Chlorine Stabilizer T-128 Enhances Efficacy of Chlorine against Cross-Contamination by *E. coli* O157:H7 and *Salmonella* in Fresh-Cut Lettuce Processing

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Abstract: During fresh-cut produce processing, organic materials released from cut tissues can rapidly react with free chlorine in the wash solution, leading to the potential survival of foodborne bacterial pathogens, and cross-contamination when the free chlorine is depleted. A reported chlorine stabilizer, T-128, has been developed to address this problem. In this study, we evaluated the ability of T-128 to stabilize free chlorine in wash solutions in the presence of high organic loads generated by the addition of lettuce extract or soil. Under conditions used in this study, T-128 significantly ($P <$ 0.001) decreased the rate of free chlorine depletion at the presence of soil. T-128 also slightly decreased the rate of free chlorine depletion caused by the addition of lettuce extract in wash solution. Application of T-128 significantly reduced the survival of bacterial pathogens in wash solutions with high organic loads and significantly reduced the potential of cross-contamination, when contaminated and uncontaminated produce were washed together. However, T-128 did not enhance the efficacy of chlorinated wash solutions for microbial reduction on contaminated iceberg lettuce. Evaluation of several produce quality parameters, including overall visual appearance, package headspace O_2 and CO_2 composition, and lettuce electrolyte leakage, during 15 d of storage indicated that iceberg lettuce quality and shelf life were not negatively impacted by washing fresh-cut lettuce in chlorine solutions containing 0.1% T-128.

Keywords: chlorine stabilizer, *E. coli* O157:H7, free chlorine, fresh-cut leafy green, *Salmonella*

Practical Application: Reported chlorine stabilizer is shown to enhance chlorine efficacy against potential bacterial cross-contamination in the presence of high organic loads without compromising product quality and shelf life.

Introduction

Pathogenic bacterial contamination of fresh-cut produce is a growing food safety threat. While such contamination could happen at various food production stages, preharvest contamination has been linked to several recent produce associated outbreaks of *Salmonella* spp. and enterohemorrhagic *Escherichia coli* (Jay and others 2007; Mitra and others 2009; Berger and others 2010). Implementation of good agriculture practices can minimize but not eliminate the possibilities of in-field contamination. An effective postharvest antimicrobial intervention is critical for ensuring fresh-cut produce safety.

In commercial fresh-cut produce operations, double or triple sanitizer washes are generally used for the reduction of potential contamination by microbial pathogens, as well as for the prevention of contamination from spreading during produce processing. Although several chemical sanitizers are commercially available for fresh-cut produce washes, including acidified sodium chlorite, peroxyacetic acid, ozone, and chlorine dioxide, chlorinated water remains the most commonly used sanitizer for fresh-cut produce processing because of its minimal impact on the nutritional and aesthetic quality of the product, its established ability to kill pathogens in suspensions, and the low cost of the applications (Gonzalez and others 2004; Gil and others 2009; Lopez-Galvez and others 2009). Hypochlorous acid (HOCl) is the most efficacious form of chlorine, known as free chlorine (Connell 1996; Zagory 2000). It is critically important that a relatively constant free chlorine level be maintained in the washing process to ensure the efficacy of the sanitizer for microbial reduction and to prevent potential cross-contamination. However, like other oxidant antimicrobial agents, free chlorine is highly reactive to organic substances and can be rapidly degraded by organic matter in the washing solution (Zagory 2000; Luo 2007; Gil and others 2009). Considerable amounts of organic latex are released into the washing solution as vegetable exudates from wounded tissues during fresh-cut processing of lettuce (Luo 2007). Consequently, the introduction of freshly cut lettuce into produce wash water often leads to sudden large surges in organic matter that rapidly depletes

MS 20101121 Submitted 9/30/2010, Accepted 12/19/2010. Authors Nou , Luo, Hollar , Yang, Millner, and Shelton are with Environmental Microbial and Food Safety Laboratory, U.S. Dept. of Agriculture, Agricultural Research Service, Beltsville, MD 20705, U.S.A. Authors Yang and Feng are with Dept. of Food Science and Human Nutrition, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801, U.S.A. Direct inquiries to author Nou (E-mail: xiangwu.nou@ars.usda.gov).

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doi: 10.1111/j.1750-3841.2011.02046.x

Journal of Food Science
A Publication of the Institute of Food Technologists

free chlorine from the washing system, creating opportunities for bacterial pathogens to survive, and cross-contaminate produce (Gil and others. 2009). This problem cannot be effectively solved by increasing the chlorine input because repeated addition of chlorine to wash water that is high in organic load also results in the increased formation of toxic chlorine by-products, and generation of harmful chlorine off-gas (Suslow 2001).

Stabilization of free chlorine in water has long been sought in many applications. For example, cyanuric acid is widely used for maintaining free chlorine in outdoor swimming pools (Sommerfeld and Adamson 1982). Chlorine reaction with cyanuric acid produces a relatively stable oxidative compound, chlorimide, which is more resistant to photodegradation than HOCl. A chlorine stabilizer that would be suitable for food-processing applications has not been developed at present. Recently, a new formula based on chemicals with generally recognized as safe (GRAS) status (FDA 2009) was developed by scientists in the produce industry that reportedly stabilized free chlorine (Lemons 2009). In this report, we examine the effectiveness of this formula, T-128, in stabilizing free chlorine in the presence of high organic loads and in reducing pathogens in chlorinated wash solutions comparable to those used for fresh-cut lettuce processing. In addition, we examine the impact of T-128 on the quality and shelf life of fresh-cut iceberg lettuce following exposure in wash solutions.

Materials and Methods

Bacterial strains and growth

Attenuated *E. coli* O157:H7 strain CDC B6–914/pGFP (ampicillin resistant) (Fratamico and others 1997), a stable spontaneous nalidixic acid resistant mutant (Yossa and others) of *E. coli* O157:H7 strain RM4406, which was isolated from a produce-associated outbreak in 2003, *E. coli* O157:H7 strain ISEHGFP (kanamycin resistant), and *Salmonella enterica* sv. Typhimurium strain ISSAGFP (kanamycin resistant) (Noah and others 2005) were used in this study. *Escherichia coli* O157:H7 and *Salmonella* strains were grown in trypticase soy broth (TSB) (Becton Dickenson, Sparks, Md., U.S.A.) supplemented with appropriate antibiotics at 37 °C overnight, followed by centrifugal harvesting and resuspension in an equal volume of phosphate-buffered saline (PBS). Resuspended cells were further diluted in PBS and used as inocula individually or as a cocktail of multiple strains.

Produce inoculation

Whole heads of iceberg lettuce (*Lactuca sativa* L.) and baby spinach (*Spinacia oleracea* L.) used in this study were purchased from local wholesale or retail establishments, stored at 4 °C and used within 24 h after purchasing. Lettuce was cored and trimmed to remove the outer most and any damaged leaves before cutting. Bacterial inocula, either as individual strains or a cocktail of multiple strains, were quantitatively inoculated on pieces of cut lettuce by depositing multiple droplets on the leaf surface. Unless otherwise specified, a typical inoculation was consisted of 10^6 to10^7 cells in 20 of 5 μL droplets deposited randomly on 10 g of iceberg lettuce cut to 1-inch wide pieces or on 10 g of baby spinach leaves. Inoculated lettuce or spinach leaves were left in a ventilated biosafety hood for 45 min to allow the droplets to dry or absorb into the leaf surface, followed by storage in sealed sample bags at 4 °C for 16 to 20 h before treatments.

Chlorine stability

Chlorine wash solutions were prepared using 6% sodium HOCl (Clorox). After mixing a calculated amount of sodium HOCl with distilled water, citric acid was used to adjust the solutions to desired pH. Chlorinated wash solution with pH adjusted to 6.4 to 6.5 was used as control solution. Chlorine stabilizer T-128 (New Leaf Food safety Solutions, LLC, Salinas, Calif., U.S.A.) was directly added to the chlorine solutions (without prior pH adjustment) to desired concentration (0.05% to 0.1%) to generate treatment solutions. Actual free chlorine in the wash solutions was measured using a chlorine photometer (HF Scientific Inc., Ft. Myers, Fla., U.S.A.). All wash solutions were used within 30 min of preparation. Water quality parameters, including turbidity, total dissolved solids (TDS), Brix, and chemical oxygen demand (COD), were determined as previously described (Luo 2007).

Lettuce extract and soils were used to quantitatively increase organic loads in wash solutions. Iceberg lettuce extract was prepared from cored and trimmed lettuce using a household juice maker and stored at −80 °C until use. Clay and sandy soils were obtained from lettuce production fields in Salinas, California, and stored at room temperature until use. Lettuce extract and soil were directly added to wash solutions at indicated concentrations and mixed for 1 min before samples were taken to determine residual free chlorine levels.

Produce wash and bacteria enumeration

To evaluate the efficacy of different washing conditions, 20 g of noninoculated lettuce (cut as $\frac{1}{4}$-inch strips) and 10 g of inoculated lettuce (cut as 1 × 1 inch squares) (or baby spinach) were sequentially submerged in 750 mL of wash solution (lettuce to solution ratio = 1:25), and washed for 1 min with moderate manual agitation. After washing, noninoculated and inoculated lettuce pieces, which were cut in easily distinguishable shapes and sizes, (or inoculated baby spinach leaves in place of inoculated cut lettuce), were sorted into different sample bags for microbial analyses. A portion of the spent wash solution was mixed with concentrated sodium thiosulfate (STS) solution (final concentration 0.1% w/v) to neutralize the chlorine residue (Kemp and Schneider 2000) before use in microbial analyses. Inoculated and noninoculated lettuce (or inoculated baby spinach) pieces were treated in a sonication water bath (Alcar industries, Belleville, N.J., U.S.A.) for 20 s, followed by stomaching for 2 min to release attached cells, in a filtered sample bag containing 5 volumes of buffered peptone water (BPW) (Neugen, Lansing, Mich., U.S.A.) with 0.1% of STS (for inoculated lettuce or baby spinach) or TSB with 0.1% of sodium pyruvate (for noninoculated lettuce). Bacterial cells were enumerated by spiral plating (for inoculated samples and wash solutions) or a microplate-based most probably number (MPN) procedures (for noninoculated lettuce samples and wash solutions) as previously described (Nou and Luo 2010). Sorbitol MacConkey (Neugen) containing cefixime (50 μg/L) and potassium tellurite (2.5 mg/L) (CT-SMAC, Invitrogen, Carlsbad, Calif., U.S.A.) and XLT4 (Neugen) plates, supplemented with appropriate antibiotics, were used for enumerate *E. coli* O157:H7 and *S. enterica* sv. Typhimurium strains, respectively.

Produce quality evaluation

Trimmed and cored iceberg lettuce was cut to $\frac{1}{4}$-inch strips in a Paxton lettuce cutter (Paxton Enterprises LLC, Shelton, Conn., U.S.A.). The lettuce shreds were washed for 30 s in the solution containing 20 ppm free chlorine with pH adjusted to 6.5 using citric acid (control; current commercial fresh-cut wash condition),

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or containing 20 ppm free chlorine and 0.1% T-128, at a lettuce to solution ratio of 1:20. The washed lettuce was drained and dewatered in a commercial produce centrifugal dryer (Meyer Machine Co., San Antonio, Tex., U.S.A.) at 650 rpm (approximately 110 g_n) for 2.5 min to remove excess water. The washed lettuce was packed in multiple retail display bags (170 g/bag) made of gas permeable package film (oxygen transmission rate 16.6 pmol/s/m^2/Pa^1), and stored at 5 °C for up to 15 d. The initial headspace O_2 partial pressure in the bags was set at 1.0 kPa. Samples were removed from the storages on indicated dates to evaluate lettuce quality parameters. Visual appearance of the packaged products was evaluated by a panel of 3 personnel familiar with lettuce and spinach quality attributes using a 9-point hedonic scale, where 9 = like extremely, 7 = like moderately, 5 = neither like nor dislike, 3 = dislike moderately, and 1 = dislike extremely (Meilgaard and others 1991; Luo and others 2009; Luo and others 2010). The headspace O_2 and CO_2 partial pressures of the packages were measured using Combi Check 9800–1 gas analyzer system (PBI Dansensor Co., Ringsted, Denmark). Tissue electrolyte leakage was determined by measuring changes in electrical conductivity (EC) of distilled water after immersing lettuce sample (50 g in 300 mL) at 5 °C for 30 min, using a conductivity meter (Orion Research Inc., Beverly City, Mass., U.S.A.). Total tissue electrolyte content was determined by measure EC change using lettuce samples frozen at −20 °C for 24 h and subsequently thawed in distilled water. Tissue electrolyte leakage (at 30 min) was expressed as relative EC (REC), which is the percentage of sample EC to total tissue electrolyte content (Luo and others 2004; Kim and others 2005; Luo 2007).

Experimental design and statistical analysis

The experiment was conducted using factorial designs with 3 to 5 replications. Data were analyzed as a linear model using the PROC MIXED procedure (SAS Inst. Inc., Cary, N.C., U.S.A.). Normality and variance homogeneity of the linear model were checked for the log-transformed data. A variance grouping technique was used to address variance heterogeneity for means comparisons. When effects were statistically significant, means comparisons were done with Sidak adjusted P-values to maintain experiment-wise error ≤0.05.

Results and Discussion

Stabilization of free chlorine in wash water

Produce processing plants typically wash freshly cut lettuce 2 to 3 times in chlorinated wash water, with water reused and recirculated. As a result, water in the wash system, especially the primary wash, has significant presence of soils and foreign debris, and a high organic load due to lettuce latex released from the cut edges. We added iceberg lettuce extract to distilled water to compare with spent water obtained from a local fresh-cut lettuce processing operation (Table 1). The values of several water quality parameters, including turbidity, TDS, and COD, increased proportionally when an increasing concentration of lettuce extract was added. We estimated that the spent wash water from the commercial operation contained organic materials corresponding to approximately 4% of lettuce extract, based on the observed COD value.

To evaluate the potential of T-128 for stabilizing free chlorine in produce wash solutions, iceberg lettuce extract was added to chlorinated wash solutions at different concentrations with or without the reported chlorine stabilizer T-128. Free chlorine levels dropped precipitously when lettuce extract concentration increased, regardless of T-128 application. However, free chlorine measurements were higher in the presence of T-128 than in the controls at higher levels of organic loads, when free chlorine in solution was approaching depletion (Figure 1A). At the presence of 1% of lettuce extract, free chlorine was depleted (0.28 ppm) in wash solution without T-128 (control). The free chlorine levels dropped to 0.96 and 1.43 ppm, respectively, for washing solutions containing 0.05% and 0.1% of T-128, when the same amount of lettuce extract was added. At the presence of 2% lettuce extract, free chlorine in all washing solutions reached near depletion (0.30, 0.31, and 0.35 ppm for 0, 0.05% and 0.1% of T-128, respectively). The degradation of free chlorine as impacted by lettuce extract concentration fits an exponential model for the control (R^2 = 0.79) and both 0.05% T-128 (R^2 = 0.95) and 0.1% T-128 (R^2 = 0.98) treatments (Figure 1B). The addition of T-128 to the wash solution reduced the chlorine degradation slightly with the power of the exponential decay changed from 2.22 for the control to 2.02 and 2.07 for 0.05% and 0.1% T-128 treatments, respectively. The significance of this slightly higher free chlorine level in affecting the survival of bacterial pathogens remains to be determined.

Soil is another major factor in the degradation of wash water quality in produce processing operations. Therefore, free chlorine stability was examined in soil contaminated wash water in the presence of T-128. Both clay and sandy soils from lettuce growth fields in Salinas, California, were directly added to chlorine wash solutions with or without T-128. Increasing soil content in wash water rapidly decreased free chlorine levels in the solutions (Figure 2A), as observed when lettuce extract was used to provide organic loads. The degradation of free chlorine as impacted by soil content fits a polynomial model, with $R^2 = 0.99$ for the control and 0.1% T-128 treatment, using clay or sandy soils (Figure 2B). The reduction in free chlorine concentration was significantly ($P < 0.001$) slower with the presence of T-128 compared to the control. When the soil level reached 2% in the chlorine wash solutions, free chlorine dropped from 20 to 11.5 ppm, whereas it only declined to 15.5 ppm in the presence of 0.1% T-128. Similarly, T-128 significantly ($P < 0.001$) retarded chlorine degradation caused by the presence of sandy soil. The addition of 2% sandy soil resulted in free chlorine dropping from 20 to 8.9 ppm for the control and from 20 to 10.2 ppm when T-128 was used.

Table 1–Quality parameters of spent wash water.

Wash water	Turbidity (NTU)	TDS (mg/L)	Brix (°Bx)	COD (mg/L)
Distilled water plus extract (%)				
0	0.31 ± 0.20	1.67 ± 0.58	0.03 ± 0.06	63.25 ± 1.77
0.25	2.15 ± 0.14	13.33 ± 2.52	0.13 ± 0.06	158.25 ± 40.66
0.5	4.76 ± 0.07	25.67 ± 1.15	0.17 ± 0.12	324.50 ± 49.50
1	8.83 ± 0.38	57.00 ± 13.08	0.13 ± 0.06	532.00 ± 31.82
2	16.83 ± 0.45	92.00 ± 2.00	0.20 ± 0.01	942.00 ± 31.82
Spent plant wash water				
Primary wash	59.8	708	0.3	1857.8

Bactericidal activity of T-128

One of the main characteristics of T-128 is its low pH as one major component is a mineral acid. To determine the bactericidal activity of T-128, *E. coli* O157:H7 strain B6–914/pGFP was directly inoculated into solutions containing different concentrations of T-128 for different length of time. Bacterial cells surviving the treatments were enumerated using an MPN procedure as described previously (Figure 3). No reduced cell counts were detected when cells were exposed to moderate concentration (up to 0.5%) of T-128 for extended times. Significant cell reduction (>0.5 $\log_{10}$ unit) was detected when exposed to 1% T-128 for over 5 min. At concentration of 2.5%, T-128 resulted in dramatic cell reduction after exposures of more than 2.5 min. Therefore, T-128 alone has weak bactericidal activity that requires either a high concentration or extended reaction time to inactivate bacteria such as *E. coli* O157:H7 in suspension. This weak bactericidal activity is not manifested under practical commercial produce washing conditions and is unlikely to play significant role in bacterial counts reduction.

Effect of T-128 on the efficacy of chlorine wash

To evaluate the effect of T-128 on chlorine wash for pathogen inactivation, iceberg lettuce inoculated with *E. coli* O157:H7 and *Salmonella* strains was washed in chlorinated wash solutions in the presence or absence of T-128, using lettuce extract to manipulate organic load levels in the solution. Bacterial pathogens surviving the washing treatments on the lettuce and in wash solutions were enumerated immediately following the treatments (Table 2). Approximately one log reduction of the inoculated strains on the lettuce was observed regardless of the wash solutions used. However, no survival of the pathogenic strains in wash solution was

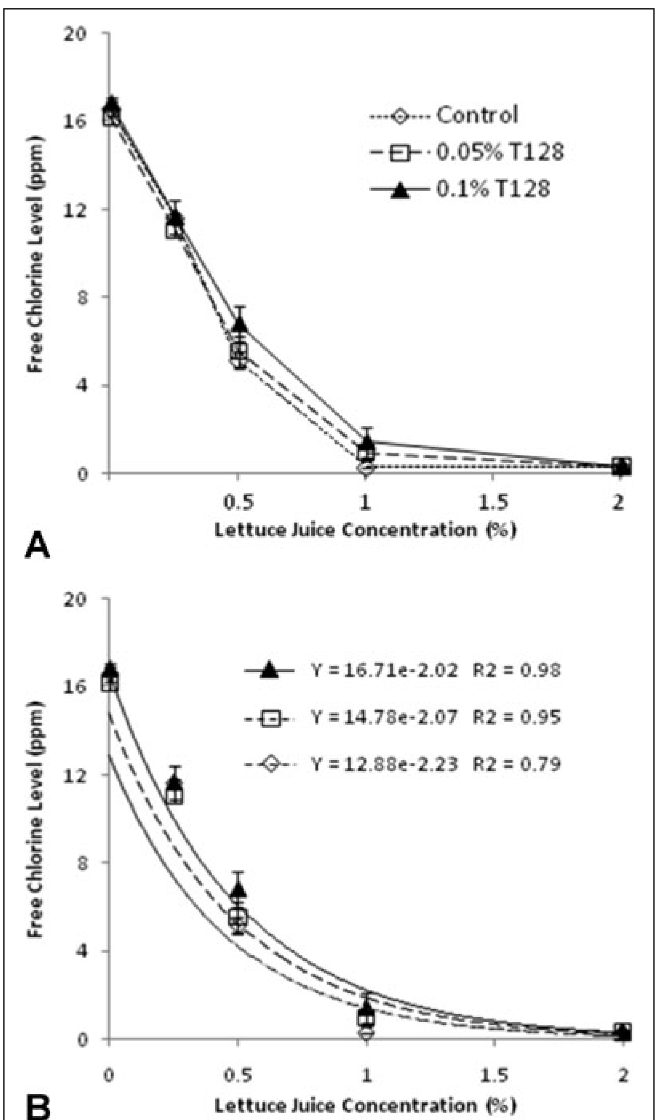

Figure 1–Effect of organic loads and T-128 (0.05% and 0.1%) on free chlorine level in wash solutions. Lettuce extract was used as the organic source. Wash solutions were exposed to the source of organic loads for 1 min before samples were taken for free chlorine measurement. (A) Data presented as line graph connecting all data points. (B) Data presented as scatter graph with trend line fitting using MS Excel.

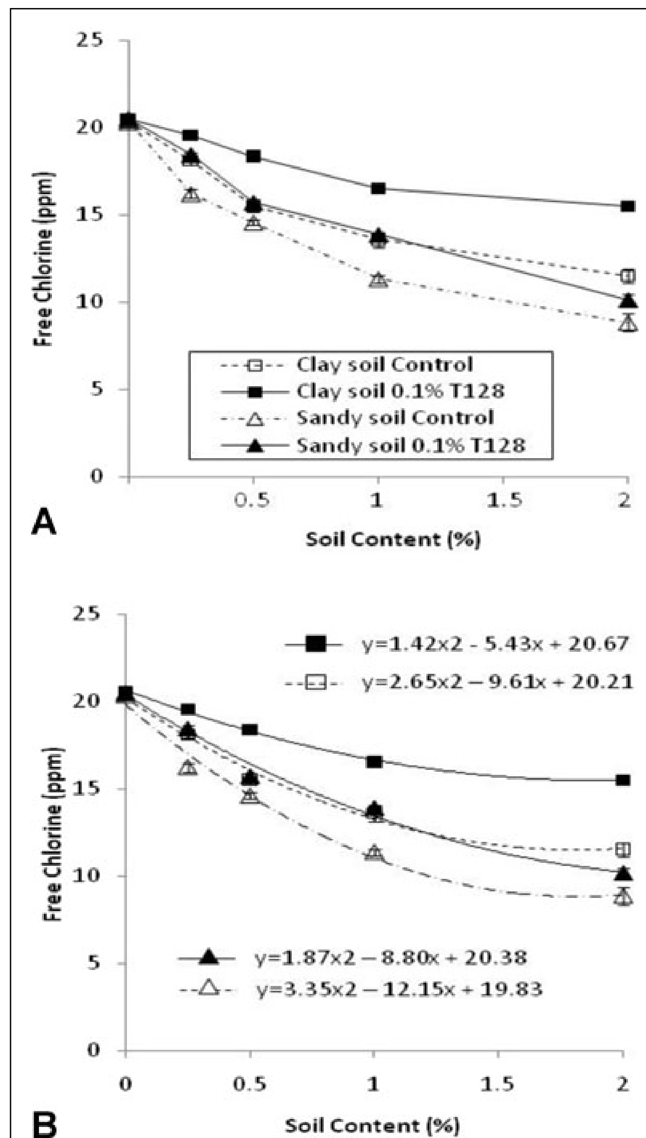

Figure 2–Effect of organic loads and T-128 (0.1%) on free chlorine level in wash solutions. Clay and sandy soils from lettuce production fields were used as the organic source. Wash solutions were exposed to the source of organic loads for 1 min before samples were taken for free chlorine measurement. (A) Data presented as line graph connecting all data points. (B) Data presented as scatter graph with trend-line fitting using MS Excel ($R^2 = 0.99$).

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detected when T-128 was added, even in the presence of high concentrations of lettuce extract and hence low chlorine levels. In contrast, significant bacterial survival in the wash solutions was detected in the presence of high concentrations of lettuce extract in the control treatment. The mechanism of the enhanced efficacy by T-128 against bacterial survival in washing solutions at high organic load levels has not been determined. Since T-128 alone at the treatment concentration (0.05% to 0.1%) and reaction time (1 min) did not exhibit any antimicrobial effect (Figure 2), this observation suggests that there might be a synergistic effect between T-128 and very low level of chlorine in washing solutions.

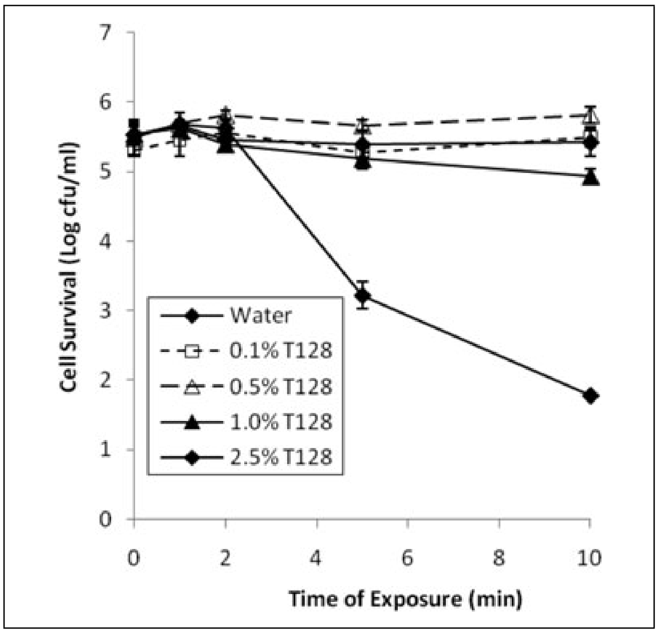

Figure 3–Bactericidal activity of T-128. *Escherichia coli* O157:H7 strain B6–914 (pGFP) was exposed to different concentrations of T-128 for indicated length of time before enumeration using an MPN procedure. Calculated detection limit used in this assay is 1.78 $\log_{10}$ CFU/mL.

Effect of T-128 on reducing cross-contamination

The failure of bacterial pathogens to survive in wash solutions containing T-128 and high organic loads suggests that the application of T-128 could significantly improve the efficacy of chlorine washing against potential cross-contamination during fresh-cut processing. To test this, baby spinach leaves were inoculated with *E. coli* O157:H7 and *Salmonella* strains and washed together with noninoculated cut iceberg lettuce in wash solutions containing different levels of lettuce extract and 0.1% of T-128. Cut iceberg lettuce pieces were sorted, drained, and the attached bacterial cells were enumerated using an MPN procedure (Table 3). As expected, significant bacterial cross-contamination of iceberg lettuce from inoculated baby spinach leaves was detected when free chlorine in wash solution was depleted by the addition of 1.0% of lettuce extract. However, the presence of 0.05% or 0.1% of T-128 effectively prevented such cross-contamination, which was consistent with the observation that T-128 prevented the survival of the pathogenic strains in wash solution even at high concentration of lettuce extract. Sporadic low level cross-contamination was observed on both T-128 treatment and control samples. The mechanisms of this sporadic cross-contamination

Table 3–Bacterial survival on noninoculated lettuce.

Wash solution	Lettuce extract (v/v %)	Bacterial survival on noninoculated Lettuce RM4406	(CFU/g)* ISSAGFP
Water	0	79.4 ± 2.0	63.1 ± 1.3
Chlorine water	0	–	<0.16
	0.5	–	<0.16
	1.0	10.0 ± 5.0	10.0 ± 4.0
Chlorine water + 0.05% T-128	0	<0.16	–
	0.5	–	<0.16
	1.0	0.2 ± 0.2	<0.16
Chlorine water + 0.1% T-128	0	–	–
	0.5	<0.31	–
	1.0	<0.16	<0.5

*Dashed lines indicate cell survival rates below the calculated limit of detection (not detected). (<) signs indicate cell survival detected at the indicated levels in one of 3 repeats.

Table 2–Bacterial survival following chlorine treatment.

Wash solution	Lettuce extract (v/v %)	Bacteria survival on inoculated lettuce (log CFU/g) ISSAGFP	ISEHGFP	RM4406	Bacterial survival in solution (log CFU/mL)* ISSAGFP	ISEHGFP	RM4406
Chlorine water	0	3.1 ± 0.6	3.5 ± 0.5	3.1 ± 0.6	–	–	–
	0.25	3.2 ± 0.4	3.0 ± 1.1	2.6 ± 0.4	–	–	–
	0.5	3.0 ± 0.7	3.0 ± 0.5	2.6 ± 0.5	–	<1.3	<1.0
	1.0	3.5 ± 0.4	3.5 ± 0.8	3.0 ± 1.0	1.2 ± 0.2	1.8 ± 0.1	1.6 ± 0.2
	2.0	3.5 ± 0.4	3.7 ± 0.4	3.4 ± 0.6	2.6 ± 0.5	2.5 ± 0.5	2.5 ± 0.4
Chlorine water + 0.05% T-128	0	2.9 ± 0.6	3.4 ± 0.6	2.9 ± 0.8	–	–	–
	0.25	3.2 ± 0.6	3.4 ± 0.4	2.7 ± 0.6	–	–	–
	0.5	3.4 ± 0.4	3.5 ± 0.4	2.9 ± 0.5	–	–	–
	1.0	3.7 ± 0.3	3.8 ± 0.1	3.4 ± 0.2	–	–	–
	2.0	3.7 ± 0.4	3.5 ± 0.5	3.2 ± 0.6	–	–	–
Chlorine water + 0.1% T-128	0	3.2 ± 0.5	2.9 ± 0.6	3.0 ± 0.3	–	–	–
	0.25	3.5 ± 0.2	3.6 ± 0.2	3.2 ± 0.3	–	–	–
	0.5	3.7 ± 0.3	3.6 ± 0.5	3.3 ± 0.1	–	–	–
	1.0	3.5 ± 0.6	3.4 ± 0.7	3.2 ± 0.1	–	–	–
	2.0	3.3 ± 0.8	3.4 ± 0.6	3.4 ± 0.5	–	–	–

Numbers of inoculated bacterial cells were determined by plating 0.1 mL of appropriate dilutions of samples prepared as described in Materials and Methods in duplicate onto selective media containing appropriate antibiotics. Theoretical detection limits for cells on lettuce is 1.4 log CFU/g and for cells in wash solution is 0.7 log CFU/mL. ISEHGFP is a *S. enterrica* sv Typhimurium strain and ISEHGFP and RM4406 are *E. coli* O157:H7 strains.
*Dashed lines indicate cell survival rates below the calculated limit of detection (not detected). (<) signs indicate cell survival detected at the specified levels in one of 3 repeats.

Table 4–Effects of T-128 on lettuce quality attributes.

Quality attributes and treatment	Storage time (d)				
	0*	4	7	11	15
O_2 partial pressure (kPa)					
Control	1.00	0.23 ± 0.25	0.50 ± 0.33	1.36 ± 0.32	7.72 ± 0.08
T-128	1.00	0.09 ± 0.03	0.35 ± 0.09	0.39 ± 0.03	9.03 ± 0.04
CO_2 partial pressure (kPa)					
Control	0.03	6.66 ± 0.21	4.17 ± 2.00	3.30 ± 1.90	4.70 ± 0.48
T-128	0.03	6.54 ± 0.88	4.37 ± 1.27	4.77 ± 0.06	4.94 ± 0.09
Visual score (1 to 9 point)					
Control	9.00	8.33 ± 0.00	8.00 ± 0.00	5.78 ± 0.38	5.58 ± 0.79
T-128	9.00	8.22 ± 0.19	7.22 ± 0.38	6.00 ± 0.00	5.40 ± 0.89
Relative EC (%)					
Control	10.75 ± 0.59	6.31 ± 0.45	4.56 ± 1.73	2.19 ± 0.20	3.48 ± 0.23
T-128	10.42 ± 1.60	4.72 ± 0.34	3.22 ± 0.30	2.98 ± 0.05	3.67 ± 0.29

*Targeted initial headspace O_2 and CO_2 partial pressure for all packages on day 0 was 1.0 and 0.03, respectively. Initial visual scores for all packages were 9.00.

were not determined, but cross-contamination due to direct contact of inoculated and noninoculated leaves during washing is hypothesized.

Effect of T-128 on lettuce quality and shelf life

The main ingredients of T-128 are chemicals with "Generally Recognized as Safe" status with characteristic low pH. Low pH often has detrimental effect on produce quality. To determine whether T-128 had similar effect on produce quality, freshly cut iceberg lettuce was washed in chlorine wash water in the presence or absence of T-128. To maximize the effect of T-128, a rinsing step to remove T-128 residue was not applied. Washed iceberg lettuce shreds were packaged and stored for up to 15 d, while produce quality attributes were evaluated periodically (Table 4). During the course of storage, the visual scores of the lettuce gradually declined as product quality deteriorated. After 15 d of storage, samples from both the control and T-128 treatment were rated within the acceptable level, with no significant ($P > 0.05$) difference between the treatments. Generally, headspace O_2 declines while CO_2 increases during storage due to O_2 consumption and CO_2 accumulation associated with lettuce tissue respiration. O_2 partial pressure significantly decreased for both control and T-128 treated samples by day 4, while CO_2 partial pressure significantly increased. O_2 partial pressure remained low, with T-128 treated samples slightly lower than in control, for up to day 11, followed by accelerated increase in O_2 partial pressure for both control and treated samples. Tissue electrolyte leakage reflected by REC is a measurement of tissue integrity (Murata 1989; Jiang and others 2001) and has been widely used as an indicator for tissue damage and product quality (Portela and Cantwell 2001; Fan and Sokorai 2002; Luo and others 2004). Tissue damage due to cutting and washing initially results in higher REC, which gradually declines as the original damage heals during storage. However, REC increases toward the end of shelf life when the product quality deteriorates. Table 4 shows that lettuce washed with solutions containing T-128 follows a typical pattern similar to that of the control, and there was no significant ($P > 0.05$) difference between the control and T-128 treatments. Overall, examination of the major produce quality parameters indicate that T-128 at the described treatment condition had no detrimental effect on packaged fresh-cut lettuce quality and shelf life. It is expected that the impact of T-128 on produce quality attributes, if any, would be further reduced by instituting a rinse step following sanitizer washing, as commonly practiced in commercial fresh-cut operations.

HOCl, referred to as free chlorine, is the most reactive species of chlorine (Connell 1996). The stability of HOCl is pH dependent. At pH above 7.0, HOCl has an increased tendency for ionization and presents predominantly as protons and less active hypochlorite ions. At low pH, while the equilibrium favors deionization, HOCl tends to revert to Cl_2 and thus escapes from solution, a phenomenon generally known as "off gassing" (Connell 1996; Suslow 2001; Delaquis and others 2004). Therefore, low pH is generally not used for produce processing with chlorine wash solutions. Within the timeframe of the experiments, we have not observed rapid free chlorine loss from wash solution when pH is dropped to as low as 2.9 by adding 0.1% T-128.

Another significant problem associated with washing fresh produce at low pH is the impact on produce quality. For example, cut lettuce washed in 1.5% of lactic acid exhibited significant deterioration of visual and sensory qualities and acceptability to consumers (Lin and others 2002; McWatters and others 2002). In the current study, no detrimental effect to produce quality was observed when cut iceberg lettuce was washed in chlorine solution containing T-128. The ability to wash leafy greens at low pH without compromising produce quality can be further explored to increase the efficacy of microbial reduction.

Conclusions

In this study, we compared washing iceberg lettuce in chlorine wash solutions with the presence or absence of a reported chlorine stabilizer, T-128. We found that T-128 had low to moderate effects on chlorine stability in the presence of lettuce extract and soils. However, T-128 significantly reduced the survival of *E. coli* O157:H7 and *Salmonella* spp. in the washing solutions, even in the presence of high organic loads that nearly depleted chlorine in the washing solutions. Thus, T-128 significantly reduced the potential of pathogen cross-contamination during produce washing. Cut lettuce washed in chlorine solutions containing T-128 did not display any detrimental effect on produce quality. The ability to wash leafy greens at much reduced pH levels without compromising produce quality is an added advantage that can be further explored to enhance the efficacy of produce wash and minimize potential cross-contamination of bacterial pathogens.

Acknowledgment

The authors thank Drs. Robert Mandrel and Pina Fratamico, USDA-ARS, for providing bacterial strains, Dr. Eric Brennan, USDA-ARS, for lettuce production soil, and James Brennan for providing chlorine stabilizer T-128. This study is partially supported by grant nr 2009–74 from Center for Produce Safety (CPS), Univ. of California, Davis, Calif., U.S.A.

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