1 Sanitizer efficacy in preventing cross-contamination of heads of lettuce during retail crisping

2
3 Yangjin Jung, Hyein Jang, Mengqi Guo, Jingwen Gao, and Karl R. Matthews*

4 Department of Food Science, Rutgers University, New Brunswick, NJ 08901, United States

5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20 * Corresponding author. Karl R. Matthews, Tel.: +1 848 932 5404.

21 E-mail address: matthews@aesop.rutgers.edu

Abstract

This study was conducted to provide information regarding mitigation of cross-contamination through the use of sanitizer during crisping at retail outlets. Seven non-inoculated heads and one inoculated head (≈ 5 log CFU/g) of lettuce were placed into commercial sink filled with 76 L of tap water (TW), electrolyzed water (EW, free chlorine: 43 ± 6 ppm), lactic acid and phosphoric acid-based sanitizer (LPA, pH 2.89), or citric acid-based sanitizer (CA, pH 2.78) and soaked for 5 minutes. Two subsequent batches (eight non-inoculated heads per batch) were soaked in the same solution. Soaking with EW significantly reduced the population of *S. enterica* (2.8 ± 1.5 log CFU/g), *E. coli* O157:H7 (3.4 ± 1.1 log CFU/g), and *L. monocytogenes* (2.6 ± 0.7 log CFU/g) inoculated on Romaine lettuce compared to TW, LPA, and CA ($p < 0.05$). On Red leaf lettuce, EW significantly reduced populations of *S. enterica* and *E. coli* O157:H7, but not *L. monocytogenes* compared to other treatments. No significant difference was noted between TW, LPA, and CA in reducing foodborne pathogens ($p > 0.05$) or preventing cross-contamination. Soaking with EW prevented cross-contamination among lettuce heads and controlled bacterial populations in crisping water for three consecutive batches. EW may be an effective option as a sanitizer to minimizing the cross-contamination of leafy greens during the retail crisping.

Key words: Heads of lettuce; cross-contamination; crisping; electrolyzed water; sanitizers; retail settings

1. Introduction

Leafy vegetables are considered to present the greatest concern with regard to microbiological hazards of fresh produce (FAO/WHO, 2008) and were identified as presenting the greatest risk for food regulated by the U.S. Food and Drug Administration (CSPI, 2009). According to the Center for Science in the Public Interest (CSPI), produce was the top food category responsible for 643 outbreaks (19% of total outbreaks) and 20,456 illnesses (24% of total illnesses) between 2004 and 2013 in the United States (CSPI, 2015). Shiga toxin-producing *Escherichia coli* (especially *E. coli* O157:H7), *S. enterica*, and *Listeria monocytogenes* are the primary pathogens of concern for leafy vegetables such as lettuce and spinach (Castillo and Rodríguez-García, 2004; Heaton and Jones, 2007; Herman *et al.*, 2015).

Microbiological safety and quality of leafy vegetables can be improved through washing. Typically produce is washed with potable water with/without chemical antimicrobial agents for the primary purpose of removing soil and debris. Washing can reduce the population of pathogenic and spoilage microorganisms, improve appearance, and potentially extend shelf-life of produce (Artés *et al.*, 2009; Herdt and Feng, 2009). According to the U.S. Food Code (section 3-302.15 Washing Fruits and Vegetables), chemical sanitizing agents approved by the FDA (e.g. sodium hypochlorite, specified in 21 CFR 173.315) and as specified under § 7-204.12 of the Food Code may be used for washing fruits and vegetables (FDA, 2013). Wash water can serve as a vehicle for microbial cross-contamination of product, especially when the water is reused without addition of sanitizing agents (Gil *et al.*, 2009). The addition of a sanitizing agent to water intended for the washing of fresh vegetables has been recommended to prevent transfer and survival of pathogens during washing (Sapers, 2001; FDA, 2009; Gil *et al.*, 2009; Luo *et al.*, 2012).

Chlorine is the most widely used sanitizer in the food industry because of its broad spectrum of antimicrobial activity, relative low cost, and minimal negative effect on product quality (Luo et al., 2012; Parish et al., 2003; Sapers, 2014). There are many alternatives to the use of chlorine including peroxyacetic acid, organic acids (lactic, citric, and acetic acids), and electrolyzed water. There are different types of EW: acidic electrolyzed water (AEW), neutral electrolyzed water (NEW), and basic electrolyzed water (BEW; alkaline electrolyzed water) (Kim et al., 2000; Hsu, 2005; Huang et al., 2008; Artés et al., 2009).). AEW has been shown to be effective in reducing or inactivating foodborne pathogens on lettuce (Park et al., 2001; Koseki et al., 2004; Park et al., 2008; Keskinen et al., 2009), spinach (Park et al., 2008), cabbage, lemons, tomatoes (Pangloli et al., 2009), and apples (Graça et al., 2011). NEW or slightly acidic electrolyzed water (pH 5.0–6.5) has received much greater attention by the food industry since skin irritation is reduced, chlorine odor limited, and increased stability at near-neutral pH compared to AEW (Len et al., 2000; Ayebah and Hung, 2005; Abadias et al., 2008; Water et al., 2014).

Although sales of fresh-cut/bagged salad has steadily increased in popularity due to its convenience, the sales of heads of lettuce are still one of the top 10 vegetables purchased in US supermarkets (Cook, 2012). Most major retail grocery stores and supermarkets employ a crisping practice for leafy greens to improve consumer acceptance. Crisping typically involves the submersion of commodities in water (with or without sanitizers) for up to ten minutes followed by refrigeration, which gives products a fresh look and crisp texture. Leafy greens such as Romaine lettuce, loose leaf lettuce, and kale may require crisping since they are easily dehydrated during storage and distribution (California Department of Public Health, 2012; Defense Commissary Agency, 2012). Crisping may result in cross-contamination through the

transfer of pathogens from contaminated commodities. Indeed, crisping has been suspected of facilitating cross-contamination of lettuce linked with an *E. coli* O157:H7 outbreak that occurred in Montana in 1995 (Ackers et al., 1998). A paucity of research has focused on the microbiological safety of retail crisping practices.

Studies have demonstrated the efficacy of sanitizers including chlorine, organic acids, and EW in reducing microbial loads on leafy vegetables (Gil et al., 2009; Nou and Luo, 2010; Barrera et al., 2012; Luo et al., 2012; Davidson et al., 2013). Most studies have focused on industry type processing and reduction of microbiological population on commodity rather the control of microbes in the wash water. Often fresh-cut/shredded lettuce is the test commodity of choice and processing done under pilot scale for evaluating a sanitizers' efficacy against foodborne pathogens. In the present study, the efficacy of commercially available electrolyzed water concentrate and acid-based sanitizers in preventing cross-contamination during crisping in a retail setting (e.g., supermarkets) was investigated. The ability of the sanitizers to reduce foodborne pathogens (*E. coli* O157:H7, *S. enterica*, and *L. monocytogenes*) on whole heads of lettuce was also investigated.

2. Materials and Methods

2.1. Bacterial strains

Nalidixic acid-resistant *S. enterica*, *E. coli* O157:H7, and *L. monocytogenes* which were associated with fresh produce outbreaks were provided by Dr. Joshua Gurtler (Eastern Region Research Center, USDA, Wyndmoor, PA): *S. enterica* subsp. *enterica* serovar Newport H1275 (sprout outbreak), *S. enterica* subsp. *enterica* serovar Stanley H0558 (sprout outbreak), *S. enterica* subsp. *enterica* serovar Montevideo G4639 (raw tomato outbreak), *L. monocytogenes* L008 (serotype 4b, Canadian coleslaw/cabbage outbreak), L2624 (serotype 1/2b, cantaloupe

outbreak), and L2625 (serotype 1/2a, cantaloupe outbreak). *S. enterica* and *E. coli* O157:H7 were maintained in tryptic soy broth (TSB; Difco, BD, Sparks, MD) containing 20% glycerol and *L. monocytogenes* in brain heart infusion (BHI; Difco, BD) broth containing 20% glycerol. Samples were held at -80°C. A single colony on tryptic soy agar (TSA; Difco, BD) plates supplemented with nalidixic acid (100 µg/mL, Alfa Aesar, England) was transferred to 30 mL of TSB (*S. enterica* and *E. coli* O157:H7) or BHI (*L. monocytogenes*) broth. All media contained nalidixic acid (100 µg/mL). Inoculum cultures were incubated at 37°C for 20 h with shaking.

2.2. Lettuce sample preparation and inoculation

Romaine lettuce (488 ± 41 g/head) and Red leaf lettuce (276 ± 38 g/head) heads were purchased from a local farmers' market and stored at 4°C prior to use. All commodities were grown in California and were not subjected to any treatments at the farmers' market. The outermost leaves and a thin slice of the butt-end of the lettuce heads were removed prior to processing.

Overnight cultures were centrifuged at 4,500 rpm for 15 minutes (Allegra™ 21R; Beckman Coulter, Palo Alto, CA), supernatant decanted, and the cell pellet washed with 0.1% of peptone water (Difco, BD). Washed cultures were then mixed in 30 mL of sterile tap-water (≈ 9 log CFU/ml). Heads of lettuce were placed butt-end down in a biosafety cabinet, and one pair of three combinations of inoculums (*S. enterica* and *E. coli* O157:H7, *E. coli* O157:H7 and *L. monocytogenes*, or *S. enterica* and *L. monocytogenes*) were separately spot-inoculated (5 µL) at 20 to 30 different locations on each head of lettuce to achieve approximately 5 log CFU/g of lettuce. After spot-inoculation, heads of lettuce were dried for 60 minutes.

Preparation of crisping solutions and submersion of lettuce

Prior to each experiment, two sets of two-compartment commercial stainless steel sinks (inside size of each sink: 60 x 60 x 35 cm³) were sanitized with a commercial sanitizer (Steramine quaternary sanitizer, Edwards-Councilor, Virginia Beach, VA). Moistened sterile cotton swab tips were used to streak two areas (100 cm² each) on the inner surface of the sink. The swabs were placed into TSB and incubated at 37°C for 18h. Aliquots were then processed for detection of *S. enterica* and *E. coli* O157:H7, and *L. monocytogenes*. Each sink was then filled with 76 L of tap-water alone (TW), commercial electrolyzed water (EW; ProduceFresh Concentration, Sterilox™, Radnor, PA), lactic acid/phosphoric acid-based sanitizer (LPA; Suma® Eden Antimicrobial Fruit & Vegetable Wash, Diversey, Charlotte, NC), or a citric acid-based sanitizer (CA; Liquid fruit & vegetable wash, Chemstar, Lithia Springs, GA). The temperature and pH values of tap water were 17.3 ± 3.6°C and 7.30 ± 0.26, respectively. Concentrated EW was automatically diluted with tap water by a manufacturer's automated dispenser (free chlorine: 43 ± 6 ppm, pH: 6.83 ± 0.11). LPA and CA were diluted in 76 L of tap water based on manufacturer's instructions; LPA and CA had pH values of 2.89 ± 0.05 and 2.78 ± 0.07, respectively. Free chlorine (Palintest 1000 Chlorometer, Palintest Ltd., England), pH (Accumet AB15, Fisher Scientific, Pittsburgh, PA), and temperature (Traceable™, Fisher Scientific, Pittsburgh, PA) were measured before and after immersion (soaking) of lettuce. A final rinse of lettuce following treatment was not required.

Three consecutive soaking processes were performed as follows. For the first batch, one head of inoculated lettuce and seven heads of non-inoculated lettuce were soaked together in 76 L of TW, EW, LPA, or CA. After five minutes of soaking, the seven heads of non-inoculated lettuce were removed from each treatment sink and placed in a perforated crisping tray. The inoculated lettuce was handled separately. The inoculated and non-inoculated heads of lettuce

were subjected to microbiological analysis. For the second and third batch, eight heads of non-inoculated lettuce per batch were soaked for five minutes in the same TW, EW, LPA, or CA crisping solutions that had been used to soak the first batch.

2.3. Microbiological analysis

Before the inoculation of lettuce, two heads of lettuce were chopped into 3 cm² pieces and two 25 g samples randomly picked from each head of lettuce. The chopped lettuce (25 g) was homogenized with 100 mL of 0.1% of peptone water for 1 minute (easyMIXTM, BioMerieux, France). An aliquot of the homogenized sample was serial diluted (1:10) with 0.1% peptone and then a 100 µL aliquot plated onto TSA for enumeration of natural flora on the lettuce.

Following inoculation the populations of *S. enterica*, *E. coli* O157:H7, and *L. monocytogenes* on lettuce were determined by plating an aliquot on XLT₄ agar (Difco, BD) supplemented with 100 µg/mL of nalidixic acid (XLT₄-N) for *S. enterica*, Sorbitol MacConkey agar (Difco, BD) supplemented with 100 µg/mL of nalidixic acid (SMAC-N) for *E. coli* O157:H7, or Palcam base agar (Difco, BD) combined with Palcam supplement (Difco, BD) and 100 µg/mL of nalidixic acid (Palcam-N) for *L. monocytogenes*. For each experiment lettuce was screened for prior contamination with nalidixic acid-resistant *S. enterica*, *E. coli* O157:H7, and *L. monocytogenes* by plating prepared lettuce samples on selective media supplemented with nalidixic acid (100 µg/mL). This was done to ensure results were attributed to only challenge strains used in the study.

Following soaking each inoculated head of lettuce was chopped with a sterile knife. Two 25 g samples of chopped inoculated lettuce were prepared. A 25 g lettuce sample was added to 100 mL of Dey/Engley neutralizing broth (D/E broth; Hardy Diagnostics, Santa Maria, CA) in a sterile plastic bag and homogenized for one minute. Samples were processed to determine

presence of *S. enterica* and *E. coli* O157:H7, or *L. monocytogenes* by plating 100 µL aliquots on XLT₄-N, SMAC-N, or Palcam-N, respectively. The change in numbers of each pathogen on lettuce following soaking in different crisping solutions was compared statistically (ANOVA-Duncan's post-hoc analysis, SAS Institute Inc., Cary, NC).

The seven non-inoculated heads in the first batch were grouped into three and four heads and then chopped on a sterile tray using a sterile knife. Two 25 g samples from each group were collected; therefore, a total four samples were analyzed. For the second and third batches, the eight heads of non-inoculated lettuce per batch were separated into two groups (four heads each), and two samples from each group of chopped lettuce were collected. Depending on the sanitizing agents, single (electrolyzed water) or double (acid based-sanitizers) strength D/E broth was dispensed into sterile plastic bags containing the chopped lettuce samples (Park and Chen, 2011). Dilutions (1:10) of the homogenized sample were made in 0.1% of peptone water and 100 µL aliquots plated on XLT₄-N, SMAC-N, or Palcam-N. All non-inoculated samples were also subjected to enrichment and then processed to determine presence of inoculated foodborne pathogens by cross-contamination. A detection limit of the direct plating method in this study was 2.09 CFU/g of lettuce.

2.4. Microbiological quality of processing waters

Samples (100 mL each) were collected from each batch of crisping water before and after every soaking process. The samples were passed through filtering apparatus with 0.2 µm size filters (Analytical Filter Units, Nalgene™, Rochester, NY) followed by rinsing with 100 mL of sterile distilled water to aid in removal of residual sanitizing agents. The filter was aseptically placed onto D/E agar (Difco, BD) plates for determination of total bacterial population.

Additionally, samples were processed to place filters on selective media for determining presence of inoculated foodborne pathogens. All plates were incubated at 37°C for 18 to 36 h.

3. Results and Discussion

3.1. Natural flora on whole heads of lettuce

Population of natural flora on whole heads of lettuce prior to treatment is shown in Figure 1. Heads of Romaine lettuce consistently harbored significantly higher ($P<0.001$) populations (4.9 ± 0.8 log CFU/g) of natural microbiota than Red leaf lettuce (4.3 ± 0.3 log CFU/g). The population of natural microbiota associated with Romaine and Red leaf lettuce in the present study is consistent with previous reports. Allende et al. (2004) reported the population of mesophilic bacteria, lactic acid bacteria, and psychotropic bacteria on non-processed Red leaf lettuce, indicating populations of 4.15 ± 0.22 log CFU/g, 2.75 ± 0.28 log CFU/g, and 3.90 ± 0.23 log CFU/g, respectively. Whole Romaine lettuce collected from a supermarket in Spain carried 5.4 to 6.6 log CFU/g of aerobic mesophilic bacteria (Abadias et al., 2008). Maffei et al. (2013) purchased Romaine and Red leaf lettuce from a farmer's market in Brazil and found no differences between the population of mesophilic aerobic bacteria associated with Romaine (6.50 ± 0.73 log CFU/g) and Red leaf (6.66 ± 0.75 log CFU/g) lettuce. It is known that vegetable microbiota and its population depends on various factors, including environmental (soil, fertilizer, and irrigation) and harvest practices, as well as leaf shape (Maffei et al., 2013).

3.2. Effect of soaking on population of foodborne pathogens

Soaking of inoculated Romaine and Red leaf lettuce for five minutes as a step in the crisping process consistently reduced the population of *S. enterica*, *E. coli* O157:H7 and *L. monocytogenes* (Table 1 and 2). The populations of *S. enterica*, *E. coli* O157:H7 and *L.*

monocytogenes associated with Romaine and Red leaf lettuce were 5.1 ± 0.3 , 5.3 ± 0.2 , and 5.4 ± 0.2 and 5.3 ± 0.2 , 4.9 ± 0.2 , and 5.1 ± 0.2 log CFU/g, respectively, prior to treatment.

Soaking Red leaf lettuce in EW significantly reduced the population of *S. enterica* (2.8 ± 1.5 log CFU/g), *E. coli* O157:H7 (3.4 ± 1.1 log CFU/g), and *L. monocytogenes* (2.6 ± 0.7 log CFU/g) compared to TW, LPA and CA ($P < 0.05$) (Table 1). Similarly, soaking of Romaine lettuce in EW resulted in reduction of 3.0 ± 1.2 log CFU *S. enterica*/g and 3.7 ± 1.5 log CFU *E. coli* O15:H7/g (Table 2). The level of reduction is significantly greater ($P < 0.05$) compared to soaking in TW, LPA, and CA. There was no significant difference ($p > 0.05$) in reduction of *L. monocytogenes* (3.4 ± 1.3 log CFU/g) between the treatments (Table 2).

No significant differences ($P > 0.05$) were noted in the control of foodborne pathogen on Romaine and Red leaf lettuce treated with EW (Tables 1 and 2). TW was equally effective in reducing populations of *E. coli* O157:H7 and *L. monocytogenes*, but proved to be less effective in reducing *S. enterica* on Red leaf lettuce ($P < 0.05$). TW was also more effective in reducing *E. coli* O157:H7 than *L. monocytogenes* ($P < 0.05$) on Romaine lettuce. LPA was more effective in reducing *E. coli* O157:H7 than *S. enterica* and *L. monocytogenes* on Romaine ($P < 0.05$). CA more effectively reduced *L. monocytogenes* than *S. enterica* on Red leaf lettuce ($P < 0.05$), but no differences were observed in pathogen reductions on Romaine lettuce ($P > 0.05$).

The evaluation of methods to reduce microbiological populations on fresh-cut lettuce has been extensively reported (Beuchat et al., 2004; Ölmez, 2010; Gil et al, 2009; Hoelzer et al., 2014). Including chlorine (20-200 free chlorine) in wash water generally reduced foodborne pathogens on fresh produce by 1-3 log CFU/g, but this method could not completely eliminate presence of foodborne pathogens (Aruscavage et al., 2006). Neutral EW (50 ppm, pH 8.6) reduced the population of *E. coli* O157:H7, *S. enterica*, *L. innocua* approximately 1–2 log CFU/g

on fresh-cut lettuce (Abadias et al., 2008). López-Gálvez et al. (2009) evaluated the efficacy of commercial sanitizers for washing fresh-cut lettuce and observed that 500 mg/L of Tsunami (15% peroxyacetic acid) and 40 mg/L of chlorine reduced the population of *E. coli* by 4 and 5 logs while Citrox (phenolic compounds and organic acids) and Purac (80% lactic acid) proved ineffective. These results are in-line with the present study in which LPA and CA were no better than water in reducing foodborne pathogens on lettuce. Other researchers showed no significant effect of commercial sanitizers including Tsunami 100 (50 ppm peroxyacetic acid) and chlorine with T-128 (chlorine stabilizer, SmartWash Solution) in reducing the population of *E. coli* O157:H7 on shredded iceberg lettuce compared to water alone (Davidson et al., 2013). The reduction of foodborne pathogens on fresh-cut/shredded lettuce differs from that of whole head lettuce since there are differences in processing of these commodities (Nou and Luo, 2010). There is a paucity of literature on the efficacy of commercial sanitizers used for washing whole leafy greens (e.g., heads of lettuce, bunches of spinach and cilantro) at retail outlets. Typical retail practices involve prolonged soaking (5 to 15 minutes) of a commodity rather than a relatively short (1 to 2 minutes) exposure associated with processing of baby and chopped leafy greens.

3.3. Cross-contamination during soaking

Many retailers soak leafy greens and other commodities often as a step in the crisping process of these products. The practice re-hydrates the commodities and improves appearance. Generally, several heads (e.g., lettuce) or bunches (e.g., cilantro) are soaked together in a large sink, removed, and the process repeated two or more times without changing the water. Lack of added sanitizer to the water may permit microbial populations in the water to increase dramatically and result in cross-contamination. Moreover, during the practice, a commodity may

increase 10 to 15% in weight as the result of absorption of water through a cut end (e.g., butt-end of a lettuce head). If pathogens are present in the water they may infiltrate the tissue of the commodity.

The contamination of heads of lettuce as the result of soaking with a contaminated head of lettuce or soaking in previously used water in which a contaminated head was processed is shown in Figure 2. The levels of *S. enterica*, *E. coli* O157:H7 or *L. monocytogenes* associated with lettuce samples were below the level of detection so enrichment results are reported. The use of EW prevented cross-contamination from occurring during processing of Romaine and Red leaf lettuce regardless of number of times the solution was re-used (Fig. 2A and 2B). TW, LPA, and CA failed to mitigate cross-contamination even during the initial soaking. Interestingly, *E. coli* O157:H7 was detected on non-inoculated Romaine and Red leaf lettuce soaked during the third use of TW. Soaking/washing of Romaine and Red leaf lettuce in TW was shown to reduce the population of *E. coli* O157:H7 on each commodity (Table 1 and 2). These results underscore the benefit of using a sanitizing agent in water intended for the soaking/submersion of a commodity.

Several studies have investigated the cross-contamination of lettuce during a washing process (Wachtel and Charkowski, 2002; Zhang et al., 2009; Holvoet et al., 2014). Unfortunately, the majority of the research utilized fresh-cut lettuce which is processed differently and has different characteristics from head lettuce. Jensen et al. (2015) simulated the cross-contamination of lettuce with *E. coli* O157:H7 during washing in a home-scale setting (single leaves of lettuce in a bowl). The researchers concluded that plain tap water will not prevent cross-contamination. López-Gálvez et al. (2009) washed 100 g of inoculated fresh-cut lettuce with 900 g of non-inoculated lettuce for one minute in commercial sanitizers. They reported that 500 mg/L of

Tsunami and 40 mg/L of chlorine prevented cross-contamination of lettuce with *E. coli* O157:H7, but Citrox and Purac failed to control cross-contamination. In the present study under conditions evaluated, the levels of a given pathogen that were found on a head of lettuce the result of cross-contamination were likely extremely low since enrichment was required for detection. However, considering the standard serving size of lettuce (approximately 100 g), the potential exists that a pathogen may be ingested. If the lettuce was improperly handled (e.g. exposure to temperature abuse) in the home by the consumer, then the population of the pathogen may increase thereby increasing the human health risk.

3.4. Accumulation of microorganisms in processing water

The microbiological quality of processing water was evaluated by conducting microbiological analysis of water samples collected prior to and after each use of the water. Table 3 shows the microbiological population of the processing water after the first, second, and third uses for soaking whole heads of lettuce. The aerobic plate counts for TW, LPA, AS were all in the TNTC range. This finding suggests that use and reuse of tap water can result in high populations of microorganisms in processing water that can essential inoculate product exposed to the water. Therefore, it is prudent that wash water used without addition of sanitizer(s) be changed regularly. EW effectively controlled the microbiological population of soak water even after the third use. The level of free chlorine in EW decreased less than 5 ppm prior to use to after the third use. Detection of *E. coli* O157:H7, *L. monocytogenes*, and *S. enterica* in water samples was attempted and yielded variable results (data not shown). Placing filters on selective media failed to inhibit growth of all commensal bacteria and made it difficult to detect colonies of a given pathogen. Monitoring the water for target values for the sanitizer, such as pH or free chlorine, should be emphasized to ensure optimal efficacy.

4. Conclusion

Very little attention has been paid to the microbial safety practices involving the submersion (washing, crisping, etc.) of produce in retail settings. This study simulated the soaking of lettuce in retail settings in association with the practice of crisping. Under conditions evaluated, a commercial concentrated electrolyzed water antimicrobial was most effective in controlling microbial populations in wash water, preventing cross-contamination, and reducing foodborne pathogens on whole lettuce. The study highlights the benefits of using a sanitizer in water in which product will be immersed for a prolonged period (5 minutes). The use of effective chemical antimicrobial agents must be considered in order to prevent the cross-contamination of product with foodborne pathogens during crisping in retail establishments.

Acknowledgments

The authors acknowledge USDA HATCH project NJ10170 and PuriCore, Inc.

Reference

- Abadias, M., Usall, J., Anguera, M., Solsona, C., Viñas, I., 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *International Journal of Food Microbiology*, 123, 121-129.
- Ackers, M.L., Mahon, B.E., Leahy, E., Goode, B., Damrow, T., Hayes, P.S., Bibb, W.F., Rice, D.H., Barrett, T.J., Hutwagner, L. and Griffin, P.M., 1998. An outbreak of *Escherichia coli* O157: H7 infections associated with leaf lettuce consumption. *Journal of infectious diseases*, 177, 1588-1593.

Allende, A., Aguayo, E., Artés, F., 2004. Microbial and sensory quality of commercial fresh processed red lettuce throughout the production chain and shelf life. *International Journal of Food Microbiology*, 91, 109-117.

Artés, F., Gómez, P., Aguayo, E., Escalona, V., Artés-Hernández, F., 2009. Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities. *Postharvest Biology and Technology*, 51, 287–296.

Aruscavage, D., Lee, K., Miller, S., LeJeune, J.T., 2006. Interactions affecting the proliferation and control of human pathogens on edible plants. *Journal of food science*, 71, R89-R99.

Ayebah, B., Hung, Y.C., 2005. Electrolyzed water and its corrosiveness on various surface materials commonly found in food processing facilities. *Journal of Food Process Engineering*, 28, 247–264.

Barrera, M.J., Blenkinsop, R., Warriner, K., 2012. The effect of different processing parameters on the efficacy of commercial post-harvest washing of minimally processed spinach and shredded lettuce. *Food Control*, 25(2), 745-751.

Beuchat, L.R., Adler, B.B., Lang, M.M., 2004. Efficacy of chlorine and a peroxyacetic acid sanitizer in killing *Listeria monocytogenes* on iceberg and romaine lettuce using simulated commercial processing conditions. *Journal of Food Protection*, 67, 1238-1242.

366 California Department of Public Health, 2012. Retail fruit and vegetable marketing guide.
367 [http://www.cdph.ca.gov/programs/cpns/Documents/Retail%20FV%20Marketing%20Guide_5_0](http://www.cdph.ca.gov/programs/cpns/Documents/Retail%20FV%20Marketing%20Guide_5_060811_FINAL.pdf)
368 [60811_FINAL.pdf](http://www.cdph.ca.gov/programs/cpns/Documents/Retail%20FV%20Marketing%20Guide_5_060811_FINAL.pdf). (accessed 16.04.03).
369
370 Castillo, A., Rodríguez-García, M.O., 2004. Bacterial hazards in fresh and fresh-cut produce:
371 Sources and control. In R. C. Beier, S. D. Pillai, & T. D. Phillips (Eds.), Preharvest and
372 postharvest food safety, Blackwell Publishing, Iowa, pp. 43–57.
373
374 Center for Science in the Public Interest (CSPI), 2009. The ten riskiest foods regulated by the
375 U.S. Food and Drug Administration. https://cspinet.org/new/pdf/cspi_top_10_fda.pdf. (accessed
376 16.04.03).
377
378 Center for Science in the Public Interest (CSPI), 2015. Outbreak Alert! 2015: a review of
379 foodborne illness in the U.S. from 2004–2013.
380 https://www.cspinet.org/foodsafety/outbreak_report.html. (accessed 16.04.03).
381
382 Cook, R., 2012. Trends in the marketing of fresh produce and fresh-cut/value-added produce. UC
383 Davis Fresh-Cut Products Short Course.
384 [http://arefiles.ucdavis.edu/uploads/filer_public/2014/10/08/freshcut20140926finalnewmarycook.](http://arefiles.ucdavis.edu/uploads/filer_public/2014/10/08/freshcut20140926finalnewmarycook.pdf)
385 [pdf](http://arefiles.ucdavis.edu/uploads/filer_public/2014/10/08/freshcut20140926finalnewmarycook.pdf). (accessed 16.04.03).
386

387 Jensen, D.A., Friedrich, L.M., Harris, L.J., Danyluk, M.D. Schaffner, D.W., 2015. Cross
388 contamination of *Escherichia coli* O157: H7 between lettuce and wash water during home-scale
389 washing. Food microbiology, 46, 428-433.
390

391 Davidson, G.R., Buchholz, A.L., Ryser, E.T., 2013. Efficacy of commercial produce sanitizers
392 against nontoxigenic *Escherichia coli* O157: H7 during processing of iceberg lettuce in a pilot-
393 scale leafy green processing line. Journal of Food Protection, 76, 1838-1845.
394

395 Defense Commissary Agency, 2012. DeCAM 40-4.1. Manual: Produce Department Operations.
396 https://www.commissaries.com/inside_deca/publications/directives/DeCAM40_4_1.pdf.
397 (accessed 16.04.03).
398

399 FAO/WHO, 2008. Microbiological hazards in fresh fruits and vegetables. Microbiological risk
400 assessment series, Meeting report.
401 http://www.who.int/foodsafety/publications/micro/MRA_FruitVeges.pdf. (accessed 16.04.03).
402

403 FDA, 2009. Draft guidance for industry: Guide to minimize microbial food safety hazards of
404 leafy greens.
405 <http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ProducePlantProducts/ucm174200.htm>. (accessed 16.04.03).
406

407 FDA, 2013. Food Code.
408 <http://www.fda.gov/downloads/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/UCM374510.pdf>. (accessed 16.04.03).
409

410

411 Gil, M.I., Selma, M.V., López-Gálvez, F., Allende, A., 2009. Fresh-cut product sanitation and
412 wash water disinfection: Problems and solutions. *International Journal of Food Microbiology*,
413 134, 37–45.

414

415 Graça, A., Abadias, M., Salazar, M., Nunes, C., 2011. The use of electrolyzed water as a
416 disinfectant for minimally processed apples. *Postharvest Biology and Technology*, 61, 172–177.

417 Heaton, J.C., Jones, K., 2008. Microbial contamination of fruit and vegetables and the behaviour
418 of enteropathogens in the phyllosphere: A review. *Journal of Applied Microbiology*, 104, 613–
419 626.

420

421 Herdt, J., Feng, H., 2009. Aqueous antimicrobial treatments to improve fresh and fresh-cut
422 produce safety. In X. Fan, B. A. Niemira, C. J. Doona, F. E. Feeherry, & R. B. Gravani (Eds.),
423 *Microbial safety of fresh produce*. Wiley-Blackwell, Oxford, pp. 169–190.

424

425 Herman, K.M., Hall, A.J., Gould, L.H., 2015. Outbreaks attributed to fresh leafy vegetables,
426 United States, 1973–2012. *Epidemiology and Infection*, 143, 3011–3021.

427

428 Hoelzer, K., Pouillot, R., Van Doren, J.M., Dennis, S., 2014. Reduction of *Listeria*
429 *monocytogenes* contamination on produce—A quantitative analysis of common liquid fresh
430 produce wash compounds. *Food Control*, 46, 430–440.

431

Holvoet, K., Sampers, I., Seynnaeve, M. Uyttendaele, M., 2014. Relationships among hygiene indicators and enteric pathogens in irrigation water, soil and lettuce and the impact of climatic conditions on contamination in the lettuce primary production. *International journal of food microbiology*, 171, 21-31.

Hsu, S.Y., 2005. Effects of flow rate, temperature and salt concentration on chemical and physical properties of electrolyzed oxidizing water. *Journal of Food Engineering*, 66, 171–176.

Huang, Y.R., Hung, Y.C., Hsu, S.Y., Huang, Y.W., Hwang, D.F., 2008. Application of electrolyzed water in the food industry. *Food Control*, 19, 329–345.

Kim, C., Hung, Y.C., & Brackett, R.E., 2000. Roles of oxidation-reduction potential in electrolyzed oxidizing and chemically modified water for the inactivation of food-related pathogens. *Journal of Food Protection*, 63, 19–24.

Keskinen, L.A., Burke, A., & Annous, B.A., 2009. Efficacy of chlorine, acidic electrolyzed water and aqueous chlorine dioxide solutions to decontaminate *Escherichia coli* O157: H7 from lettuce leaves. *International Journal of Food Microbiology*, 132, 134-140.

Koseki, S., Yoshida, K., Kamitani, Y., Isobe, S., Itoh, K., 2004. Effect of mild heat pre-treatment with alkaline electrolyzed water on the efficacy of acidic electrolyzed water against *Escherichia coli* O157:H7 and *S. enterica* on lettuce. *Food Microbiology*, 21, 559–566.

455 Len, S.V., Hung, Y.C., Chung, D., Anderson, J.L., Erickson, M.C., Morita, K., 2002. Effects of
456 storage conditions and pH on chlorine loss in electrolyzed oxidizing (EO) water. Journal of
457 Agricultural and Food Chemistry, 50, 209–212.

458
459 López-Gálvez, F., Allende, A., Selma, M.V., Gil, M.I., 2009. Prevention of *Escherichia coli*
460 cross-contamination by different commercial sanitizers during washing of fresh-cut lettuce.
461 International Journal of Food Microbiology, 133, 167-171.

462
463 Luo, Y.G., Nou, X.W., Millner, P., Zhou, B., Shen, C.L., Yang, Y., Wu, Y.P., Wang, Q., Feng,
464 H., Shelton, D., 2012. A pilot plant scale evaluation of a new process aid for enhancing chlorine
465 efficacy against pathogen survival and cross-contamination during produce wash. International
466 Journal of Food Microbiology, 158, 133–139.

467
468 Maffei, D.F., de Arruda Silveira, N.F., Catanozi, M.D.P.L.M., 2013. Microbiological quality of
469 organic and conventional vegetables sold in Brazil. Food Control, 29, 226-230.

470
471 Nou, X., Luo, Y., 2010. Whole-leaf wash improves chlorine efficacy for microbial reduction and
472 prevents pathogen cross-contamination during fresh-cut lettuce processing. Journal of food
473 science, 75, M283-M290.

474
475 Ölmez, H., 2010. Effect of different sanitizing methods and incubation time and temperature on
476 inactivation of *Escherichia coli* on lettuce. Journal of food safety, 30, 288-299.

Palma-Salgado, S., Pearlstein, A.J., Luo, Y., Park, H.K. and Feng, H., 2014. Whole-head washing, prior to cutting, provides sanitization advantages for fresh-cut Iceberg lettuce (*Lactuca sativa* L.). *International journal of food microbiology*, 179, 18-23.

Pangloli, P., Hung, Y.C., Beuchat, L.R., King, C.H., Zha, Z.H, 2009. Reduction of *Escherichia coli* O157:H7 on produce by use of electrolyzed water under simulated food service operation conditions. *Journal of Food Protection*, 72, 1854–1861.

Parish, M.E., Beuchat, L.R., Suslow, T.V., Harris, L.J., Garrett, E.H., Faber, J.N., Busta, F.F., 2003. Methods to reduce/eliminate pathogens from fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety*, 2, 161–173.

Park, C.M., Hung, Y.C., Doyle, M.P., Ezeike, G.O. I., Kim, C., 2001. Pathogen reduction and quality of lettuce treated with electrolyzed oxidizing and acidified chlorinated water. *Journal of Food Science*, 66, 1368–1372.

Park, E.J., Alexander, E., Taylor, G.A., Costa, R., Kang, D.H., 2008. Effect of electrolyzed water for reduction of foodborne pathogens on lettuce and spinach. *Journal of Food Science*, 73, M268–M272.

Park, Y.J., Chen, J., 2011. Mitigating the antimicrobial activities of selected organic acids and commercial sanitizers with various neutralizing agents. *Journal of Food Protection*, 74, 820-825.

Sapers, G.M., 2001. Efficacy of washing and sanitizing methods for disinfection of fresh fruit and vegetable products. *Food Technology and Biotechnology*, 39, 305–311.

Sapers, G.M., 2014. Disinfection of contaminated produce with conventional washing and sanitizing technology. In K. R. Matthews, G.M. Sapers, & C. P. Gerba (Eds.), *The produce contamination problem: Causes and solutions*. Elsevier, San Diego, pp. 389–431.

Wachtel, M.R., Charkowski, A.O., 2002. Cross-contamination of lettuce with *Escherichia coli* O157: H7. *Journal of Food Protection*, 65, 465-470.

Waters, B.W., Tatum, J.M., Hung, Y.C., 2014. Effect of chlorine-based sanitizers properties on corrosion of metals commonly found in food processing environment. *Journal of Food Engineering*, 121, 159–165.

Zhang, G., Ma, L., Phelan, V.H., Doyle, M.P., 2009. Efficacy of antimicrobial agents in lettuce leaf processing water for control of *Escherichia coli* O157: H7. *Journal of Food Protection*, 72, 1392-1397.

Figure legends:

Figure 1. Natural flora of whole heads of Romaine and Red leaf lettuce. Asterisk indicates a significantly different mean value between Romaine and Red leaf lettuce (PROC TTEST, $p < 0.001$).

Figure 2. Cross-contamination of non-inoculated Romaine (A) and Red leaf (B) lettuce associated three consecutive uses of soaking water. Colonies were not detected following direct plating of samples, so all samples were subjected to enrichment and processed for presence of *S. enterica*, *E. coli* O157:H7 and *L. monocytogenes*. Positive samples from all experiments are reported.

Table 1. Log reduction of *S. enterica*, *E. coli* O157:H7 and *L. monocytogenes* on Red leaf lettuce following a five-minute soak.

Treatment ¹	<i>S. enterica</i> ²	<i>E. coli</i> O157:H7	<i>L. monocytogenes</i>
TW	1.1 ± 0.6 _B ^b	1.8 ± 0.5 _B ^a	2.3 ± 0.7 _B ^a
EW	2.8 ± 1.5 _A ^a	3.4 ± 1.1 _A ^a	2.6 ± 0.7 _A ^a
LPA	1.2 ± 0.7 _B ^a	1.4 ± 0.6 _B ^a	1.8 ± 0.6 _B ^a
CA	1.6 ± 0.4 _B ^b	1.9 ± 0.1 _B ^{ab}	2.2 ± 0.1 _B ^a

¹Tap water alone (TW), electrolyzed water (EW), citric acid-based sanitizer (CA), and lactic acid and phosphoric acid-based sanitizer (LPA).

²Population on inoculated lettuce of *S. enterica*, *E. coli* O157:H7, and *L. monocytogenes* were 5.3 ± 0.2, 4.9 ± 0.2, and 5.1 ± 0.2 log CFU/g, respectively, prior to treatment.

^AAverages compared between soaking treatments on each foodborne pathogen with the same capital letter are not significantly different ($P>0.05$).

^aAverages followed by the same lower case letter indicate no significant difference between foodborne pathogens on the same soaking treatment ($P>0.05$).

Table 2. Log reduction of *S. enterica*, *E. coli* O157:H7 and *L. monocytogenes* on Romaine lettuce following a five-minute soak.

Treatment ¹	<i>S. enterica</i> ²	<i>E. coli</i> O157:H7	<i>L. monocytogenes</i>
TW	1.8 ± 0.3 _B ^{ab}	2.2 ± 0.6 _B ^a	1.5 ± 0.5 _A ^b
EW	3.0 ± 1.2 _A ^a	3.7 ± 1.5 _A ^a	3.4 ± 1.3 _A ^a
LPA	1.2 ± 0.1 _B ^b	1.7 ± 0.3 _B ^a	0.9 ± 0.1 _A ^c
CA	1.8 ± 0.2 _B ^a	1.9 ± 0.2 _B ^a	1.9 ± 0.3 _A ^a

¹Tap water alone (TW), electrolyzed water (EW), citric acid-based sanitizer (CA), and lactic acid and phosphoric acid-based sanitizer (LPA).

²Population on inoculated lettuce of *S. enterica*, *E. coli* O157:H7, and *L. monocytogenes* were 5.1 ± 0.3, 5.3 ± 0.2, and 5.4 ± 0.2 log CFU/g, respectively, prior to treatment.

^AAverages compared between soaking treatments on each foodborne pathogen with the same capital letter are not significantly different ($P>0.05$).

^aAverages followed by the same lower case letter indicate no significant difference between foodborne pathogens on the same soaking treatment ($P>0.05$).

Table 3. Microbiological quality of soaking water.

		Soaking Event ^B	Treatment ^A			
			TW	EW	LPA	CA
Romaine Lettuce	Aerobic Filter Counts ^C	1 st	>250	0	>250	>250
		2 nd	>250	0.1 ± 0.3 ^D	>250	>250
		3 rd	>250	0.1 ± 0.3	>250	>250
Red leaf lettuce	Aerobic filter counts	1 st	>250	0.1 ± 0.3	>250	>250
		2 nd	>250	0.5 ± 0.8	>250	>250
		3 rd	>250	0.5 ± 0.8	>250	>250

^ATap water alone (TW), electrolyzed water (EW), citric acid-based sanitizer (CA), and lactic acid and phosphoric acid-based sanitizer (LPA).

^BThree consecutive soakings were processed without changing crisping water.

^CAerobic filter count: Total colony count associate with 100 mL soaking water.

^DValues are the mean colony count from samples for all experiments (n=12)

Figure 1.

620

621 Figure 2.

622

623

624