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A pilot plant scale evaluation of a new process aid for enhancing chlorine efficacy against pathogen survival and cross-contamination during produce wash

Yaguang Luo [a,*], Xiangwu Nou [a], Patricia Millner [a], Bin Zhou [a], Cangliang Shen [a], Yang Yang [a], Yunpeng Wu [b], Qin Wang [b], Hao Feng [c], Dan Shelton [a]

[a] Environmental Microbial and Food Safety Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD 20705, United States
[b] Department of Nutrition and Food Science, University of Maryland, College Park, MD 20740, United States
[c] Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL 61801, United States

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ABSTRACT

Developing food safety intervention technology that can be readily adopted by the industry often requires test conditions that match as closely as possible to those of commercial food processing operations; yet biosafety risks inherent in pathogen studies constrain most experiments to laboratory settings. In this study, we report the first semi-commercial pilot-scale evaluation of a new process aid, T128, for its impact on enhancing the antimicrobial efficacy of chlorinated wash water against pathogen survival and cross-contamination. A non-pathogenic, BSL-1, strain of *Escherichia coli* O157:H7 was inoculated onto freshly harvested baby spinach leaves and washed with large amounts of freshly cut un-inoculated iceberg lettuce shreds in wash water with free chlorine periodically replenished, in the presence or absence of T128. Changes in water quality and pathogen survival and cross-contamination were monitored at every 2 min intervals for up to 36 min for each treatment during the wash operation. Results indicated that the use of T128 did not significantly ($P>0.05$) influence the rate of wash water deterioration, nor the pathogen populations remaining on the inoculated spinach leaves. However, in the absence of T128 (control), survival of *E. coli* O157:H7 in wash water and cross-contamination of un-inoculated lettuce frequently occurred when free chlorine in solution dropped below 1 mg/l during the wash process. In contrast, the use of T128 significantly reduced the occurrence of *E. coli* O157:H7 surviving in wash water and of cross-contamination to un-inoculated shredded iceberg lettuce under the same operational conditions, suggesting that the application of T128 in a chlorine-based fresh produce sanitization system could increase the safety margin of process control on fresh-cut operations.

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1. Introduction

Fresh fruits and vegetables are nutrient-rich foods with high levels of minerals, vitamins, and phytochemicals. Sufficient consumption of fresh produce is a key element of a healthy diet and contributes to prevention of chronic diseases such as heart disease, cancer, diabetes and obesity (WHO, 2005). Nonetheless, recent outbreaks of food-borne illness associated with fresh produce have negatively impacted consumer confidence in the safety of fresh and fresh-cut produce (Arnade et al., 2009; Lynch et al., 2009). Thus, improving produce safety is an urgent, critical task for the produce industry and public health researchers.

In the absence of practical technologies that provide a "kill step" to eliminate pathogens without significantly diminishing produce quality, a sanitizing wash is critically important in fresh-cut produce processing, as it provides a crucial opportunity to focus on pathogen inactivation. However, washing also has the potential to cause pathogen cross-contamination, especially when water is re-circulated and re-used (Gil et al., 2009; Luo et al., 2011a). Processing water is an ideal medium for the potential spread of bacterial pathogens during fresh-cut processing. Therefore, the presence of a sanitizing agent such as sodium hypochlorite in the wash water is critical to preventing pathogen survival and transfer. Presently, chlorine is widely used by the produce industry because of its ability to reduce microbial content, its economical efficiency, and its minimal adverse impact on product quality. Extensive studies have shown that hypochlorous acid is the most efficacious form of chlorine for inactivation of pathogens. However, maintaining a stable level of hypochlorous acid in wash water during commercial fresh-cut wash operations is a technical challenge. Deterioration of wash water quality is the result of accumulation of soil, debris, and plant exudates in the washing system and is manifested by the increase in turbidity, chemical oxygen demand (COD), and decrease in the efficacy of sanitizers. When produce is introduced into the wash system, the organic matter surges and binds with the chlorine. This may create a situation in which chlorine demand may exceed the available concentration, thus resulting in a rapid depletion of free chlorine available for disinfection in the process wash water. Bacterial pathogens, if present due to pre-harvest contamination, will be able to survive and spread

* Corresponding author. Tel.: +1 301 504 6186; fax: +1 301 504 5107.
E-mail address: Yaguang.Luo@ars.usda.gov (Y. Luo).

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throughout the processing chain once the sanitizer efficacy drops below a critical level. Although periodic monitoring and replenishment of chlorine is a common practice on fresh produce processing lines, repeated addition of chlorine into high organic wash water generates noxious chlorine by-products and chlorine off-gassing (Connell, 1996; Suslow, 2001) into the processing environment.

Recently, produce industry scientists have developed a novel chemical mixture, T128, to stabilize hypochlorous acid in wash water containing high concentrations of organic matter (Lemons and Taylor Fresh Food, Inc., 2009). The wash is composed of chemicals with GRAS (generally recognized as safe) status, including propylene glycol and phosphoric acid. T128 is currently being used by several fresh-cut produce companies (Brennan, 2010). For commercial fresh-cut leafy green processing, T128 is added to chlorinated wash water using a pH-coupled automated dosing system, with acidity settings of pH 5.0–5.5. We have previously reported that T128 significantly attenuated the depletion of free chlorine in washing solutions containing up to 2% of soil, and, to a lesser extent, in washing solutions containing high concentration of lettuce extract (Nou et al., 2011). In comparison to traditional chlorinated wash water solutions, the application of 0.05–0.1% of T128 with chlorinated water significantly reduced the potential for cross contamination by *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium, without negatively impacting the market quality parameters of fresh-cut iceberg lettuce (Nou et al., 2011). However, all these previous studies were conducted under laboratory conditions with a single application of chlorine. Therefore, the objectives of this current study were to evaluate the dynamic changes of wash water quality and the survival and cross-contamination of *E. coli* O157:H7 during typical commercial fresh-cut lettuce wash operations with periodic replenishment of chlorine and adjustment of wash solution pH using either citric acid or T128. The main production parameters, including wash system and operations, material preparation, material input speed, water utility and exposure time, closely mimicked a typical commercial fresh-cut iceberg lettuce packaging production line and thus truly reflected the dynamics of wash water quality deterioration in commercial operations.

2. Materials and methods

2.1. Pilot plant wash system

This study was carried out in a commercial pilot plant operated by New Leaf Food Safety Solutions, LLC., a Taylor Fresh Foods Inc. subsidiary, in Salinas, California (USA). A commercial double wash system (Fig. 1) manufactured by FTNON USA, Inc. (Salinas, CA, USA) was used for the pilot-plant scale studies. This wash system consists of two separate water tanks, primary and secondary, each holding approximately 3200 l of water. A water chiller is also connected with the second tank. Air is pumped into the wash tanks to create turbulence to wash the product, and the wash tanks are equipped with rotating screens to facilitate produce submersion. The average dwell time for leaves in each wash tank is approximately 26 s. After the washing stage, the product exits the tank on a dewatering conveyor. The process water is constantly screened and re-circulated into stage specific reservoir tanks, allowing continuous debris (soil, plant material, fines, etc.) removal from the system. The two wash tanks are equipped with separate SmartWash™ analytic controllers that are used to automatically dispense T128 or other acids to maintain pH in the wash tanks to preset values. No constant water replacement is set for the washing system, but operational water loss in the primary wash tank is replenished from the secondary tank, which in turn is replenished from the water chiller. A commercial produce cutter (model H-A, Urschel Laboratory, Valparaiso, IN), with selectable slicing modes is linked to the primary tank through a loading conveyor belt.

Prior to the start of each test, the wash tanks were filled with chilled (5 °C) tap water, and pH was adjusted to 6.5 with citric acid (control), or 5.0 with T128, using the attached SmartWash™ analytic controller. Preliminary studies showed that the chlorine concentration of the secondary tank water was relatively stable. Therefore, studies were focused on the primary tank only.

2.2. Bacterial strain preparation

A spontaneous nalidixic acid resistant mutant of a BSL-1 non-pathogenic *E. coli* O157:H7 strain, ATCC 700728, was used throughout the experiments. This bacterial strain was previously prepared by repeated subculturing on a nutrient plate containing a gradient of nalidixic acid (Sigma, St. Luis, MO) from 0 to 100 mg/l. This mutant was used in this research after tests showed that its growth pattern was indistinguishable from that of the parental strain, and that resistance was stable following multiple subculturing in the absence of nalidixic acid. Cultures of *E. coli* O157:H7 were grown in tryptic soy broth (TSB) (Becton Dickenson, Sparks, MD) overnight at 37 °C. Cells were harvested by centrifugation and washed once in sterile phosphate buffered saline (PBS), followed by dilution of 2 ml of cell suspension in 1 l of sterile distilled water.

2.3. Sample inoculation

Baby spinach (*Spinacia oleracea* L.) leaves were harvested from Huron, CA, hydro-vacuum cooled to 5 °C, and shipped by refrigerated truck to the pilot plant. Spinach leaves were spread in a very thin

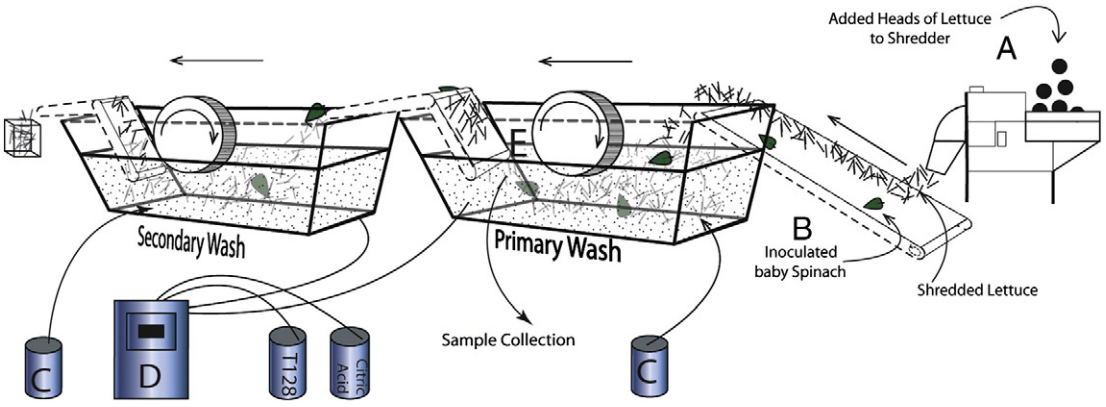

Fig. 1. Schematic diagram of the pilot plant wash system including produce and chemical inputs and flows. A: Un-inoculated pre-cored lettuce heads were shredded and directly added to the conveyer belt at a rate of approximately 45 kg/min. B: Previously inoculated spinach leaves were manually added to the conveyer belt, adjacent to but separate from the lettuce. C: Concentrated sodium hypochlorite was manually added to the primary wash tank. D: T128 or citric acid was added automatically to the wash tank via an automated controller. E: Water, lettuce and spinach samples were taken periodically from the upper water level in the primary wash tank.

layer on a 50 cm wide motorized conveyor belt, at an estimated rate of 2.5 kg/min, while inoculum was sprayed onto them, at an estimated rate of 500 ml/min using a pressurized garden sprayer with fine nozzle. Inoculated spinach leaves were collected in a large biohazard bag and stored at 5 °C for 48 h to allow the inoculum to attach to leaf surfaces before being used for the washing experiments. The targeted inoculation level was 2×10^5 CFU/g of spinach. Prior to washing, spinach leaves were randomized, and pre-weighed into 90-g portions so that inoculated spinach could be proportionally added along with lettuce shreds to the washing system.

2.4. Washing of lettuce and spinach mix

Pre-cored Iceberg lettuce (*Lactuca sativa* L.) heads harvested from Huron, CA, USA, were vacuum cooled to 5 °C within 4 h, then shipped in a refrigerated truck to the pilot plant facility. The lettuce was stored at 5 °C and used within 24 h of harvesting. Before the start of the test, the lettuce heads were pre-weighed to approximately 22.5 kg per tote. Lettuce was shredded using an Urschel slicer (Urschel Laboratories, Inc., Valparaiso, IN) to 0.6 cm-thick shreds at a preset rate and discharged onto the conveyor belt linked to the primary wash tank. Inoculated spinach leaves were manually spread onto the conveyer belt in parallel to the shredded lettuce, simulating sporadic contamination during washing.

Each test run consisted of three 12-min segments, simulating a continuous fresh-cut lettuce washing operation with periodic replenishment of sodium hypochlorite. Before the start of the first segment, 700 ml of concentrated (12.5%) sodium hypochlorite (BCS Chemicals, Redwood City, CA, USA) was added to the primary wash tank to achieve approximately 20 mg/l free chlorine in the wash water. The pH was simultaneously adjusted to 6.5 using citric acid (control) or to 5.0 using T128. Water quality and free chlorine concentration were monitored and the test run started within 5 min of chlorine solution addition. Shredded lettuce was immediately discharged onto the conveyer belt at a rate of two totes (45 kg) per min. Pre-weighed spinach leaves were manually spread onto the conveyer belt adjacent to but separated from the lettuce at a 0.2% spinach to lettuce ratio. The spinach leaves and lettuce shreds were submerged simultaneously and thereby were mixed upon entry into the wash solution in the primary tank. Water and lettuce samples were collected before starting the run and every 2 min during washing. Spinach leaves were collected before the run, and randomly during each segment of the test run. Separate strainer baskets were used to collect lettuce and spinach samples from the primary wash tank and care was taken to avoid collecting spinach leaves in the lettuce sample basket. After 12 min or approximately 540 kg of lettuce were washed, the operation was paused and a second dose of sodium hypochlorite (1050 ml) was added to the primary wash tank to compensate depleted free chlorine. The second segment of the wash process started as soon as the solution with added chlorine reached equilibrium. After another 12 min of processing and sample collection, a third dose of sodium hypochlorite (1400 ml) was similarly added and the third segment of produce was processed and sampled over another 12 min. The equipment and the pilot plant were thoroughly sanitized by a professional sanitation team between each test run and at the end of the day. The facility was also randomly swab tested for the inoculated *E. coli* O157:H7 to verify the effectiveness of the sanitation.

2.5. Evaluation of water quality

The free chlorine was measured immediately based on a DPD (N, N-Diethyl-p-Phenylenediamine) method using Chlorine Photometer (CP-15, HF Scientific Inc., Ft. Myers, FL). The pH and turbidity were measured on-site using a digital pH meter, and a turbidity meter (Aquafast, Thermo Orion, Beverly, MA), respectively. The chemical oxygen demand (COD) was determined using a reactor digestion method 10236 (HACH, 2002; Jirka and Carter, 1975; Luo, 2007).

2.6. Enumerating E. coli O157:H7 in wash water and produce

The water samples collected from the wash tank for microbial testing were immediately poured into filter (to remove debris) bags containing sodium thiosulfate to neutralize the residual chlorine (Kemp and Schneider, 2000). Duplicate 25-g samples of lettuce from each sampling point were weighed into sterile, filter bags (WhirlPak®, Nasco, Modesto, CA) and macerated with 125 ml of sterile TSB at 230 rpm for 2 min with a stomacher blender (Seward Stomacher 400, London, UK). The inoculated *E. coli* O157:H7 in the filtrate or water were enumerated using a modified MPN method previously described (Luo et al., 2011a; Nou and Luo, 2010). Briefly, eight aliquots (3 ml each) of filtrate were added to a 8×6 deep-well micro-plate (5.0 ml/well), followed by 10-fold serial dilutions with TSB. The micro-plates were covered with gas permeable sealing membrane (Diversified Biotech, Boston, MA) and incubated overnight. The turbidity of each well after incubation was recorded and 3 μl droplets of the cultures were arrayed on sorbitol MacConkey agar plates supplemented with 50 μg/l nalidixic acid and 2.5 mg/l potassium tellurite (ctSMAC; Invitrogen, Carlsbad, CA). Serological testing using a rapid RIM™ *E. coli* O157:H7 latex agglutination assay (Remel Inc., Lenexa, KS) was used for further confirmation of characteristic *E. coli* O157:H7 colonies. An MPN calculator (Curiale, 2004) was used to compute the MPN cell count for each sample. Detection limits were defined as the MPN value when a single undiluted well was determined positive. Spinach leaves were weighed into sterile, WhirlPak® filter bags and macerated with sterile PBS at 230 rpm for 2 min with the stomacher blender. The filtrate was spread plated onto ctSMAC supplemented with 50 μg/l nalidixic acid. The plates were incubated at 37 °C overnight and colonies were counted manually. The inoculated *E. coli* O157:H7 colonies were confirmed using the rapid RIM™ *E. coli* O157:H7 latex agglutination assay (Remel Inc., Lenexa, KS).

2.7. Experimental design and statistical analysis

The experiments were conducted using factorial designs, and all experiments were repeated three times. *E. coli* O157:H7 populations were subjected to log transformation before statistical analysis. Data were analyzed as a 2-factor linear model with treatment and sample number as the 2 factors. Because of the iterative nature of washing–sampling process, a repeated statement was included in the model using a compound symmetry (cs) covariance structure to relate the wash samples in each run. In each replication, sample numbers 6, 11, 12, 17 and 18 (which corresponded to the last sample in the first time series, and the last two samples in both the second and third time series) were the only wash water samples for which bacterial survival was found for both treatments; hence, the statistical analysis focused on these 5 samples. The cross-contamination data were analyzed similarly, but bacterial survival on lettuce occurred for both treatments in the last three samples in each time series, a total of nine out of 18 samples in each test run. The turbidity data was analyzed as a 2-factor model with chlorine addition (before vs. after) and time series (two levels) as the two factors. For all studies, assumptions of normality and variance homogeneity of the linear model were checked and the variance grouping technique was used to correct for variance heterogeneity. When effects were statistically significant, means comparisons were done with Sidak adjusted p-values to maintain experiment-wise error of ≤0.05.

3. Results and discussions

3.1. Water quality and free chlorine concentration changes during lettuce washing

The wash solutions initial turbidity and COD ranged from 0.5 to 0.6 NTU and 301 to 366 mg/l, respectively before introduction of

shredded iceberg lettuce and spinach (Fig. 2). As shredded lettuce was continuously discharged into the wash system, COD and turbidity increased rapidly in both control (Fig. 2A) and T128 (Fig. 2B) wash solutions. At the end of the test run (36 min, or 1620 kg of lettuce washed), the water turbidity increased to 25 NTU, while the COD reached 1600 mg/l. Under the operation conditions present in this experiment, the increases in both water turbidity and COD linearly corresponded to the amount of lettuce washed and hence the amount of tissue exudates released into the wash system. Wash water turbidity was also affected by the presence of free chlorine, as a steep decrease in measured water turbidity occurred immediately following chlorine replenishment. This pilot-plant data parallels our previous laboratory test results in which 2-lb (907 g) batches of shredded lettuce were consecutively washed in 40-l of chlorinated water (Luo, 2007). Results also show no significant differences between control and T128 treatments for COD and turbidity, suggesting that T128 has no direct effect on the organic loads in the water.

As shredded lettuce was continuously introduced into the washing system, the rapid increases in COD and turbidity were accompanied by a rapid decline in residual free chlorine concentration (Fig. 3A and B). The latter decreased from the initial concentration of ~20 mg/l to near depletion at the end of the first wash segment, when approximately 1/6 of the weight equivalent of shredded lettuce was washed in the solution (540 kg in 3200 l). Adding fresh sodium hypochlorite very briefly increased the solution free chlorine levels. However, as increasing amounts of organic materials accumulated in the wash solution, an increasingly greater quantity of sodium hypochlorite was needed to restore the solution free chlorine to the initial ~20 mg/l target. Although increasing amounts of concentrated sodium hypochlorite (700 ml before 540 kg of lettuce washed, 1050 ml after 540 kg lettuce washed, and 1400 ml after 1080 kg lettuce washed) were added to the wash solution, decreased residual free chlorine levels (15 mg/l, and 17 mg/l respectively) were measured in the control wash solution immediately after the chlorine replenishments at the start of wash segments two and three. Following chlorine replenishment between wash segments, chlorine levels decreased extremely rapidly to below 1 mg/l. The application of T128 did not significantly affect the rate of free chlorine consumption, although slightly higher residual free chlorine remained in the T128 system (Fig. 3B) than in the control system (Fig. 3A) after 36 min or 1620 kg of lettuce was washed.

3.2. E. coli O157:H7 survival in wash water

The antimicrobial quality of wash water is a strong indicator of the integrity of the washing operation. In this study, a non-pathogenic *E. coli* O157:H7 strain was inoculated on spinach and washed with un-inoculated lettuce shreds to simulate sporadic contamination. *E. coli* O157:H7 was not recovered from the wash water when the residual free chlorine >1.0 mg/l, indicating that the *E. coli* O157:H7 strain in suspension is highly susceptible to free chlorine. In a previous study (Luo et al., 2011a), we reported that *E. coli* O157:H7 did not survive in solutions with targeted free chlorine concentrations ≥0.5 mg/l with a 30 s to 1 min exposure. However, *E. coli* O157:H7 was recovered in the control wash water solution (Fig. 3C), in concentrations ranging from 0.2 to 8.1 MPN/ml, when solution was with free chlorine of <0.5 ppm. *E. coli* O157:H7 survival was frequently detected in the later stages of all three control washing segments (without T128). In contrast, application of T128 significantly reduced the survival of *E. coli* O157:H7 in wash water ($P=0.0003$). Very few sporadic incidences of *E. coli* O157:H7 survival were detected in the wash water containing T128 during the whole wash process, even when free chlorine concentration decreased to below 0.5 mg/l (Fig. 3D). This finding is in agreement with our previous observations in a laboratory-scale study (Nou et al., 2011); namely, that application of T128 significantly enhanced the inactivation of *E. coli* O157:H7 in chlorinated wash solutions containing high organic loads.

3.3. Effect of T128 on cross-contamination

The consequence of pathogen survival in sanitizer-depleted wash water could be the widespread cross contamination throughout the entire wash system (Luo et al., 2011a). *E. coli* O157:H7 was frequently detected on un-inoculated lettuce after washing in the same wash tank with inoculated baby spinach, suggesting significant pathogen transference from contaminated to un-contaminated produce when they are washed together (Fig. 3E and F). Similar findings were reported by Allende et al. (2008) and Luo et al. (2011a) in laboratory-scale studies.

In the absence of T128, pathogen survival was highly dependent on solution residual chlorine levels. When concentrations were maintained well above 1 mg/l level, no *E. coli* O157:H7 were detected

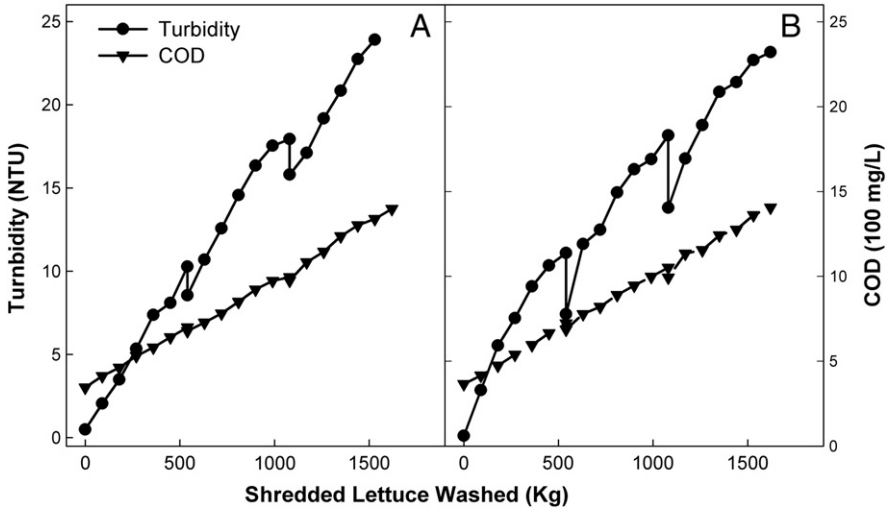

Fig. 2. Dynamic changes in wash water chemical oxygen demand (COD) and turbidity of the wash solution with control (A; no T128) and T128 (B) treatments. Lettuce shreds and spinach were continuously fed into the wash system at a rate of 45 kg/min. Arrows indicate that points when the process was paused and sodium hypochlorite was replenished to the wash solution. Data are the average of three replicate experiments.

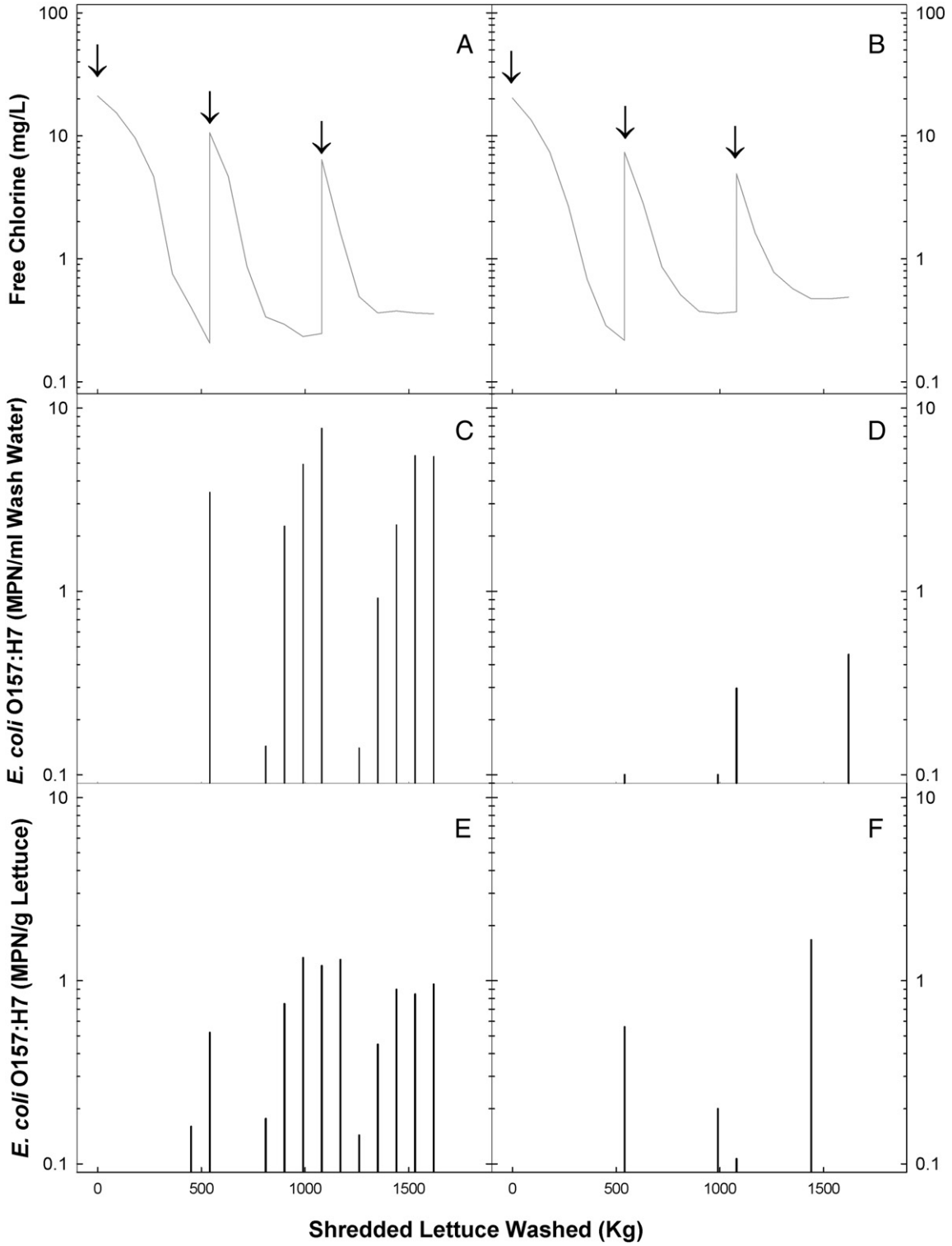

Fig. 3. Dynamic changes in residual free chlorine concentration in the wash solution (A — Control and B — T128), and the recovery of *E. coli* O157:H7 in the solution (C — Control and D — T128) and un-inoculated iceberg lettuce (E — Control and F — T128). Un-inoculated shredded lettuce and inoculated baby spinach (1000:2, gram basis, respectively) were continuously introduced into the wash system at a rate of 45 kg/min. Arrows indicate points when aliquots of 700 ml, 1050 ml, and 1400 ml of concentrated sodium hypochlorite were added to the wash system. Data are the average of three replicate experiments.

on lettuce. However, *E. coli* O157:H7 were increasingly detected when chlorine in the wash solutions were near or below 1 mg/l (Fig. 3E). Similar results were reported by Luo et al. (2011b) in another laboratory study. The disparity between pathogen survival in wash solution and cross-contamination on lettuce may be primarily attributable to the two following scenarios: 1) contamination via direct contact between inoculated spinach and un-inoculated lettuce; and 2) *E. coli* O157:H7 in the wash solution reached/adhered/attached to lettuce before they were inactivated by the free chlorine in the solution. Our follow up tests showed that *E. coli* O157:H7 survival in the solution was a function of chlorine concentration and contact time. At low chlorine concentration, an extended contact time is necessary for inactivating *E. coli* O157:H7 (data not shown). Water samples for pathogen survival evaluation in this study were collected after approximately 30 s of

co-immersion of inoculated spinach and un-inoculated lettuce leaves, while bacterial pathogens could instantly attach to lettuce surface. Once attached to produce surfaces, especially the cut edges of lettuce, disinfection of bacterial pathogens by chlorine becomes increasingly difficult as pathogens are relatively protected against effects of the sanitizer.

In the presence of T128 (Fig. 3F), the occurrence of cross-contamination was significantly ($P=0.0005$) reduced, and no incidence of cross-contamination was detected at free chlorine above 1 mg/l. Furthermore, in those sporadic instances where *E. coli* O157:H7 contamination of lettuce was detected in the presence of T128, the concentrations were extremely low.

3.4. Reduction of E. coli O157:H7 on spinach leaves

Initial *E. coli* O157:H7 populations on inoculated spinach leaves before washing averaged 4.9 ± 0.4 log CFU/g. The washing of inoculated spinach leaves and un-inoculated lettuce leaves reduced pathogen counts on spinach by 0.8 to 0.9 log CFU/g for the three wash segments (Fig. 4). No difference was observed in *E. coli* O157:H7 populations remaining on spinach leaves after washing in chlorinated water in the presence or absence of T128 (Fig. 4). Our previous laboratory study also showed similar *E. coli* O157:H7 survival on inoculated lettuce after washing in chlorine solutions with and without T128 (Nou et al., 2011).

For fresh-cut produce processing, the primary purpose of wash water sanitization is to prevent potential widespread cross contamination of produce by foodborne pathogens. Currently available interventions are generally ineffective or impractical for eliminating foodborne pathogens attached to leafy green surface, especially wounded surfaces (Takeuchi and Frank, 2000; McEvoy et al., 2008). For most chemical sanitizers, a one to two $\log_{10}$ unit reduction of inoculated bacterial pathogens, including *E. coli* O157:H7 and *Salmonella* spp., are typically reported in laboratory-scale studies. The reduction efficacy is often reduced in commercial-scale operations since many operational parameters of the process are much less controlled in industrial implementation than in laboratory studies. We observed a less than one $\log_{10}$ unit reduction of *E. coli* O157:H7 when inoculated spinach leaves were washed along with shredded lettuce in this pilot-plant study, highlighting the critical importance of preventing pre-harvest contamination.

Chlorinated water, one of the most widely used sanitizing solutions, is especially sensitive to the rapid accumulation of organic materials from fresh-cut tissue. Although it is recognized that free chlorine depletion in wash water is conducive to pathogen cross contamination, maintaining high free chlorine levels in wash water is costly and impractical. Many fresh-cut leafy green processors have implemented HACCP programs that require minimal free chlorine of 1 mg/l in wash water. Data obtained in this study indicate that survival of pathogens in the wash solution was effectively prevented when wash water free chlorine was maintained above 1 mg/l. However, frequent surges of organic matter into the washing system made it difficult to maintain an adequate stable free chlorine level using citric acid at pH 6.5. Lacking a reliable automated on-line pH and sanitizer monitoring and dispensing system, fresh-cut processors often rely on laborious intermittent monitoring and frequent manual interventions to maintain an effective sanitizer level, which can fluctuate widely between monitoring events. Maintaining wash water at a constant slightly acidic pH also helps to keep free chlorine in wash water in the most efficacious form, i.e. hypochlorous acid, rather than the less efficacious form of hypochlorite ions. However, as demonstrated in our previous studies, simply maintaining low pH using citric acid does not result in significant improvement of antimicrobial efficacy of chlorinated wash water.

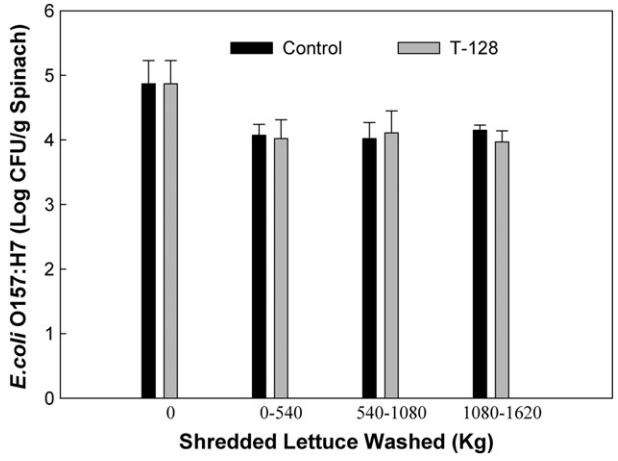

Fig. 4. Recovery of *E. coli* O157:H7 on inoculated baby spinach leaves after continuously washing un-inoculated lettuce with inoculated baby spinach in chlorinated water with periodic replenishment of sodium hypochlorite. Baby spinach samples were collected immediately before the run, and during the first, second and third segments when 0–540, 540–1080, and 1080–1620 kg of lettuce was washed.

Data obtained in the current semi-commercial scale pilot-plant study indicate that the application of T128 in a chlorine-based leafy green processing system significantly ($P=0.0003$) reduced the likelihood of survival by bacterial pathogens, hence the potential of cross contamination, even when the free chlorine level dropped below the currently accepted critical limit, 1.0 mg/l. In addition, since one of the main components of T128 is an inorganic acid, it can be easily dispensed using a pH controlled automated dispensing system.

No attempt was made to investigate the mechanism of T128 on pathogen cross-contamination or the pH difference in pilot scale. The pH of the control solution in this pilot plant study was maintained at 6.5 since chlorine is most effective in this pH level, and also this pH level was used by the majority of the fresh-cut processors using chlorine as sanitizers. The pH value of 5.0 used for the T128 treatment was based on the specifications provided by the manufacturer (Brennan, 2010). Previous laboratory studies compared the effect of control and T128 at pH values of 3.0, 5.0, and 6.5 showed that the improvement in preventing pathogen survival in the wash solution by T128 was consistent among all these pH levels tested. Furthermore, laboratory studies also indicate that T128 exerted a synergistic effect with low levels of chlorine (Luo et al., 2011b). The application of T128 significantly reduced survival of *E. coli* O157:H7, when free chlorine in solution decreased to levels approaching depletion. In the presence of T128, no survival of *E. coli* O157:H7 in the solution was observed with the free chlorine concentration as low as 0.05 mg/l, while a much higher concentration (at least 0.4 mg/l) of free chlorine was required to kill pathogens in suspension in the absence of T128 (Luo et al., 2011b).

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