhesion of *Salmonella* Typhimurium to mangoes as a multifactorial process, in which the roughness and hydrobhobicity of the fruit surface did not affect the efficiency of sanitation treatments. Treatment of 1 x 1 cm pieces of mango peel by submersion in different sanitizers and surfactant mixtures for 10 min does not represent practical packinghouse treatments to remove pathogens from the surface of mangoes, and little practical knowledge can be gleaned from this study to inform the validation of packinghouse processes.

Research Methods and Results
Objective 1. Evaluate the fate of foodborne pathogens on the surfaces of whole and fresh-cut mangoes.

The primary focus of objective 1 was to evaluate persistence of foodborne pathogens on the whole, intact surface of mangoes, where limited published works exist when stored at different temperatures. The effect of inoculum level on the survival of pathogens on whole mangoes was addressed as feedback from stakeholders has been received that levels of microorganisms used in the laboratory are often unrealistically high to be of practical value. To compare inoculum, *Salmonella* or *L. monocytogenes* were inoculated onto the surface of 'Tommy Atkins' mangoes at either High (6 log) or Low (3 log) concentrations and stored for 14 days at 25 ± 5°C. Pathogen populations were enumerated following a rub (60 s)- shake (60 s)- rub (30 s) protocol. Serial dilutions were made in 0.1% peptone water and surface plated (0.1 ml) in duplicate onto selective and nonselective media, with antibiotics as appropriate. To increase the limit of detection an additional 1 ml of the lowest dilution will be plated onto four plates each (0.25 ml/plate) of nonselective and selective media. Figure 1 shows populations of *Salmonella* exhibit similar growth trends for high and low levels of inoculation, both reaching a maximum growth at day 3 (high 6.4 and low 5.8 Log CFU/mango). Thereafter levels decline slightly by day 14 (High 6.1 and 5.6 Log CFU/mango). Populations of *L. monocytogenes* also show similar trends for high and low inoculum (Figure 2) with maximum growth at day 7 (6.5 and 5.9 Log CFU/mango, respectively) and showing a slight decline at day 14 (6.1 and 5.6 Log CFU/mango, respectively). Both *Salmonella* and *L. monocytogenes* grew on the surface of mangoes, an unexpected result.

Studies to evaluate the persistence of cocktails of *Salmonella* and *L. monocytogenes*, on the surface of whole 'Ataulfo', 'Tommy Atkins', and 'Kent' mangoes were been completed. Whole fruit were inoculated by spotting 100 µl of the inoculum cocktail at 6 log CFU/mango onto the appropriately marked sample area on the smooth mid-section of the fruit. Inoculated intact fruit will be dried for 1 h before storage at the appropriate temperature. The temperatures selected for evaluation were 12, 20 and 30 ± 2°C. These temperatures were selected based on the lowest safe temperature for long-term exposure of mangoes (12°C), the minimum forced-air cooling temperature (10°C), the recommended storage room, marine container, or truck trailer temperatures for shipping (10-12°C), the temperature for storage following ripening (10 to 13°C), the temperature mangoes are cooled to in the hydrocooler (21 to 22°C), the optimum temperature for ripening mangoes (20-22°C), the approximate ambient temperature of retail display (ca. 20°C), and the highest temperature for exposure to prevent poor ripening and flavor (30°C) temperatures mangoes may experience between being unloaded at a packinghouse and being processed (Brecht, 2014). Mangoes were stored up to 28 days. Pathogen populations were enumerated as described above. Figures 2-6 show both pathogen populations increased on the surface of all varieties of mangoes at all temperatures. These results are similar to preliminary experiments evaluating inoculum level, where both pathogens grew on the surface of mangoes.

Although a relatively minor part of this objective, we built upon our research and limited published works on the fresh-cut mangoes and mango pulp, we will evaluate the fate of foodborne pathogens inoculated cut surfaces of mango pulp and stored at different temperatures. As this work has already been published for *Salmonella* and *Escherichia coli* O157:H7 (Strawn and Danyluk, 2010), this objective was carried out using only *L. monocytogenes* and only 'Tommy Atkins' mangoes. Cut fruit (10 g) was placed in a petri dish and inoculated with 20 µl at 3 log CFU/g and held in a biosafety cabinet for 20 min to allow the inoculum to dry. Following the drying period, samples were placed into a sterile filtered Stomacher® bag. Bags were not sealed to allow air movement and to prevent creation of an anaerobic environment due to fruit respiration. Cut samples at 4, 12 and 20 ± 2°C for up to 28 days. The temperatures selected for evaluation were 4, 12, and 20 ±

2°C. These temperatures represent the optimum temperature for fresh-cut storage (4°C), common open case refrigerated storage at retail (10-12°C), and ambient temperatures (20°C). Pathogen populations were enumerated as described above, with one exception; samples were stomached for 2 min at high speed, rather than undergoing a rub-shake-rub. At 4 and 12°C *L. monocytogenes* populations remained stable over 14 days with a slight decline (0.8 log CFU/g) at day 28 (Figure 7). When stored at 20°C, *L. monocytogenes* populations increased slightly at day 3 (0.6 log CFU/g) and then declined throughout the 7 days of storage (Figure 7).

Objective 2. Assessing the risks of contamination with foodborne pathogens from practices that are common in mango packinghouses.

The primary focus of this objective will be to evaluate control of foodborne pathogens by various steps currently in mango production. After the fruit leave the initial dump tank, they run through a spray operation to remove soil, latex, and other material adhering to the fruit. Here we will evaluate the efficacy of antimicrobial chemicals approved by the US Environmental Protection Agency (EPA) for contact with food products in a spray operation on mangoes using a pilot scale operation with both brush and PVC rollers. Four different sanitizers, including chlorine, chlorine dioxide, ozone, and peroxyacetic acid (PAA), were evaluated. 'Tommy Atkins' mangoes were used for all studies. When necessary, mangoes were stored at 12°C, and warmed to ambient temperatures prior to use. *Salmonella* cocktails, inoculation of whole mangoes, and enumeration of *Salmonella* following treatment were performed as described above. Four different overhead spray sanitizer solutions will be prepared in ground water meeting the microbiological standards for potable water, including: (i) chlorine, 100 ppm, pH 6.5-7.5; (ii) chlorine dioxide, 5 ppm; (iii) ozone, 2 ppm; and (iv) PAA, 80 ppm. A water control will also be evaluated. Both brush and PVC rollers were evaluated. Mangoes were onto the spray and roller pilot system, and be treated for 0, 5, 15, 30 and 60 s. Three mangoes were treated on the brush bed at one time and the experiment was replicated five times (n=15). As Unwashed/waxed mangoes that had not undergone a USDA APHIS Hot Water Treatment were not always available, a small trial (n=3) was run to determine if differences in *Salmonella* removal existed between mangoes that had previously been heat treated, and not heat treated mangoes, as described above, with chlorine, PAA, water, and chlorine dioxide.

When using brush rollers, there were statistically significant differences (*P*<0.05) in *Salmonella* reductions between water, chlorine and chlorine dioxide, and PAA treatment regardless of washing time (Figure 8A). There were no significant differences (*P*>0.05) between ozone and water. The highest log reduction with PAA treatment was at 60 s (5.13±1.03), compared with chlorine (4.43±0.95), chlorine dioxide (3.81±0.64), ozone (3.20±0.50) and water treatment (3.27±0.50). In all cases, log reductions increased with longer treatment times. Chlorine, chlorine dioxide, ozone and water, and PAA wash achieved at least 3-log and 4-log CFU/mango reduction, respectively.

Results for PVC rollers are shown in Figure 8B. There are no significant differences (*P*>0.05) between water, chlorine dioxide, and ozone. The highest log reduction with PAA treatment was at 60 s (4.65±1.04), compared with chlorine (4.46±0.83), chlorine dioxide (3.77±0.41). ozone (2.70±0.10) and water treatment (3.27±0.50). In all cases, log reductions increased with longer treatment times. Chlorine and PAA wash achieved at least 4-log, chlorine dioxide and water achieved at least a 3-log, and ozone achieved at least a 2.5 log-CFU/mango reduction.

Results from sanitizer treatments on mangoes that had not previously been heat-treated are shown in Figure 9. On brush rollers (Figure 9A), the highest log reduction with PAA treatment was at 60 s (5.2±1.1), compared with Chlorine (4.1±0.0), Chlorine dioxide (4.2±0.0), and water treatment (2.9±0.4). Results for PVC rollers are shown in Figure 9B. The highest log reduction with

chlorine treatment was at 60 s (4.8±1.2), compared with PAA (3.4±0.3), chorine dioxide (3.8±0.5), and water treatment (3.4±0.3). In all cases, log reductions increased with longer treatment times, and reductions are similar to larger experiments on heat-treated mangoes.

Corresponding experiments to evaluate the efficacy of the four antimicrobial chemicals (chlorine, chlorine dioxide, ozone, and PAA) in a spray operation on mangoes using a pilot scale operation with both brush and PVC rollers against *L. monocytogenes* were also evaluated following similar protocols described above; one important difference was the sample size.(n=3 fruit instead of n=15). Results for *L. monocytogenes* removal from mangoes by different sanitizers on brush and PVC rollers are shown in Figure 10. Results for brush rollers are shown in Figure 10A. The highest log reduction with chlorine and PAA treatments was at 60 s (4.3), compared with chorine dioxide (2.7±0.3), ozone (2.5±0.7) and water treatment (3.9±0.5). On PVC rollers (Figure 10B), the highest log reduction with PAA treatment was at 60 s (4.3±0.3), compared with Chlorine (4.0±0.0), Chlorine dioxide (3.5±0.0), Ozone (2.0±0.0) and water treatment (3.0±0.5). In all cases, log reductions increased with longer treatment times.

<u>Objective 3. Evaluating the ability of *Salmonella* spp. to infiltrate into mangoes and to determine the fate of internalized cells after infiltration.</u>

Hot water treatment (46.1°C) for the control of fruit flies varies based on the general shape and weight of the fruit. 'Tommy Atkins' and 'Kent' mangoes are rounded varieties, that may be submerged either 75 min (<500 g) or 90 min (501-700 g). 'Ataulfo' mangoes are a flat variety and submersion time is either 65 min (<375 g) or 75 min (376-570 g). Following heat treatment, mangoes are typically hydrocooled. Hydrocooling is approved immediately following the hot water treatment if 10 min is added to the heat treatment times (Brecht, 2014). Alternatively, fruit may be hydrocooled after a waiting period of at least 30 min at ambient temperature with no changes to the heat treatment (USDA, 2010). The water of the hydrocooler can be no colder than 21°C. 'Tommy Atkins', 'Ataulfo' and 'Kent' mangoes will be used for studies. Unwashed/waxed mangoes that have not undergone a USDA APHIS Hot water treatment were sourced from Florida for this study. Mangoes were separated by weight prior to use into two size categories: <500 g or 501-700 g for 'Tommy Atkins' and 'Kent' and <375 g and 375-570 g for 'Ataulfo'. Mangoes at ambient temperatures were submerged in ground water meeting the microbiological standards for potable water heated to 41.6°C for the appropriate time based on mango shape, weight, and hydrocooling time (USDA, 2010). All 'Tommy Atkins' and 'Kent' mangoes evaluated were <500 g, and were submerged for 85 min. 'Ataulfo' mangoes that were <375 g were submerged for 75 min and those that were 376-570 g were submerged for 85 min.

The inoculum cocktail was prepared as described above. Ground water meeting the microbiological standards for potable water was adjusted to 21°C, and the *Salmonella* cocktail was added to the water to achieve an inoculum level of 6 log CFU/ml. Mangoes, were submerged at a depth of 12 cm into the inoculated cooling water, either immediately following heat treatment or after 30 min at ambient temperature. Mangoes were removed from the hydrocooling treatment following 0, 30, 40, and 50 min. Immediately following removal from the hydrocooling treatment, mangoes were surface sanitized by spraying with 95% ethanol and wiping with a paper towel soaked in 95% ethanol. Once surface sterilized, mangoes were allowed to dry for up to 10 min on sterile Petri plates.

Salmonella populations were enumerated from the mango stem, mid-section and blossom-end. On a sterilized cutting board, the blossom end skin, stem scar end skin, and a section of the mid-section skin (ca. 3-5 mm), was removed using a sterilized serrated knife. A sterilized corer (1.9 cm in diameter) was pushed into the blossom end, stem scar end or mid-section to remove a core of

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Factors that influence the introduction, fate and mitigation of foodborne pathogens on mangoes throughout the production chain

mango flesh. A sterile cotton swab was used to remove the core segment from the corer. Each core section will be weighed, and placed into a sterile filtered Stomacher® bag using sterilized tongs. Following preparation of core segments, 25 ml of lactose broth will be added to each bag and samples macerated for 60 s on the highest setting of a Stomacher®. Once stomached, an additional 15 ml of lactose broth will be added and the bag shaken by hand for 30 s. *Salmonella* populations were enumerated from these cores by two methods, by either plate count as described above and Most Probable Number (MPN), with a lower limit of detection of 0.1 log MPN/segment. A 3 x 3 MPN was set up for the homogenized tissue mixture by extracting 3 x 10 ml, 1 ml and 0.1 ml of each sample and adding each to a sterile test tube, 9 ml of lactose broth, and 9.9 ml of lactose broth, respectively. Tubes will be incubated for 24 ± 2 h at 35 ± 2°C. Following incubation, 1 ml from each tube will be transferred to 9 ml of tetrathionate broth (TT) and 0.1 ml transferred to 9.9 ml of Rappaport-Vassiliadis R10 broth (RV). TT broth tubes will be incubated overnight (24 ± 2 h) at 35 ± 2°C and RV tubes will be incubated for 48 ± 2 h at 42 ± 2°C. Following incubation, TT and RV tubes are streaked onto Hektoen enteric agar (HE) and xylose lysine desoxycholate agar (XLD) supplemented with antibiotics, as appropriate. Plates will be incubated for 24 ± 2 h at 35 ± 2°C, and presumptive positive *Salmonella* colonies confirmed by stabbing and streaking onto triple sugar iron agar (TSI) and lysine iron agar (LIA) incubated at 35 ± 2°C for 24 ± 2 h. All experiments used triplicate samples on untreated mangoes (n=3).

Salmonella infiltration was significantly ($P \leq 0.05$) impacted by variety, fruit region, hydrocool time, and addition of rest period between heating and hyrdocooling. Mangoes hyrdrocooled for 50 and 40 min resulted in greater infiltration (3.2 and 3.1 log CFU/segment) compared to 30 min (2.7 log CFU/segment). 'Tommy Atkins' and 'Atualfo' varieties (3.1 and 3.2 log CFU/segment, respectively) were more susceptible to infiltration than Kent with 2.7 log CFU/segment. Greater concentrations of *Salmonella* were present in flesh sampled from the stem ends for all three varieties (4.0 – 4.7 log CFU/segment) than from the blossom or middle sections (1.9-2.8 log CFU/segment). The addition of a 30 minute rest period resulted in a significant reduction of internalized *Salmonella* from 3.1 to 2.8 log CFU/sample ($P < 0.05$) across all varieties and flesh regions.

Results from experiments were mangoes were immediately submerged into ground water can be seen in Figure 11. Higher levels of *Salmonella* internalization were seen into the stem scar, than into the mid-section or blossom end for all mango types at all cooling times. Internalization into the stem-scar, mid-section and blossom-end, were generally higher in 'Ataulfo' mangoes (Figure 11C), than into 'Tommy Atkins' (Figure 11A) or 'Kent' (Figure 11B).

Results from experiments where mangoes rested at ambient temperature for 30 min prior to cooling can be seen in Figure 12. Higher levels of *Salmonella* internalization were seen into the stem scar, than into the mid-section or blossom end for all mango types at all cooling times with the exception of 'Ataulfo' mangoes cooled for 50 minutes. Internalization into the stem-scar, mid-section and blossom-end, were generally higher in 'Ataulfo' mangoes (Figure 12C), than into 'Tommy Atkins' (Figure 12A) or 'Kent' (Figure 12B).

In order to determine what happens to *Salmonella* populations that have internalized into mango flesh during the APHIS heat treatment, *Salmonella* populations were internalized into 'Ataulfo' mangoes as described above. Once dry, mangoes were stored at 12, 20 and 30 ± 2°C. *Salmonella* populations will be enumerated from the blossom end, stem end and mid-section as described above on days: 12 ± 2°C, days 0, 1, 3, 5, 7, 10, 14, 21, and 28; 20 ± 2°C, days 0, 1, 3, 7, 10, 14, and 21; 30 ± 2°C, days 0, 1, 3, 5, 7, 10, and 14. Due to limited availability of not heat treated 'Ataulfo'; mangoes in FL, three replications of with single samples in each replication were performed (n=3).

The fate of *Salmonella* populations internalized into 'Ataulfo' mangoes and stored at 12, 20, or 30°C is shown in Figure 13. Starting concentrations of internalized *Salmonella* varied in stem scar, mid-section, and blossom end tissues. Internalized populations, in general, remained consistent during storage at 12°C for up to 28 days (Figure 13A). When storage temperatures increased to 20 or 30°C, internalized *Salmonella* in stem scar tissues increased over the first 3 days, then remained stable for the remainder of the storage (21 days at 20°C, Figure13B; 14 days at 30°C Figure 13C). Internalized populations in mid-sections increased at both 20 and 30°C within the first 24 h, but the increase was not sustained, and populations remained stable at levels similar to populations internalized into the blossom end (Figure 13 B and C) for the remainder of the storage.

In the late summer of 2017, Danyluk travelled to a Mango packinghouse where hot water treatment was being used to treat mangoes prior to arrival in the mainland USA. This trip was extremely valuable for Danyluk, and CPS should encourage similar activities between researchers and the industry whenever possible.

Outcomes and Accomplishments
Initial studies were performed to address the concerns of Stakeholders that levels of microorganisms used in the laboratory are unrealistically high to be of practical value. The results demonstrate that initial inoculum concentration does not have an impact on the survival of pathogens on the surface of whole mangoes. Therefore; the high inoculum were used in subsequent studies. No meaningful reductions of *Salmonella* or *L. monocytogenes* were observed on the surface of whole mangoes. These results imply that postharvest microbial reduction rates should not be relied on as a Corrective Measure to meet the produce safety rule requirements of mangoes.

Though *L. monocytogenes* did not grow substantially under storage temperatures tested it has the potential to survive for the shelf life of fresh-cut mangoes. However, when compared to previously published work on *Salmonella* and *E. coli* O157:H7 (Strawn and Danyluk, 2010), *Salmonella* populations at 12 and 20°C, and STEC populations at 20°C, grew significantly more (p≤0.05) than *Listeria monocytogenes.* If control of bacterial hazards is identified during the cold chain as a Process Preventive Control in the Hazard Analysis of a Food Safety Plan under the Preventive Controls for Human Foods Rule of FSMA, *Salmonella*, not *L. monocytogenes* should be identified as the bacterial hazard, as it has the fastest growth rate.

The use of sanitizers can significantly reduce *Salmonella* populations on the surface of mangoes with both PVC and brush rollers. Significant reductions are apparent after 5 s, and continue for up to 60 s. Following 60 s, reductions exceed 3.5 log CFU/mango with all sanitizers except ozone, and exceed 4 log CFU/mango with 100 ppm free chlorine and 80 ppm PAA.

Salmonella internalizes into whole mangoes with some mango varieties and regions on the mango surface having greater susceptibility to internalization. The addition of the rest period between heat treatment and hydro cooling significantly reduces *Salmonella* infiltration. Once internalized, *Salmonella* populations may increase at higher storage temperatures, and persist at all storage temperatures evaluated.

Summary of Findings and Recommendations
- No meaningful reductions of *Salmonella* or *L. monocytogenes* were observed on the surface of <u>whole mangoes</u>. These results imply that postharvest microbial reduction rates should not be relied on as a Corrective Measure to meet the produce safety rule requirements of mangoes.

- If control of bacterial hazards is identified during the cold chain as a Process Preventive Control in the Hazard Analysis of a Food Safety Plan <u>for fresh-cut mangoes</u> under the Preventive Controls for Human Foods Rule of FSMA, *Salmonella*, not *L. monocytogenes* should be identified as the bacterial hazard, as it has the fastest growth rate.
- Use of an overhead spray wash, with either PVC or brush rollers, can be used as a Corrective Measures as defined by the Produce Safety Rule and as a Process Preventive Controls as defined by the Preventive Controls for Human Food Rule.
  - Highest reductions are seen following 60 s with 100 ppm free chlorine and 80 ppm PAA.
  - Specific reductions needed in validations will be based on the roller type, sanitizer type, and contact time.
- *Salmonella* populations present in hydrocooling water can internalize into mango flesh, and is variable depending on variety and if mangoes are cooled at ambient temperature prior to hydrocooling. Proper control of sanitizer in hydrocooling water is needed to prevent internalization.

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Danyluk, M.D. 2018. Factors that influence the introduction, fate, and mitigation of foodborne pathogens on mangoes throughout the production chain. Center for Produce Safety Research Symposium, Charlotte, NC, 2018

Danyluk, M.D. 2017. Factors that influence the introduction, fate, and mitigation of foodborne pathogens on mangoes throughout the production chain. Center for Produce Safety Research Symposium, Denver, CO, 2017.

Wang, X., and M.D. Danyluk. 2017. Assessing the effectiveness of sanitizer spray and brush roller treatment on reducing the population of a five-strain *Salmonella enterica* cocktail on mango surfaces. International Association for Food Protection Annual Meeting Abstract P3-43.

Wang, X., and M.D. Danyluk. 2017. Assessing the effectiveness of sanitizer spray and PVC roller treatment on reducing the population of a five-strain *Salmonella enterica* cocktail on mango surfaces. Florida State Horticultural Society Annual Conference, HP-13.

Wang, X., and M.D. Danyluk. 2017. Assessing the effectiveness of sanitizer spray and roller treatment on reducing the population of a five-strain *Salmonella enterica* cocktail on mango surfaces. Citrus Research and Education Center, University of Florida, Posters and Pastries 2017 Research Symposium, P. 25.

Danyluk, M.D. 2016. Factors that influence the introduction, fate and mitigation of foodborne pathogens on mangoes throughout the production chain. CPS Research Symposium Lightning Round, Seattle, WA, 2016 – presentation given by M. Roberts.

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No publications have been submitted as of today.
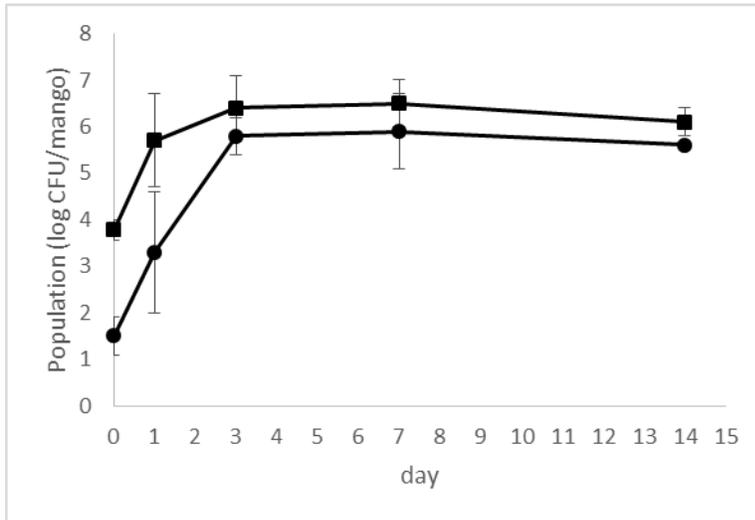
Tables and Figures (optional)

Figure 1. Behavior during storage at 25 ± 5°C of *Salmonella* spp. inoculated onto whole mangoes at levels of 4 log CFU (■), and 2 log CFU (●) per mango. Values are the average of duplicate samples from each of three experiments (n = 6)
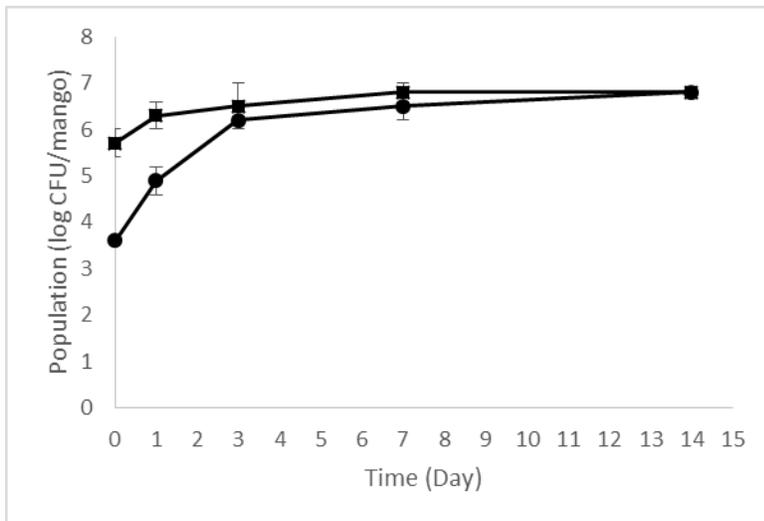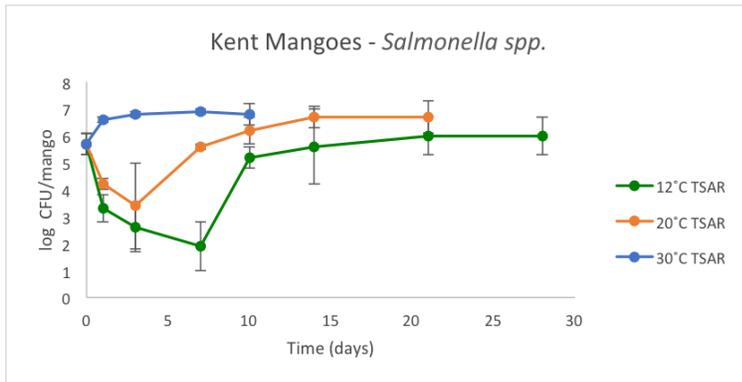

Figure 2. Behavior during storage at 25 ± 5°C of *Listeria monocytogenes* inoculated onto whole mangoes at levels of 6 log CFU (■), and 3 log CFU (●) per mango. Values are the average of duplicate samples from each of three experiments (n = 6).

Figure 3. Survival of a 5-strain *Salmonella* cocktail on the surface of Kent, mangoes stored at 12 ± 2°C for 28 days, 20 ± 2°C for 21 days, and 30 ± 2°C for 14 days. Values represent the average and standard deviations of three replications with duplicate samples (n=6).
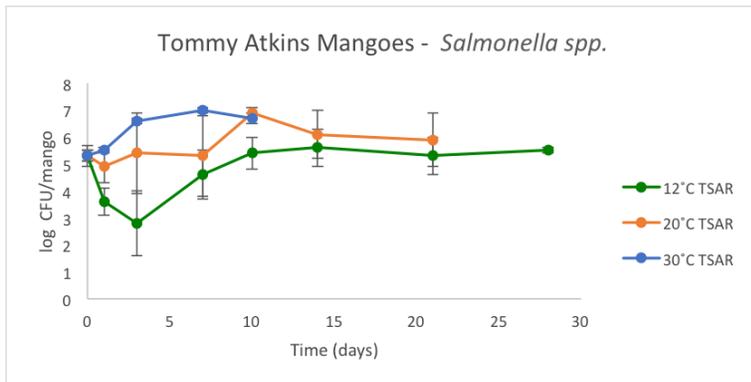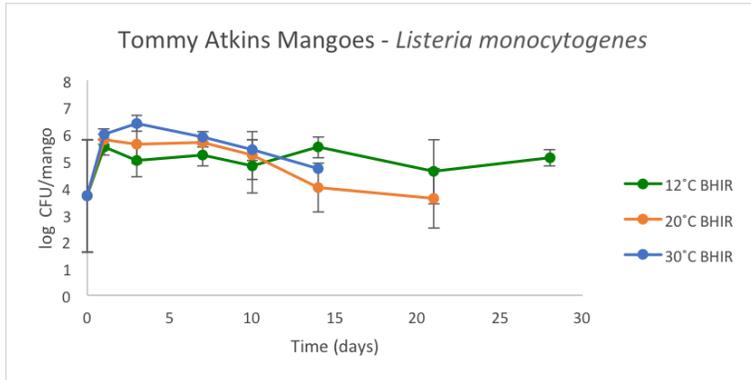

Figure 4. Survival of a 5-strain *Salmonella* cocktail on the surface of Tommy Atkins mangoes stored at 12, 20, and 30 ± 2°C for 14 days. Values represent the average and standard deviations of three replications with duplicate samples (n=6).
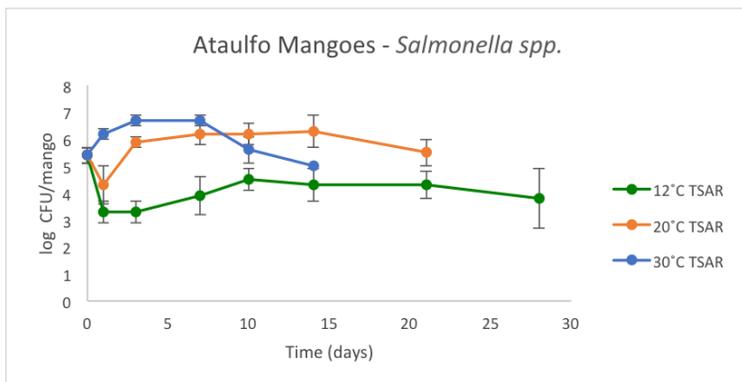
Figure 5. Survival of a 5-strain *Salmonella* cocktail on the surface of Ataulfo mangoes stored at 12, 20, and 30 ± 2°C for 7 days. Values represent the average and standard deviations of three replications with duplicate samples (n=6).

Figure 6. Survival of a 5-strain *Listeria monocytogenes* cocktail on the surface of Tommy Atkins mangoes stored at 12, 20, and 30 ± 2°C for 7 days. Values represent the average and standard deviations of three replications with duplicate samples (n=6).
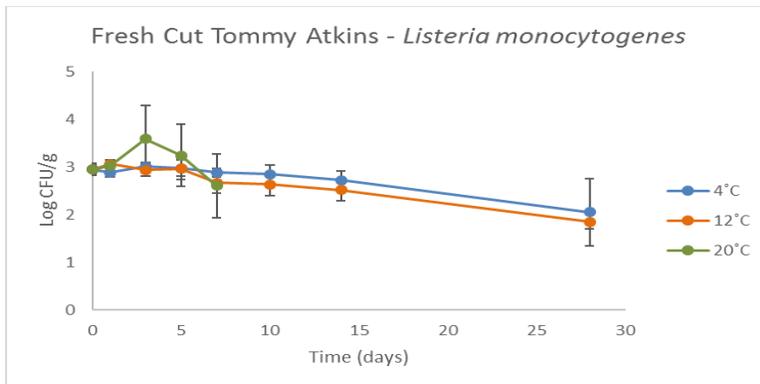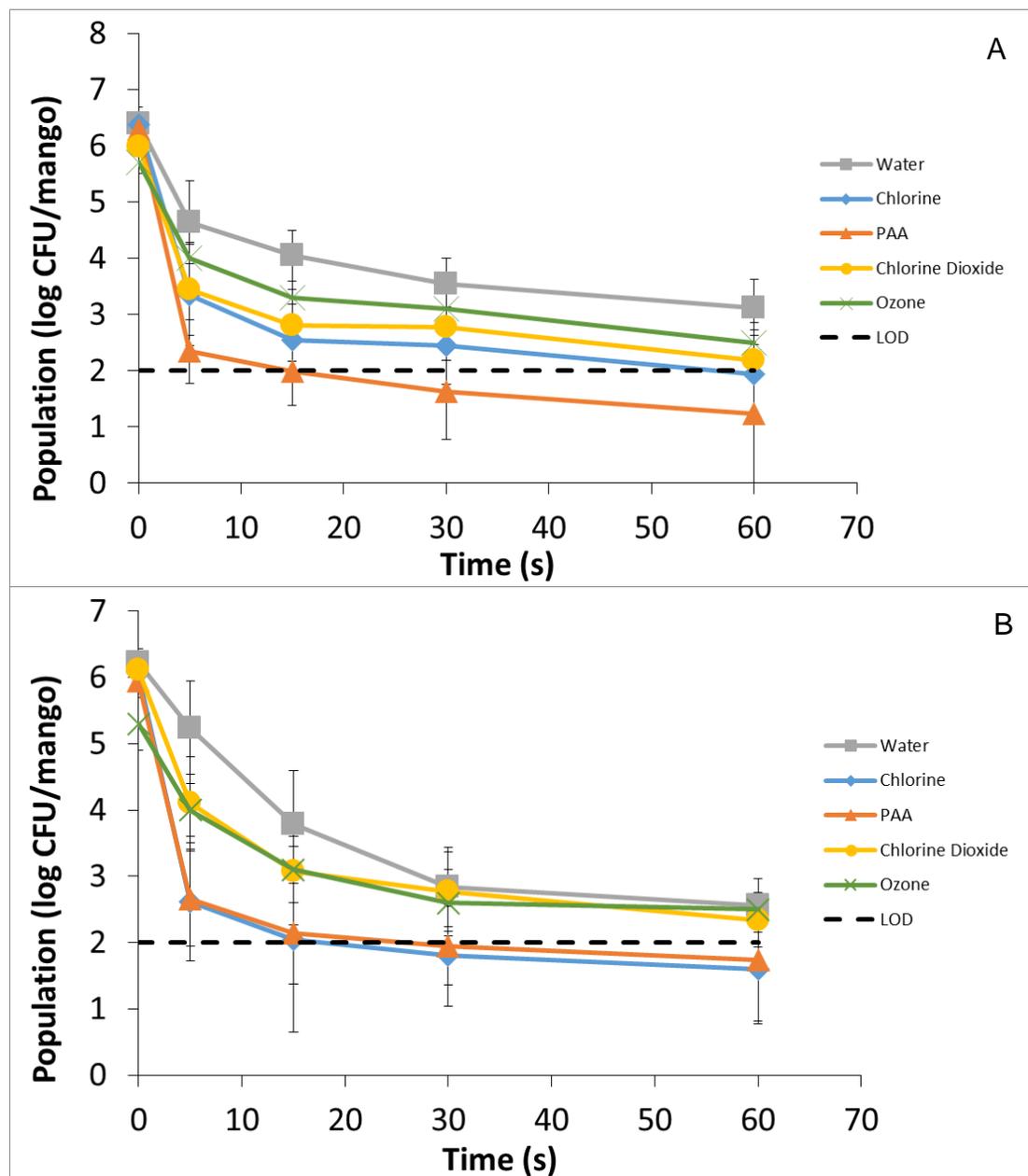

Figure 7. Survival of a 5-strain *Listeria monocytogenes* cocktail inoculated onto cut Tommy Atkins mangoes stored at 4, 12, and 20 ± 2°C for 28 days. Values represent the average and standard deviations of three replications with duplicate samples (n=6).

Figure 8. Reduction of a 5-strain *Salmonella* cocktail on the surface of 'Tommy Atkins' mangoes treated for up to 60 s with 100 ppm free chlorine (pH 6.5), 80 ppm PAA, 5 ppm chlorine dioxide, 2 ppm ozone, or ground water on a brush roller overhead spray wash system (A) or a PVC roller overhead spray wash system (B). Values represent the average and standard deviations of five replications with triplicate samples (n=15), the dashed line represents the limit of detection (2 log CFU/mango).
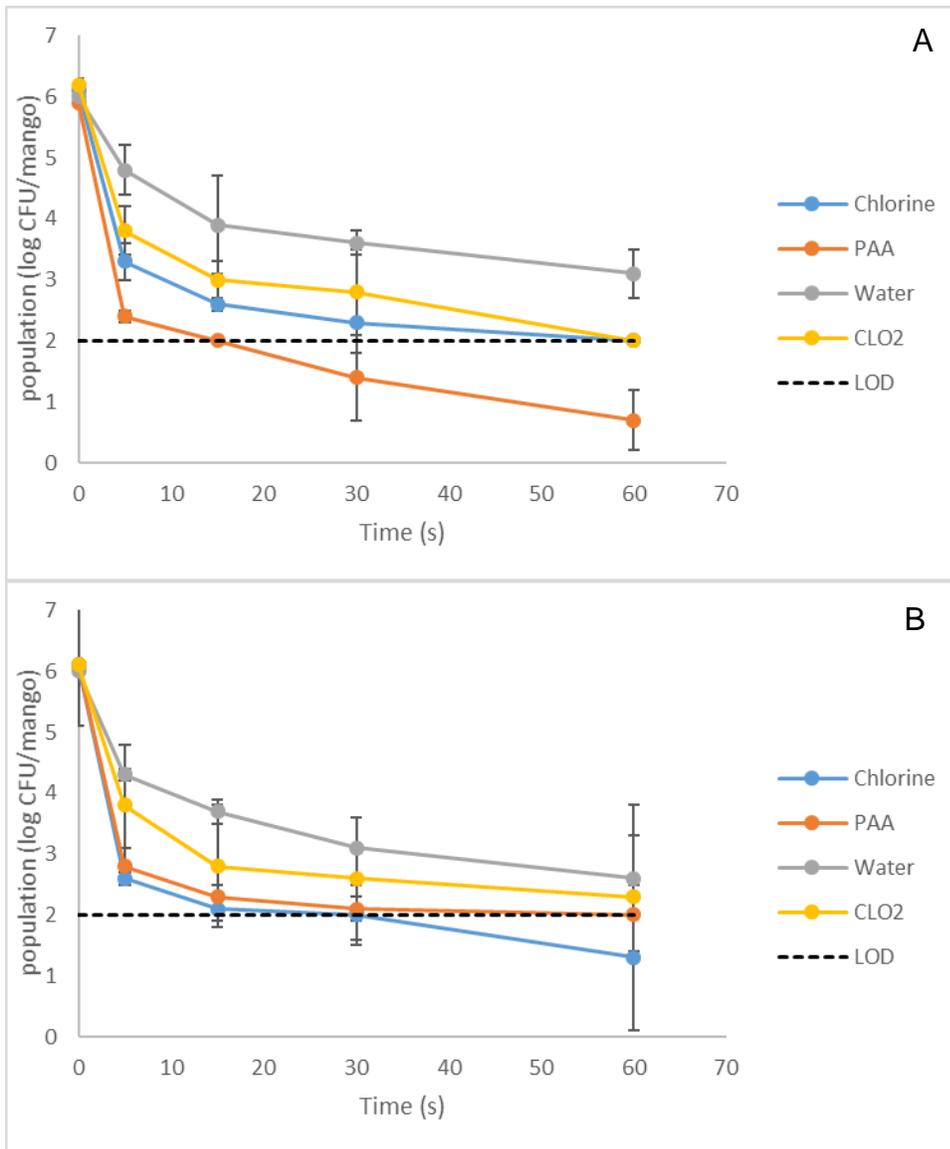
Figure 9. Reduction of a 5-strain *Salmonella* cocktail on the surface of non heat treated 'Tommy Atkins' mangoes treated for up to 60 s with 100 ppm free chlorine, 80 ppm PAA, 5 ppm chlorine dioxide, or ground water on a brush roller overhead spray wash system (A) or a PVC roller overhead spray wash system (B). Values represent the average and standard deviations of triplicate samples (n=3), the dashed line represents the limit of detection (2 log CFU/mango).
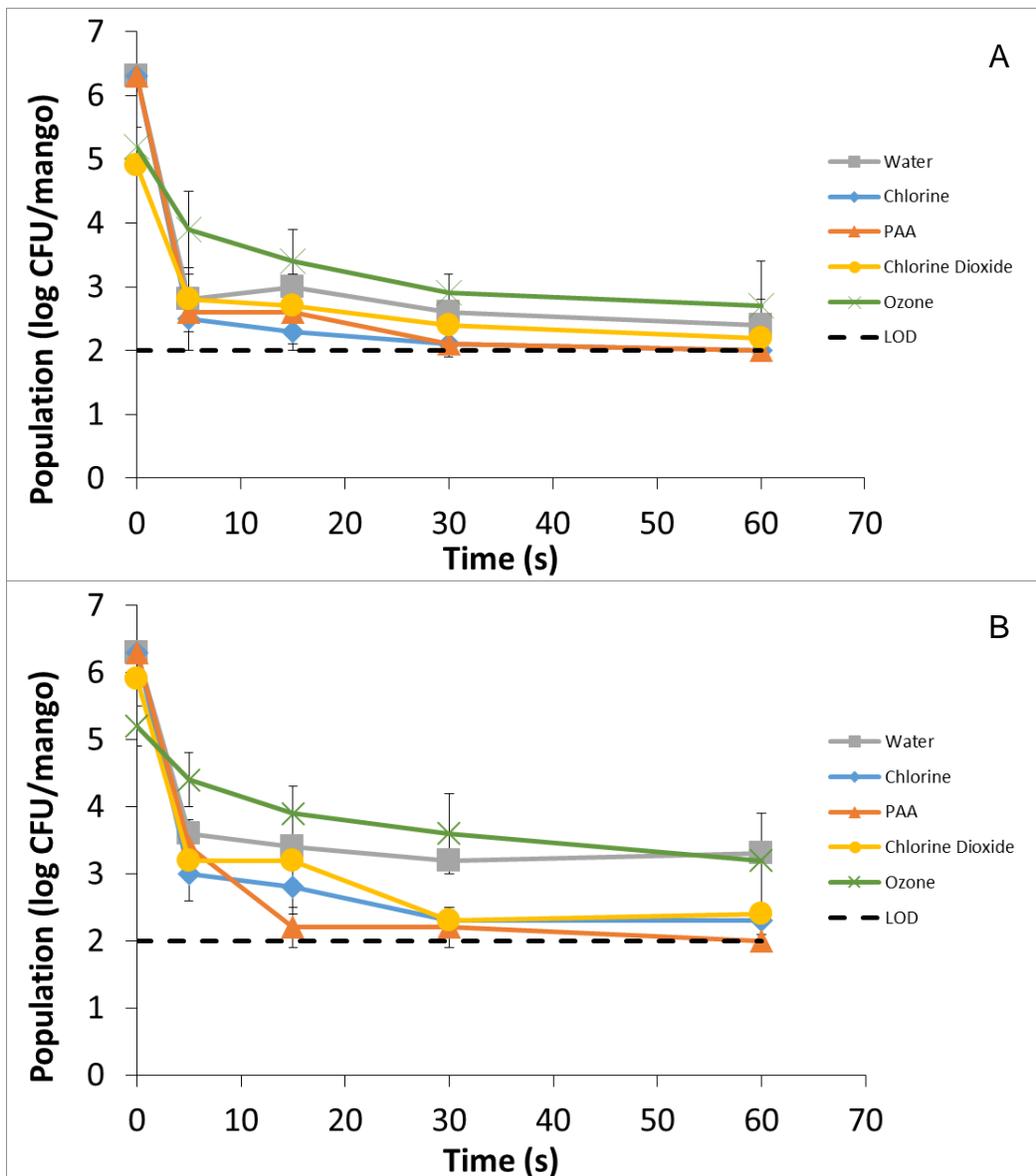
Figure 10. Reduction of a 5-strain *L. monocytogenes* cocktail on the surface of 'Tommy Atkins' mangoes treated for up to 60 s with 100 ppm free chlorine (pH 6.5), 80 ppm PAA, 5 ppm chlorine dioxide, 2 ppm ozone, or ground water on a brush roller overhead spray wash system (A) or a PVC roller overhead spray wash system (B). Values represent the average and standard deviations of five replications with triplicate samples (n=3), the dashed line represents the limit of detection (2 log CFU/mango).
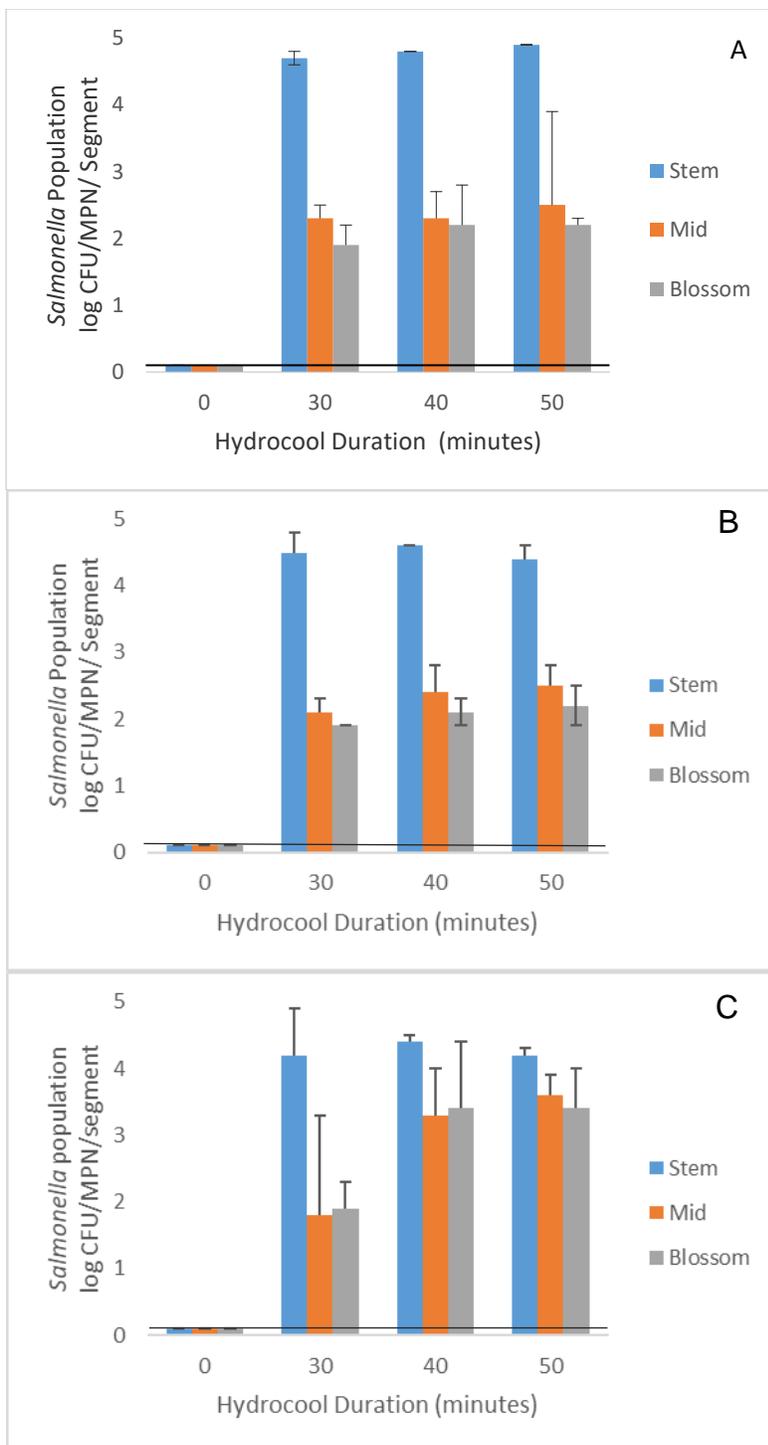
Figure 11. Internalization of a 5-strain *Salmonella* cocktail into (A) 'Tommy Atkins', (B) 'Kent', and (C) 'Ataulfo' mangoes submerged into 21°C water at a depth of 12 cm for up to 50 min, immediately following heat treatment at 46.1°C. Values represent the average and standard deviations of three samples (n=3), the solid line represents the lower (0.1 log MPN/segment) limit of detection.
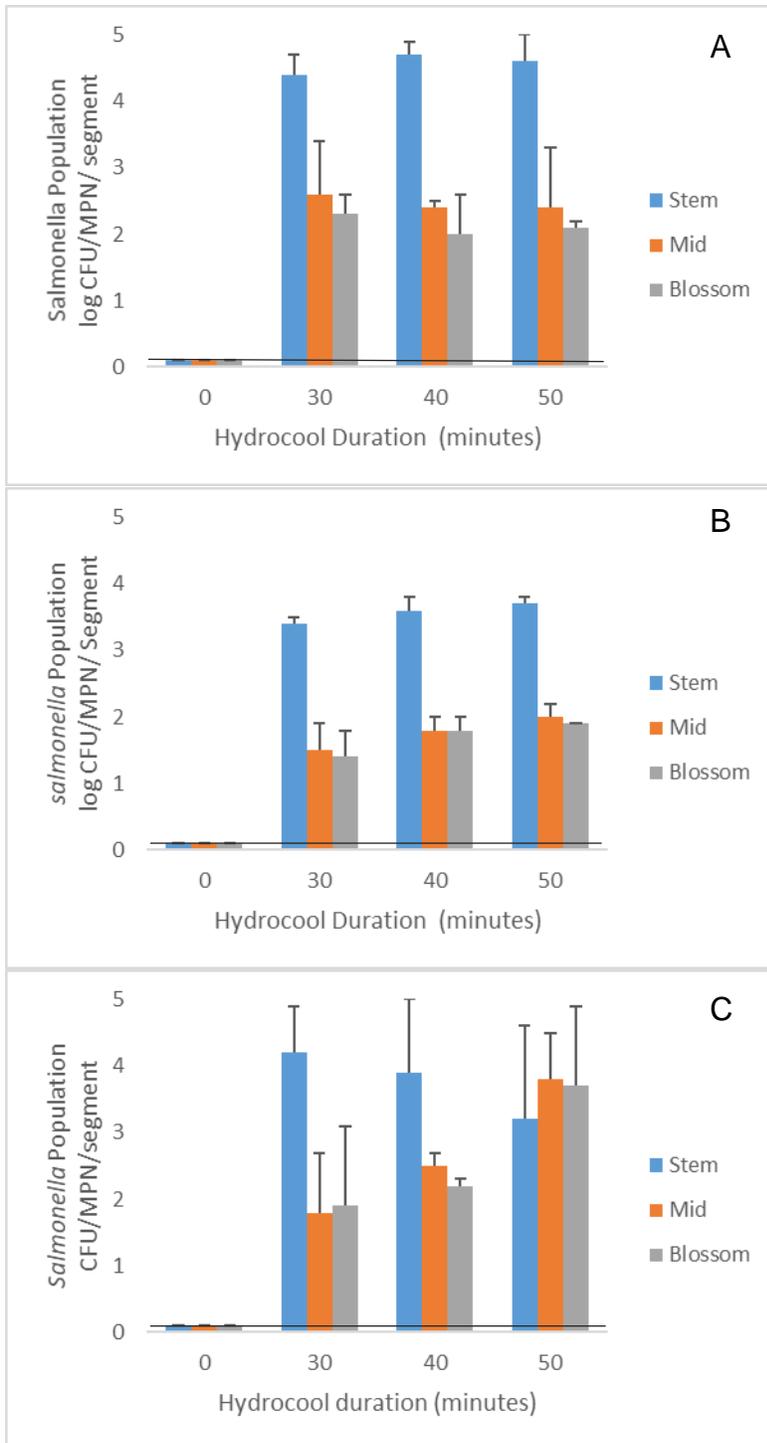
Figure 12. Internalization of a 5-strain *Salmonella* cocktail into (A) 'Tommy Atkins', (B) 'Kent', and (C) 'Ataulfo' mangoes. Mangoes were heat treated at 46.1°C, followed by 30 minutes rest at ambient temperature, and then submerged into 21°C water at a depth of 12 cm for up to 50 min. Values represent the average and standard deviations of three samples (n=3), the solid line represents the lower (0.1 log MPN/segment) limit of detection.
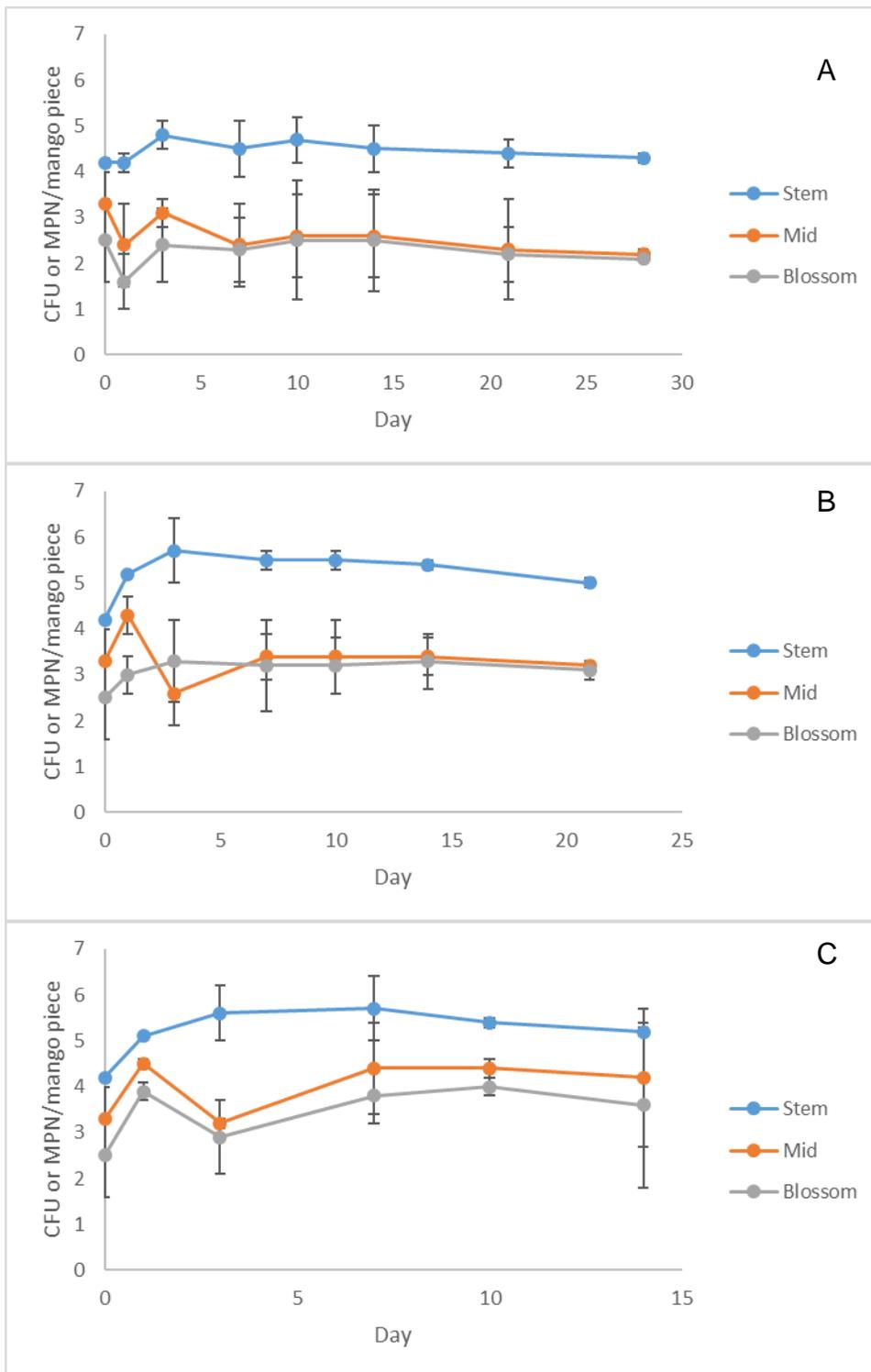
Figure 13. Persistence of a 5-strain *Salmonella* cocktail internalized into 'Ataulfo' mangoes and stored at (A) 12°C, (B) 20°C, or (C) 30°C. Values represent the average and standard deviations of three samples (n=3).

Suggestions to CPS (optional)

None.