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Final Report

Risk assessment and mitigation of foodborne pathogen cross transfer during Hydrocooling and Processing

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Executive Summary:

The goal of this study was to evaluate the antimicrobial efficacy of these sanitizers in preventing cross contamination of *E. coli* O157: H7 from a contaminated batch of iceberg lettuce to subsequent iceberg lettuce heads being cooled in the same water. The potential for survival and pathogen regrowth on stored lettuce was also evaluated. The results from this study indicate that sanitizer use and selection play an important role in reducing *E. coli* O157: H7 cross contamination among lettuce during immersion hydrocooling. All the sanitizers evaluated in this study prevented *E. coli* O157: H7 presence in the water used for hydrocooling of lettuce. Chlorine was the most efficacious commercially available sanitizer in preventing cross contamination among heads of lettuce. The use of chlorine also resulted in a decrease in *E. coli* O157: H7 population on lettuce during storage for a duration of a week at 4°C. The use of ozone during hydrocooling prevented cross contamination among batches 3, 4 and 5. Chlorine dioxide was less effective than chlorine and ozone in preventing cross contamination as batches 2 and 4 were positive for the presence of *E. coli* O157: H7. The experimental sanitizer consisting of pelargonic emulsions was highly efficacious in reducing *E. coli* O157: H7 population from the highly contaminated first batch. Pelargonic acid emulsions also displayed a residual antimicrobial effect during storage and could be considered for the improvement of produce

safety operations. Oxidizers such as chlorine, chlorine dioxide and ozone have limited antimicrobial activity in presence of organic matter. Growers should use FSMA recommendations for the optimal use of these sanitizers. Further, none of the sanitizers were able to reduce the population of *E. coli* O157: H7 below detectable levels in highly contaminated product indicating that pre-harvest food safety practices should be adhered to, to reduce the risk of produce contamination during post-harvest operations.

Introduction:

The risk of foodborne pathogen contamination due the consumption of raw produce is a public health concern(Bennett et al., 2018;Carstens et al., 2019). Vegetable row crops such as leafy greens have served as vehicles for foodborne pathogens and have been responsible for 38% of all produce associated outbreaks between 1998-2013(Bennett et al., 2018). Over 20 multistate outbreaks associated with vegetable row crops have been caused by Shiga Toxin producing *E. coli* occurred between the same period(Bennett et al., 2018). Recent *E. coli* O157:H7 outbreaks associated with romaine lettuce further highlight the risks of leafy greens being contaminated and the gaps in current mitigation strategies(Bottichio et al., 2019).

E. coli O157: H7 has been isolated from animals, animal production environments, dung, soils, irrigation water and many other matrices that are commonly encountered in agriculture(Kumar et al., 2017a;Kumar et al., 2017b). The human pathogen can survive on plants through attachment to plant tissues and internalization into structures such as stomata and intravascular cavities(Crozier et al., 2016;Wright et al., 2017). Attachment and internalization of *E. coli* O157:H7 to plant tissue is facilitated by the presence of curli fimbriae, pili, flagella and the Type 3 Secretion System (Saldaña et al., 2011;Macarisin et al., 2012).

Once on the surface or in sub-surface cavities of plants, *E. coli* O157: H7 can persist and proliferate under certain conditions(Hartmann et al., 2017;Scott et al., 2017). Contaminated produce can serve not only as a vehicle for human illness but also as a source of cross contamination(Pang et al., 2017;Fu et al., 2018). Pre-harvest practices such as hydrocooling and washing involve contact with water which can in turn serve as a medium for microbial cross transfer(Fu et al., 2018;Abnavi et al., 2019). During hydrocooling, harvested produce commodities are cooled to remove " field heat" and increase shelf life of the product using cold water (Macarisin et al., 2017). Hydrocooling has several advantages such as low costs and efficient cooling but also could result in microbial internalization due to temperature differential and cross transfer of foodborne pathogens from contaminated produce to uncontaminated product (Warning et al., 2016;Macarisin et al., 2017). Hence, the use of antimicrobial sanitizers is important in mitigating contamination risk during hydrocooling.

The use of oxidizers such as chlorine, ozone and chlorine dioxide has been recommended to reduce cross contamination during hydrocooling and washing(Kumar et al., 2016;Kumar et al., 2017a;Praeger et al., 2018). The lower temperature of the water during hydrocooling could reduce off gassing and better retention of antimicrobial activity of these sanitizers(Kumar et al., 2017a). While effective when used in optimal conditions, quenching and reduction in antimicrobial activity could occur due to the presence of organic matter, which could increase over multiple use. The objective of this study was to evaluate the antimicrobial efficacy of these sanitizers in preventing cross contamination of *E. coli* O157: H7 from a contaminated batch of iceberg lettuce to subsequent iceberg lettuce heads being cooled in the same water. The potential for survival and pathogen regrowth on stored lettuce was also evaluated. Further a novel antimicrobial, pelargonic acid, a fatty acid component of the tomato cuticle (Dev Kumar and Micallef, 2017;Kumar et al.,

2019;Kumar et al., 2020) was also tested for its ability to prevent cross contamination during the hydrocooling of lettuce. Infrared imaging technology was used to evaluate the efficiency of hydrocooling and to detect non-optimally cooled samples. The results from this study is intended to help optimize safe hydrocooling practices for leafy greens such as iceberg lettuce.

Materials and Methods

Bacterial Culture: A human isolate of *E. Coli* O157: H7 (H1730) implicated in a lettuce outbreak was used for this study. The frozen glycerol stock was revived in BHI by incubating it for 24h at 37℃. The culture was then streaked on to Sorbitol MacConkey agar and Eosin Methylene Bile agar. Typical colonies were isolated and streaked on to Tryptic Soy agar, The isolates were then conferred fluorescence and ampicillin resistance (100µg/ml) by tagging with gfp plasmid. Transformants were then made resistant to streptomycin (100µg/ml) by incremental exposure to streptomycin (20, 40, 60, 80 and 100 µg/ml). The *E. coli* gfp *E. Coli* O157: H7 (H1730) ampicillin and streptomycin resistant strain was streaked on TSA plates with ampicillin and streptomycin (100µg/ml) and used for the study.

Lettuce: Iceberg lettuce heads: Iceberg lettuce grown in the Yuma valley in Arizona was shipped to the laboratory in coolers. The iceberg lettuce heads were stored in a walk-in refrigerator at 4℃ until they were used for the study.

Lettuce inoculation: The gfp *E. Coli* O157: H7 (H1730) ampicillin and streptomycin resistant strain was streaked on to TSA with 100µg of ampicillin and streptomycin and grown for a duration of 18h at an incubation temperature of 37℃. The colonies were scraped and suspended in PBS (Phosphate Buffered Saline) to obtain a final concentration of 8 log CFU/ml. One ml of the suspension was used to inoculate iceberg lettuce heads around the core. The iceberg lettuce

heads were then transferred to an incubator and stored at 42℃ for a duration of 20h. The storage conditions resulted in a 2 log CFU/ml decrease in population. The external and internal temperature was approximately 35℃.

Hydrocooling Protocol: Five batches of lettuce heads consisting of 6 lettuce heads per batch (n=25) were cooled consecutively in a tank with 15 L of 4℃ sterile deionized water for a duration of 30 min each to mimic immersion hydrocooling. The first batch was inoculated with the gfp *E. Coli* O157: H7 (H1730). While all the other batches (2, 3, 4, 5) were not inoculated and did not contain pathogen.

Sanitizer treatments: The sanitizers evaluated in this study consisted of sodium hypochlorite, chlorine dioxide, ozone and pelargonic acid. The sanitizers were added to the water and measured for their potency (concentration) as described below:

- 200 ppm Free Chlorine: 43 ml sodium hydrochloride (4.75-5.25% w/w, RICCA Chemical Company, Arlington, TX) was added to 13 liters sterile DI water. Stirred to completely dissolved. Free chlorine was measured using a DR900 colorimeter with the USEPA DPD Method (Method 10245, HACH, Company, Loveland, CO) before lettuce was immersed and after each batch.
- 5 ppm Chlorine Dioxide: 0.975 grams MB-10 Tablet (AquaPulse Systems, Camarillo, CA) in 13 liters sterile DI water. Stirred until completely dissolved. Chlorine dioxide was determined by a Hach DPD Method (Method 10126, Hach Company)
- Ozone: A laboratory scale ozone generator (Enaly 5000BF Ozone Generator, Oxidation Technologies, LLC, IA) equipped with an oxygen tank (OX20, Airgas, Randor, PA) was used to produce ozone water (6 mg l^{-1}). The concentrations of ozone in water used to treat

seeds and sprouts were determined by OZ-2 Ozone Test Kit (Hach, Hach Company, Loveland, CO).

- One hundred ml of 1 molar Pelargonic acid (Nonanoic acid ≥96% FG, MilliporeSigma, St. Louis, MO) was added to 900 ml sterile DI water to make 100 mM.

Hydrocooling of lettuce in presence of sanitizer: Immersion hydrocooling was performed in chilled water containing the sanitizers as previously described. Each batch of produce was hydrocooled by immersion in chilled water for a duration of 30 min.

Post Hydrocooling storage: After hydrocooling, three heads of lettuce from each batch were stored in a walk-in refrigerator at a temperature of 4℃ for a duration of a week.

Microbiological Analysis: The outer most leaves of the immersed iceberg lettuce were excised to obtain 25g samples. The 25g samples were added to 335 ml of sterile peptone water (0.1% peptone) supplemented with 0.001% of sodium thiosulfate. The leaves were pummel in a stomacher for a duration of 1min at normal speed. The suspension was then enumerated on TSA with 100µg of ampicillin and streptomycin with appropriate dilutions.

Iceberg lettuce heads that had been stored for a week at 4℃ were sampled similarly to enumerate surviving *E. coli* O157: H7

Statistical Analysis: Data were compiled, log transformed, and used to create histograms, using Microsoft Excel (Microsoft, Redmond, Washington). The number of bacterial populations transferred on subsequent lettuce samples was plotted on the y-axis to visualize variability in log percent transfer rates during the different transfer events. *E. coli* O157:H7 counts were converted to log CFU per gram and subjected to analysis of variance using JMP 11.0 (SAS Institute Inc., Cary, NC). For all tests, a = 0.05.

Results

Effect of antimicrobial use in reducing initial contamination

After hydrocooling the population of *E. coli* O157: H7 was 5.62±0.1 log CFU/g. The use of 200-ppm chlorine ad n6 ppm ozone in water resulted in a reduction of *E. coli* O157: H7 population to 3.71±0.24 log CFU/g and 4.37 log CFU/g respectively. The use of chlorine dioxide did not result in significant reductions of *E. coli* O157: H7 population from the surface of the contaminated iceberg lettuce. Dipping iceberg lettuce in pelargonic acid emulsions before hydrocooling resulted in the highest decrease in *E. coli* O157:H7.

Effect of antimicrobial in reducing cross contamination of *E. coli* O157: H7

No cross contamination was detected among the 4 consecutively cooled batches of iceberg lettuce when chlorine or pelargonic acid was used. The use of ozone during hydrocooling resulted in batches 3, 4 and 5 being free of *E. coli* O157: H7 was positive for the presence of *E. coli* O157: H7, though the population of the pathogen was significantly lower than the initial contaminated batch of hydrocooled lettuce. The use of chlorine dioxide during hydrocooling resulted in batches 2 and 4 being positive for *E. coli* O157: H7. Without the use of a sanitizer, all five batches of lettuce that were hydrocooled were positive for the presence of pathogen.

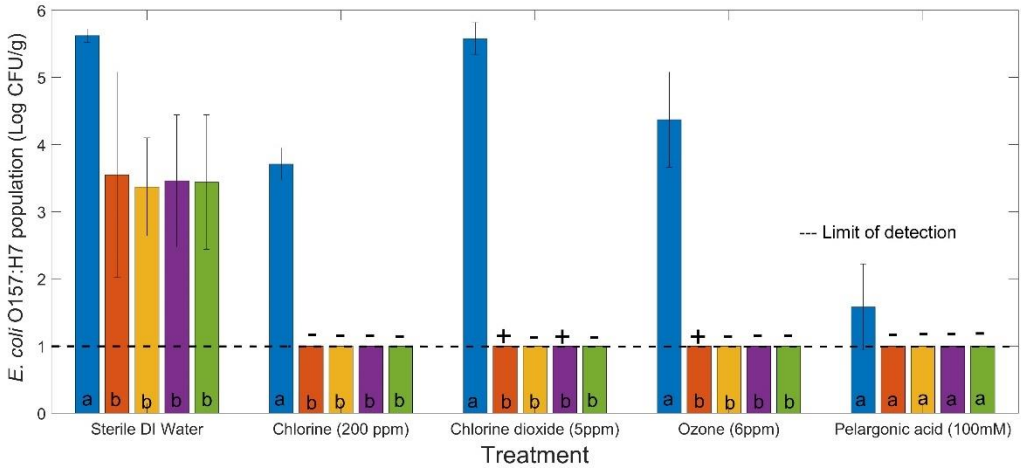

Figure 1: *E. coli* O157:H7 populations (Day 0, immediately after treatment) on Iceberg lettuce samples subsequently washed with different treatments. Different letters represent significant difference of populations within same treatment. Positive (+) and negative (-) represent positive and negative enrichment for bacterial populations below limit of detections (dashed line).

Presence of *E. coli* O157: H7 on hydrocooled lettuce after 1-week storage

The heads of lettuce that were inoculated with *E. coli* O157: H7 (batch 1) did not have a decrease in *E. coli* O157: H7 population when the water that was used for hydrocooling did not contain a sanitizer. A decrease in *E. coli* O157: H7 population was observed from batch 1 lettuce heads when chlorine, ozone and pelargonic emulsions were used in the water for hydrocooling. None of the other batches of lettuce that was cooled consecutively were positive for the presence of *E. coli* O157: H7 after a week of storage at 4°C when chlorine or pelargonic acid was used in the water for hydrocooling. Batch 2 of lettuces that were hydrocooled with ozonated water and batch 4 of the lettuce that was hydrocooled with chlorine dioxide as a sanitizer were positive for the presence of *E. coli* O157: H7.

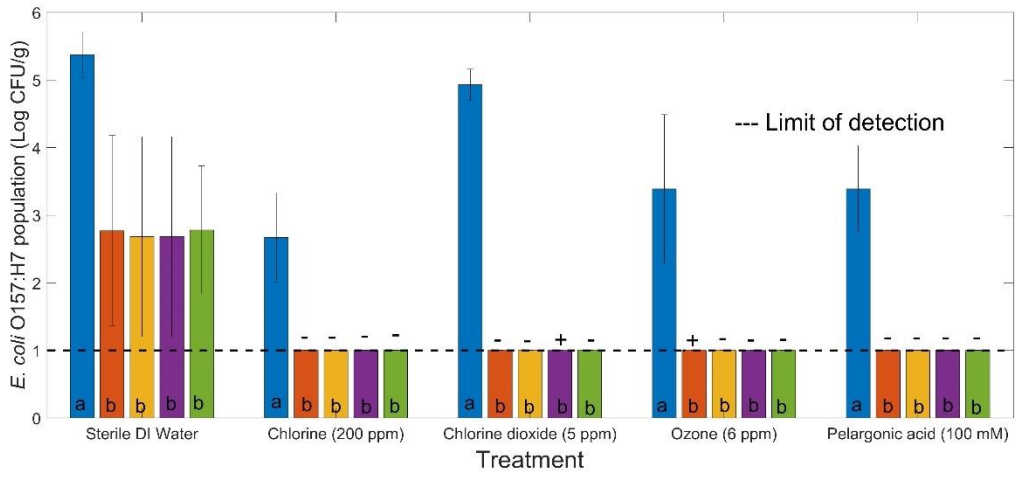

Figure 2: *E. coli* O157:H7 populations (Day 7) on Iceberg lettuce samples subsequently washed with different treatments. Different letters represent significant difference of populations within same treatment. Positive (+) and negative (-) represent positive and negative enrichment for bacterial populations below limit of detections (dashed line).

Effect of sanitizer use on *E. coli* O157: H7 presence in water for hydrocooling

Without the presence of a sanitizer, 4.95 log CFU/ml of *E. coli* O157: H7 was detected in the water used for hydrocooling over the duration of 150 min of the experiment. The addition of the sanitizers to the water reduced the population of *E. coli* O157: H7 below detectable limits. None of the water samples were positive for the presence of pathogen even after enrichment.

Summary:

- Chlorine (200ppm) and ozone (6 ppm) can serve as an effective sanitizer in preventing lettuce cross contamination during hydrocooling.
- None of the sanitizers were effective in eliminating the presence of *E. coli* O157: H7 from heavily contaminated lettuce.

- Pelargonic acid was most effective in reducing *E. coli* O157:H7 populations from heavily contaminated lettuce samples.
- The use of a sanitizer in water during immersion hydrocooling is an essential and effective mitigation strategy to prevent iceberg lettucc cross contamination and contamination of the water used for hydrocooling.

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