CENTER for PRODUCE SAFETY

CPS 2009 RFP
FINAL PROJECT REPORT

Project Title
Improving produce safety by stabilizing chlorine in washing solutions with high organic loads

Project Period
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Objectives
1. Evaluate the effect of T-128 on the stability of hypochlorous acid in the presence of high organic matter load; determine the influence of common produce wash operation conditions on the effectiveness of T-128.
2. Determine the efficacy of T-128 in enhancing pathogen reduction, reducing pathogen survival and transference in chlorinated wash water processing of leafy greens.
3. Determine the efficacy of T-128 in enhancing pathogen reduction, reducing pathogen survival and transference in chlorinated wash water processing of herbs, tomatoes, and cantaloupes.
4. Evaluate the impact of T-128 on produce quality and shelf life, and analyze the residue levels on finished products.

Abstract

Chlorine is widely used by the fresh and fresh-cut produce industry to reduce microbial populations and to prevent pathogen cross-contamination during produce washing. However, free chlorine degrades rapidly by reacting with organic materials released from cut produce. A novel food-grade (GRAS) chemical mixture, T-128 (previously known as F86-128), formulated by processing industry scientists and used under commercial wash conditions was purported to stabilize hypochlorous acid in fresh-cut leafy green wash systems with high organic load. Preliminary industry data also indicated that T-128 reduced surface microbial populations by more than 2 logs**.** Funded by the Center for Produce Safety, a USDA-ARS research team evaluated the effect of T-128:

1) on the stability of chlorine concentration in wash solutions containing large organic loads;
2) on enhancing pathogen reduction, reducing pathogen survival and preventing cross-contamination in chlorinated wash water used for processing of leafy greens;
3) on reducing pathogen survival and transference during tomato washing, and on determining the conditions needed to reduce pathogens in biofilms on stainless steel and on cantaloupe rinds, conditions which are characteristically resistant to pathogen inactivation;
4) on produce quality and shelf life.

Extensive laboratory studies and commercial pilot processing plant trials were conducted. Results show that:

1) T-128 is beneficial in maintaining a more stable level of free chlorine in the fresh-cut leafy green wash solutions containing a typical high level of organic material; and T-128 also significantly reduced chlorine degradation caused by foreign materials such as sandy or clay soils;
2) T-128 significantly reduced the potential for *E. coli* O157:H7 and *Salmonella enterica* to survive in the wash solution and cross-contaminate produce during washing when chlorine was nearly depleted, although the benefit in reducing pathogen populations on the leafy green produce *per se* is limited;
3) Significant reduction on *Salmonella enterica* on tomato stem-scar was achieved with T-128 in a reaction time dependant manner, and significant reductions in *Salmonella enterica* and *Pseudomonas fluorescens* survival in biofilms on netted surface cantaloupe rinds and on stainless steel surfaces were achieved with T-128 in a concentration dependent manner; and
4) T-128 at the recommended application rate showed no detrimental effect on fresh-cut lettuce quality, on samples processed either in our pilot plant or in commercial operation.

These results suggest that T-128 can be used to improve the safety of fresh produce safety at the washing stage of processing without compromising product quality.

Background

Fresh fruits and vegetables are nutrient-rich foods with high levels of minerals, vitamins, and phytochemicals. Encouraging the consumption of fresh produce is a key element of the produce industry's economic well-being, as well as national policy goals for nutrition and public health. However, recent outbreaks of food-borne illness associated with fresh produce have negatively impacted consumer confidence in the safety of fresh and fresh-cut produce. Thus, improving produce safety and restoring consumer confidence is an urgent, critical task for the produce industry and public health researchers.

In the absence of practical technologies that provide a “kill step” for eliminating pathogens without significantly diminishing produce quality, a sanitizing wash is critically important in fresh-cut produce processing, as it is a uniquely suitable stage to focus on pathogen inactivation. However, washing also has the potential to cause pathogen cross-contamination, especially when water is re-used and re-circulated. Therefore, the presence of a sanitizing agent in the wash water is critical for preventing pathogen survival and transfer. Currently, 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 the inactivation of pathogens. However, maintaining a stable level of hypochlorous acid in wash water during commercial fresh-cut wash operations is a technical challenge. When produce is introduced to the wash system, the sudden surge of organic matter creates a situation in which chlorine demand may exceed its addition, thus resulting in a rapid depletion of free chlorine in the wash system. Although replenishing chlorine periodically in wash solutions is common practice in fresh produce processing lines, repeatedly adding chlorine into high organic wash water generates unwanted chlorine by-products and chlorine off-gas into the processing environment.

Recently, produce industry scientists developed a novel chemical mixture, T-128 (originally named as F86-128), to stabilize hypochlorous acid in wash water in the presence of a high organic load and to improve microbial reduction during commercial operations. The purpose of this project was to provide independent third party verification and quantitative evaluation of the effectiveness of T-128 under diverse conditions and uses, as well as determining best practices for its use.

Research Methods and Results

Objective 1. Evaluate the effect of T-128 on the stability of hypochlorous acid in the presence of high organic matter load; determine the influence of common produce wash operation conditions on the effectiveness of T-128

Methodology:

Laboratory studies and pilot plant trials were conducted to evaluate the effect of T-128 on chlorine stability. In the laboratory studies, solutions initially containing 10-20 ppm free chlorine were prepared, with pH adjusted to 6.5, 5.0, or 2.9 by T-128 or citric acid (as a control). Challenge with organic loads and foreign materials were simulated using either lettuce juice extract (LJE, 0-2%) or soils from lettuce growing fields, respectively. The dynamic changes in free chlorine concentration and water quality were monitored following repeated addition of shredded lettuce or LJE and replenishment of sodium hypochlorite. In the pilot plant trials, shredded lettuce was continuously added to the commercial wash system equipped with a pH controlled automatic T-128 dosing system. Lettuce was washed at approximately 100 lb/min rate for 36 min per treatment per replication (3600 lb of lettuce processed). Water quality in both primary and secondary tanks was monitored every 2 min. Testing parameters include pH, free and total chlorine, turbidity, Brix, total dissolved solids (TDS), and chemical oxygen demand (COD). A detailed description of the pilot plant trial is included under objective 2. All data presented in this report are the average of at least three replications.

Key findings:

- Free chlorine degrades rapidly during fresh-cut wash operations, when shredded lettuce (Fig.1) or lettuce latex (Appendix 1) was incrementally introduced into the wash solution. Wash solutions in the presence of T-128 had more stable free chlorine concentrations than in the presence of citric acid, although the magnitude of this difference was variable. The benefit of T-128 in stabilizing chlorine was more evident during the later stage of processing when large amounts of organic materials were present in the wash solution.
- Laboratory studies were further validated by the pilot plant trials. Water and lettuce samples from both primary and secondary wash tanks were tested in our early trials. Water quality degenerated at a much slower rate in the secondary tank than in the primary tank. Therefore, our studies focused on the primary tanks. T-128 has no effect on the tested water quality parameters, including COD, TDS, and turbidity of the wash water, which increased dramatically with the increase in the amount of lettuce washed. No difference in these parameters was found between T-128 and the citric acid control (Fig. 2). However, higher levels of free chlorine were noted in the wash tank containing T-128, especially towards the latter part of the run, when very high organic load was present in the wash solution.
- Free chlorine was also significantly degraded by soils during whole and fresh-cut produce washing. When soils were introduced into chlorinated wash water, T-128 application resulted in significantly slower chlorine degradation than the control (Fig. 3). The benefit of T-128 in maintaining stable chlorine concentrations is more pronounced with soil than with lettuce.

Objective 2. Determine the efficacy of T-128 in enhancing pathogen reduction, reducing pathogen survival and transference in chlorinated wash water processing of leafy greens.

Methodologies:

Laboratory studies:

- *E. coli* O157:H7 strains ISEH-GFP, RM4406, and *Salmonella* strain ISSA-GFP were inoculated on lettuce surface and stored at 4 °C for overnight and the lettuce leaves were cut and washed in a series of wash solutions with varying LJE levels, in the presence and absence of T-128. Bacterial survival on the washed lettuce pieces and in spent wash water was determined using a microplate based modified most probable number (MPN) procedure. The effect of T-128 on the wash water antimicrobial efficacy was also tested using shredded lettuce as source of organic loads. Increments of 2 lb shredded lettuce were sequentially washed in 40 L of chlorinated water, with or without T-128. *E. coli* O157:H7 ATCC700728 was inoculated into aliquots of spent wash water after each washing and the surviving cells enumerated after 30 sec exposure.

- To test the effect of T-128 on pathogen cross-contamination, baby spinach leaves were inoculated with *E. coli* O157:H7 and *Salmonella* strains. The samples were washed together with uninoculated shredded iceberg lettuce in chlorinated wash solutions containing different concentration of LJE and T-128. Iceberg lettuce pieces were drained, sorted, and surviving bacterial cells were enumerated using the modified MPN procedure.

Pilot plant trials:

- Pilot plant trials were conducted in Salinas CA in 2010 and 2011, in collaboration with New Leaf Food Safety Solutions, LLC. The studies were carried out using a FTNON wash system consisting of primary and secondary wash tanks (each holds approximately 850 gallon of water). Pathogen survival and cross-contamination during produce wash were investigated by washing inoculated baby spinach (0.25% of the total load) together with un-inoculated shredded lettuce.
- Prior to the wash test, freshly harvested/cooled baby spinach leaves were spray inoculated with a nalidixic acid resistant strain of a BSL-1 non-pathogenic E. coli O157:H7 strain, ATCC 700728. The spinach was stored at 5 °C for 48 hrs to allow the inoculum to attach to leaf surfaces. Pre-cored iceberg lettuce heads were weighed to approximately 50 lb per tote.
- 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 aliquots of sodium hypochlorite were added to the wash solution to achieve approximately 20 ppm free chlorine initially in the wash water. The pH was simultaneously adjusted to pH 5.0 using either citric acid (Control) or T-128. Water quality and free chlorine concentration were monitored and the test run started within 5 min of adding the chlorine solution. Pre-cored iceberg lettuce was shredded and immediately discharged to the conveyer belt at a rate of 2 totes (100 lb) per minute. Pre-weighed spinach leaves were manually spread onto the conveyer belt adjacent to but separated from the lettuce at a 0.25% spinach to lettuce ratio. The spinach and lettuce shreds were mixed and submerged upon entry into the wash solution. Lettuce and spinach samples were collected separately using strainer baskets. Water, spinach and lettuce samples were collected before starting the run and every 2 min during washing. After 12 min. or approximately 1200 lb lettuce/spinach washed, the operation was paused. Additional sodium hypochlorite (1050 ml) was added to the primary washer and the 2nd segment of wash process was started. After 12 min, the process was paused, and additional sodium hypochlorite (1400 ml) was added. The 3rd segment was started, and lasted for another 12 min.
- The water quality parameters tested include free chlorine, pH, turbidity, and COD. Chlorine neutralizer reagent was added to the water samples used for pathogen enumeration upon sample collection. Water samples and duplicate 25-g samples of washed lettuce from each sampling point were tested for *E. coli* O157:H7 using a modified MPN method (Appendix 1). Survival of *E. coli* O157H7 on washed spinach leaves was examined using a direct plating method.

Key findings:

1. Application of T-128 resulted in a significant reduction in bacterial pathogen survival in wash solution (Table 1) and hence the potential for cross-contamination (Table 2), when free chlorine levels declined to near depletion as increasing concentrations of lettuce juice was added to the wash water. Similar improvement for the reduction of bacterial cells attached to lettuce was not observed (Table 1). The effect of T-128 on reduction of pathogen survival in wash solution was also observed when increments of shredded lettuce were washed in chlorine waters to provide organic loads (Fig. 4).

2. Pilot test results indicated that pathogen survival and cross-contamination frequently occurred when free chlorine in solution dropped below 1 ppm during the wash process; T-128 significantly reduced the potential for *E. coli* O157:H7 to survive in wash water (Fig. 5) and to cross-contaminate un-inoculated iceberg lettuce when the lettuce was washed together with inoculated baby spinach (Fig. 6). 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 T-128 (Fig. 7).
3. When used alone, T-128 at either a high concentration or an extended reaction time was able to inactivate *E. coli* O157:H7 in suspension (Fig. 8, left). Additionally, when combined with low level of free chlorine, there appears to be a significant increase in the bactericidal effect of the combination (Fig. 8, right), suggesting a synergistic interaction between T-128 and chlorine may be responsible for the increased bactericidal activities.

Objective 3. Determine the efficacy of T-128 in enhancing pathogen reduction, reducing pathogen survival and transference in chlorinated wash water processing of herbs, tomatoes, and cantaloupes.

Methodology:

- **Tomatoes:** Increasing levels of tomato juice were added to chlorinated wash solutions to simulate free chlorine depletion during tomato washing. *S. enterica* sv. Thompson was inoculated into chlorinated wash solutions, with and without T-128, and *Salmonella* inactivation was determined after 0.5 and 2 min exposure. Effect of T-128 in preventing infiltration by *Salmonella* was evaluated by examining the presence of *Salmonella* cells in tomato internal tissues following washing in chlorinated solution in the presence or absence of T-128.
- **Biofilms on cantaloupes and stainless steel coupons**: Bacterial biofilms of *Salmonella enterica* or *P. fluorescens* formed on cantaloupes or stainless steel coupons were washed in chlorine solutions with or without T-128. Biofilm cell populations on coupons were dispersed and enumerated. Biofilm cell responses to fluorescent viability stains after treatment with washing solutions were examined using confocal laser-scanning microscopy after a live-dead bacterial stain.
- **Herbs**: The effect of T-128 on pathogen reduction on herbs was not tested. The industry partner informed us after the grant was awarded that their recently completed preliminary tests with T-128 on cilantro and parsley resulted in unacceptable discolorations. Therefore, they recommended that we not pursue the herb experiments with T-128 at this point.

Key findings:

1. **Tomatoes:** Similar to leafy green vegetables, T-128 showed significant benefit in preventing pathogen survival and cross-contamination when washing inoculated (with *S. enterica* sv. Thompson) and un-inoculated tomatoes in the solution when chlorine was degraded by high organic load (Table 3). Additionally, T-128 also showed benefit in reducing pathogen infiltration (Table 4), and population reduction on stem-scars when the tomatoes were treated with a combination of T-128 and chlorine for 2 min, but not for 30 sec.

2. **Cantaloupes**: Use of T-128 significantly reduced the natural microflora and *Salmonella* bacterial biofilms at chlorine levels of 500-1000 ppm, resulting in ~1-2 log CFU/cm^2 less survival of these microbes on rind than when melons were washed in the same chlorinated wash without T-128 (Fig. 9). No significant reduction in pathogen populations resulted from use of T-128 on cantaloupe with < 500 ppm free chlorine in pH 5 wash water; the benefit of microbial reduction on rinds was enhanced with 2000 ppm free chlorine and T-128 at pH 2.8 (data not shown).
3. **Biofilm on stainless steel surfaces**: For both *Salmonella* and *Pseudomonas* biofilm cells on stainless steel coupon, the sanitizing effect of free chlorine (1.0-5.0 ppm) was enhanced when combined with T-128. Image analysis of surfaces further corroborated the cultural assay results (Fig. 10)

Objective 4. Evaluate the impact of T-128 on produce quality and shelf life, and analyze the residue levels on finished products.

Methodologies:

Shredded iceberg lettuce was washed in chlorine solutions with pH 6.5 adjusted with citric acid, or with 0.1% T-128. Washed lettuce was drained, dewatered and packaged, and stored at 15°C for 15 days. Samples were evaluated periodically for O_2/CO_2 partial pressures, visual quality, and lettuce tissue integrity etc.

Key findings:

The visual scores of iceberg lettuce from citric acid or T-128 treatment were similar and rated within acceptable quality range after 15 days storage. Up to day 4, O_2 partial pressure decreased and CO_2 partial pressure increased in both citric acid and T-128 treated samples. Up to day 15, the variance tendency of O_2 and CO_2 partial pressure, and tissue electrolyte leakage REC values were similar in both citric acid and T-128 treatments (Table 5).

Outcomes and Accomplishments

From 09/2009 to 09/2011, USDA-ARS research scientists and post-doctoral research associates successfully evaluated the performance of T-128 as a produce wash aid for improving fresh-cut produce safety. All of the proposed research activities were fully completed. In addition to the extensive laboratory studies, large scale pilot plant trials were conducted in Salinas, CA, working closely with our industry collaborator. Our research results quantitatively evaluated the efficacy of T-128 on chlorine stabilization, pathogen reduction, cross-contamination prevention, and biofilm disinfection with different types of fresh produce and washing conditions. This research initiative provides commercial scale results to fresh produce processors who are reviewing the technology and making decisions regarding T-128. Results provide valuable insights for produce safety research that addresses the real-world contamination problem at the washing stage of product processing. One manuscript has been published, and three additional papers are being reviewed by journals. Beyond the direct scientific and commercial impacts, the accomplishments of this project also strongly attest to the benefits and success of the strategic collaborations fostered by CPS between fresh produce industry processors and the USDA-academia research partners.

Summary of Findings and Recommendations

In summary, our research provides an objective evaluation of the effect of T-128 on chlorine stabilization, pathogen reduction, cross-contamination prevention, and pathogen biofilm sanitization in high organic load washing conditions. The extensive laboratory and pilot-scale tests addressed the major questions posed in the initial CPS-RFP for the project:

a) How does the adjuvant T-128 in combined use with hypochlorous acid compare to other common (hypochlorous acid, etc) wash water sanitizers in the presence of high organic loads?
   - Enhanced maintenance of the active antimicrobial in solution
   - Ability to maintain microbial control of the wash solution
   - Prevention of leaf to leaf cross-contamination

b) At what level of organic material, pH or temperature does the enhanced stabilization of hypochlorous acid by T-128 breakdown?

c) Is T-128 effective in wash systems designed for vegetables and fruits other than leafy-greens?

Our results show that:

- T-128 is beneficial in maintaining a more stable chlorine concentration under the conditions of typical high organic loads in process wash water (RFP focus a and b above; Objective 1);
- T-128 significantly reduced the potential for pathogen survival in solution and cross-contamination when free chlorine in the process wash water was near depletion (RFP focus a and b; Objective 2);
- T-128 significantly reduced survival of pathogens on tomatoes and infiltration through the stem scars; and also reduces pathogen populations in biofilms formed on either cantaloupe rind or stainless steel surfaces in a concentration, pH, and exposure time dependent manner (RFP focus c; Objective 3);
- T-128 at the recommended application rate showed no detrimental effect on fresh-cut lettuce quality (Objective 4). We did not test for T-128 residuals on the produce or in the wash water because the industry partner provided us a letter from the FDA indicating that they had no objection to the use of the T-128 formulation for washing fresh-cut produce.

These results suggest that T-128 can be used to improve the safety of fresh produce at the washing stage of processing without compromising product quality.

Disclaimer

Mention of a company name or product by the USDA does not imply approval or recommendation of the product to the exclusion of others that also may be suitable for the intended purpose and conditions.

Tables and Figures

Table 1. Recovery of *Salmonella* enterica and *E. coli* O157:H7 Populations on Inoculated Lettuce Surfaces and in Wash Water after Chlorine Treatment

Wash Solution	Lettuce Extract (v/v %)	Bacterial Population Remaining on Inoculated Lettuce (log CFU/g)			Bacterial Recovery in Solution (log CFU/ml)*		
		ISSA-GFP*	ISEH-GFP	RM4406	ISSA-GFP	ISEH-GFP	RM4406
Chlorine Water	0	3.1 ± 0.6	3.5 ± 0.5	3.1 ± 0.6	ND	ND**	ND
	0.25	3.2 ± 0.4	3.0 ± 1.1	2.6 ± 0.4	ND	ND	ND
	0.5	3.0 ± 0.7	3.0 ± 0.5	2.6 ± 0.5	ND	< 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	ND	ND	ND
	0.25	3.2 ± 0.6	3.4 ± 0.4	2.7 ± 0.6	ND	ND	ND
	0.5	3.4 ± 0.4	3.5 ± 0.4	2.9 ± 0.5	ND	ND	ND
	1.0	3.7 ± 0.3	3.8 ± 0.1	3.4 ± 0.2	ND	ND	ND
	2.0	3.7 ± 0.4	3.5 ± 0.5	3.2 ± 0.6	ND	ND	ND

** ISSA-GFP: S. enterica sv Typhimurium strain; ISEH-GFP and RM4406: E. coli O157:H7 strains.*
***ND: Cell count below detectable limit.*

Table 2. Recovery of *E.coli* O157:H7 and *Salmonella* on Non-Inoculated Lettuce after Washing with Inoculated Baby Spinach

Wash Solution	Lettuce Extract (v/v %)	Bacterial Survival on Non-Inoculated Lettuce (CFU/g)	
		RM4406	ISSA-GFP
Water	0	79.4 ± 2.0	63.1 ± 1.3
Chlorine water	0	ND*	< 0.2**
	0.5	ND	< 0.2
	1.0	10.0 ± 5.0	10.0 ± 4.0
Chlorine water + 0.05% T-128	0	< 0.2	ND
	0.5	ND	< 0.2
	1.0	0.2 ± 0.2	< 0.2
Chlorine water + 0.1% T-128	0	ND	ND
	0.5	< 0.3	ND
	1.0	< 0.2	< 0.5

*ND-cell count below detection limit; RM4406-*E.coli* O157:H7; ISSA-GFP: *Salmonella* enterica.

Table 3. Effect of T-128 on the Survival of *S. enterica* sv. Thompson in Wash Solution

Free Chlorine (ppm)*	*Salmonella* Survival (log MPN/ml)		
	Control	T-128, pH 3.0	T-128, pH 5.0
0	5.5 ± 0.2	5.5± 0.1	5.5 ± 0.3
1/8	2.0 ± 0.1	ND**	ND
1/4	1.4 ± 0.1	ND	ND
1/2	ND	ND	ND
1	ND	ND	ND
5	ND	ND	ND

** Chlorine level was achieved by adding tomato juice to wash solution containing 10 ppm free chlorine.*
***ND indicates cell survival rates below the detection limit.*

Table 4. Effect of T-128 on *Salmonella enterica* infiltration

Free chlorine (ppm)*	No. of Positives with Infiltration**				Average MPN/g Internal Tissue			
	Control		T-128		Control		T-128	
	Strain 1987	Strain 2757	Strain 1987	Strain 2757	Strain 1987	Strain 2757	Strain 1987	Strain 2757
0	10	10	10	10	66.9 ± 29.1	52.0 ± 21.2	58.8 ± 22.3	51.1 ± 12.3
0.02	8	8	3	3	28.9 ± 22.5	15.2 ± 15.3	2.8 ± 2.2	3.8 ± 3.5
0.1	5	5	0	0	6.6± 4.9	7.1 ± 1.3	ND***	ND
0.5	2	2	0	0	0.6 ± 0.3	1.1 ± 0.3	ND	ND
1	0	0	0	0	ND	ND	ND	ND
5	0	0	0	0	ND	ND	ND	ND

**Chlorine level was achieved by adding tomato juice to wash solution containing 10 ppm free chlorine.*
*** Each treatment had a total of 15 tomatoes (three replicates of 5 tomatoes each).*
**** ND indicates non-detectable. Calculated detection limit is 0.38 MPN/ml.*

Table 5. Effects of T-128 on Lettuce Quality Attributes

Quality Attributes and Treatment		Storage Time (days)				
		0*	4	7	11	15
O_2 (%)						
	Control	1.0	0.2 ± 0.3	0.5 ± 0.3	1.4 ± 0.3	7.7 ± 0.1
	T-128	1.0	0.1 ± 0.0	0.4 ± 0.1	0.4 ± 0.0	9.0 ± 0.0
CO_2 (%)						
	Control	0.0	6.7 ± 0.2	4.2 ± 2.0	3.3± 1.9	4.7 ± 0.5
	T-128	0.0	6.5 ± 0.9	4.4 ± 1.3	4.8 ± 0.1	4.9 ± 0.1
Visual Score						
(1-9)	Control	9.0	8.3 ± 0.0	8.0 ± 0.0	5.8 ± 0.4	5.6 ± 0.8
	T-128	9.0	8.2 ± 0.2	7.2 ± 0.4	6.0 ± 0.0	5.4 ± 0.9
Relative EC						
(%)	Control	10.8 ± 0.6	6.3 ± 0.5	4.6 ± 1.7	2.2 ± 0.2	3.5 ± 0.2
	T-128	10.4 ± 1.6	4.7 ± 0.3	3.2 ± 0.3	3.0 ± 0.1	3.7± 0.3

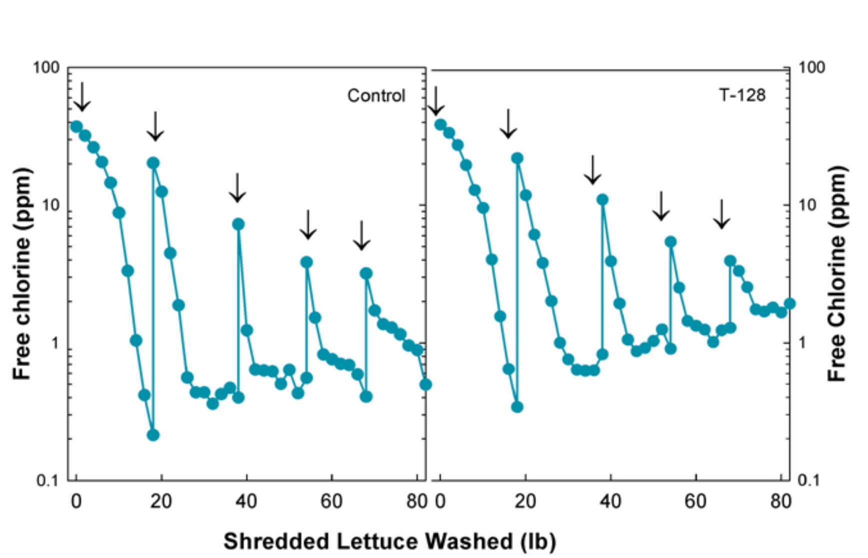

Figure 1. Dynamic changes in free chlorine in wash solution with repeated addition of shredded lettuce and replenishment of sodium hypochlorite (arrows).

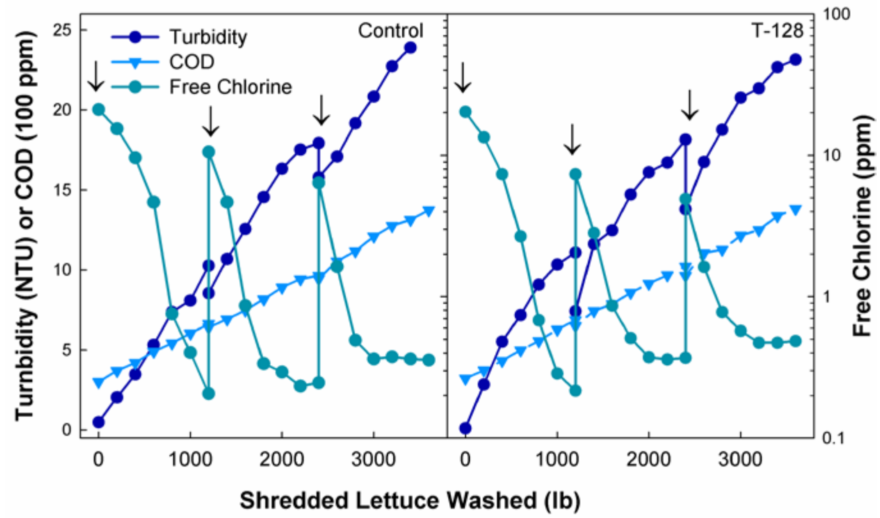

Figure 2. Pilot plant trial: dynamic changes in wash water COD, turbidity, and free chlorine during washing of shredded iceberg lettuce. Lettuce shreds were continuously introduced to the wash system at a 100 lb/min. An increased amount of sodium hypochlorite was added at the beginning of the run, and after 1200, and 2400 lb of lettuce processed (arrows).

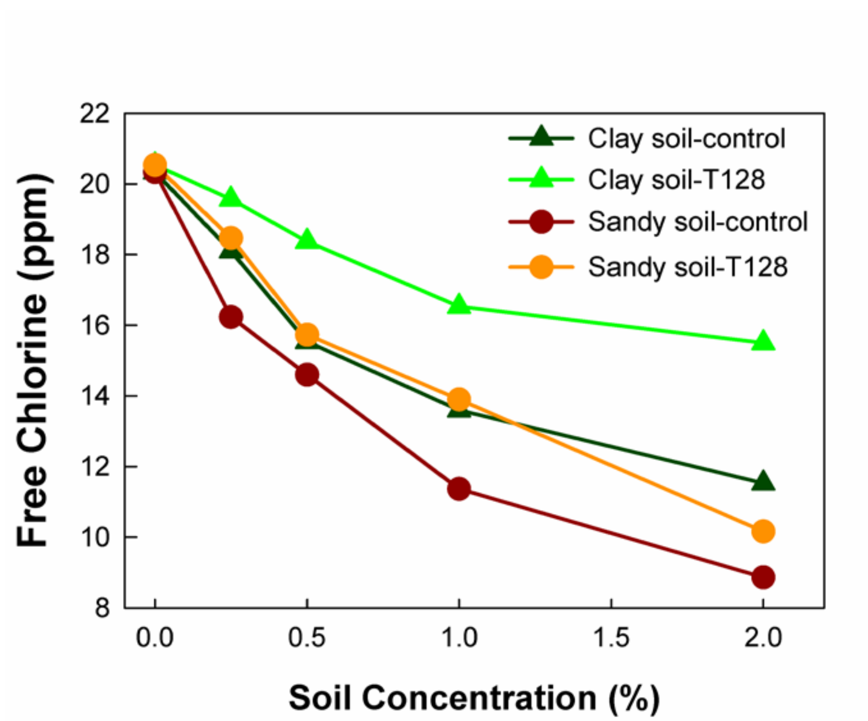

Figure 3. Changes in free chlorine in the wash solution as impacted by soils in the presence or absence of T-128. Clay and sandy soils were obtained from lettuce production fields in Salinas CA.

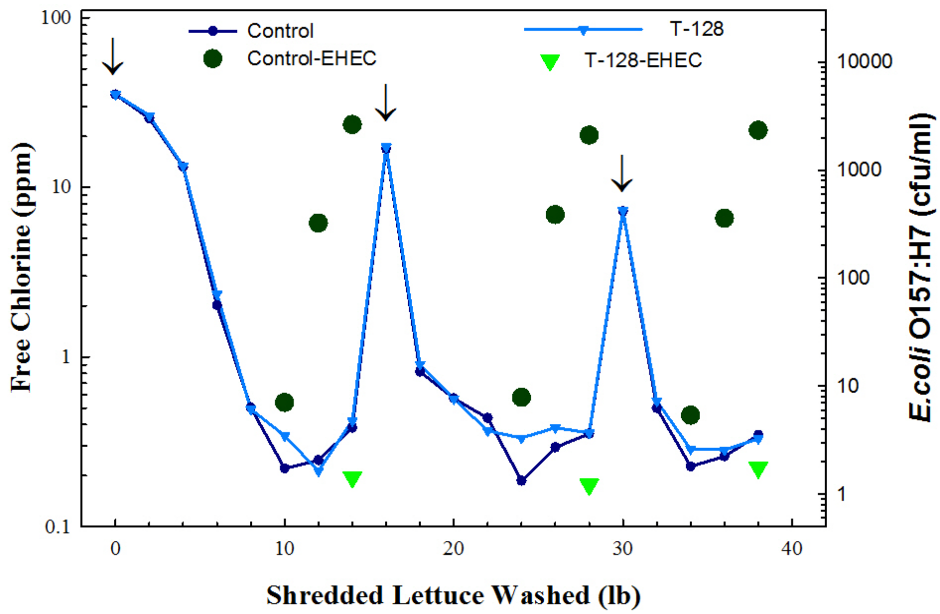

Figure 4. The effect of T-128 on wash water chlorine levels (blue lines) and on the inactivation of *E. coli* O157:H7 in spent wash water (green circles and triangles). Arrows indicate points of sodium hypochlorite addition. Data points with cell counts below the detection limit are not shown.

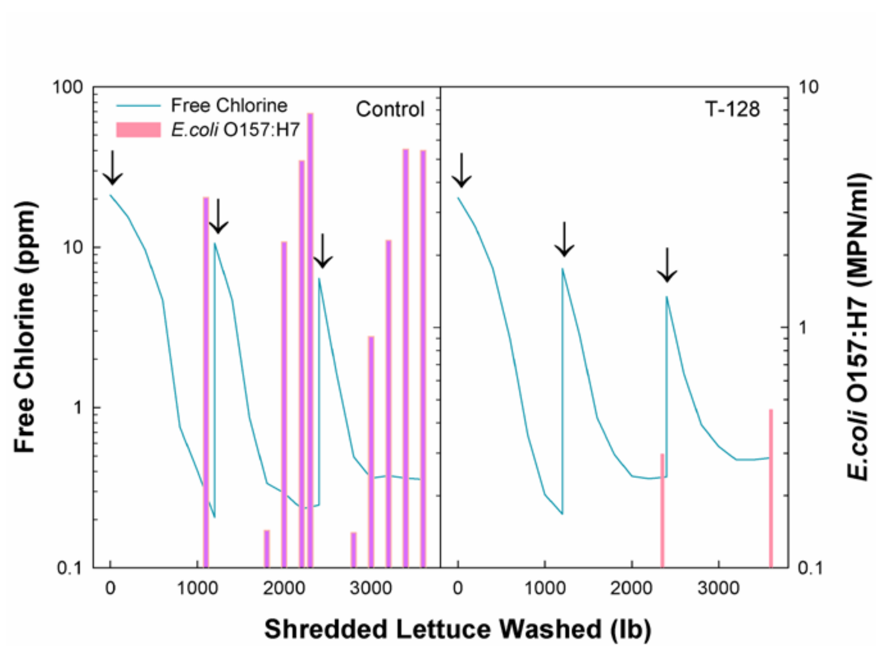

Figure 5. Pilot plant trial: The effect of T-128 on recovery of *E.coli* O157:H7 in the process wash water after continously washing un-inoculated lettuce with inoculated baby spinach in chlorinated water and replenishing sodium hypochlorite (arrows).

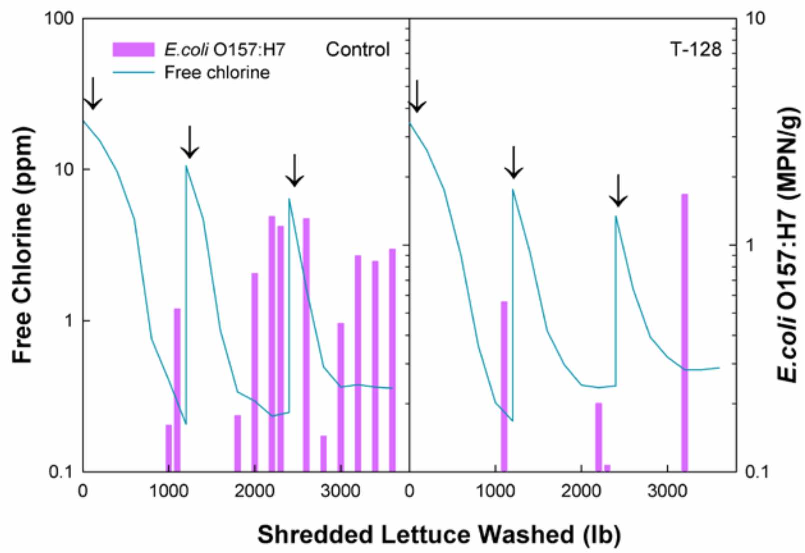

Figure 6. Pilot plant trial: The effect of T-128 on the recovery of *E.coli* O157:H7 in un-inoculated iceberg lettuce after continously washing un-inoculated lettuce with inoculated baby spinach in chlorinated water. Arrows denote the point when sodium hypochlorite was added.

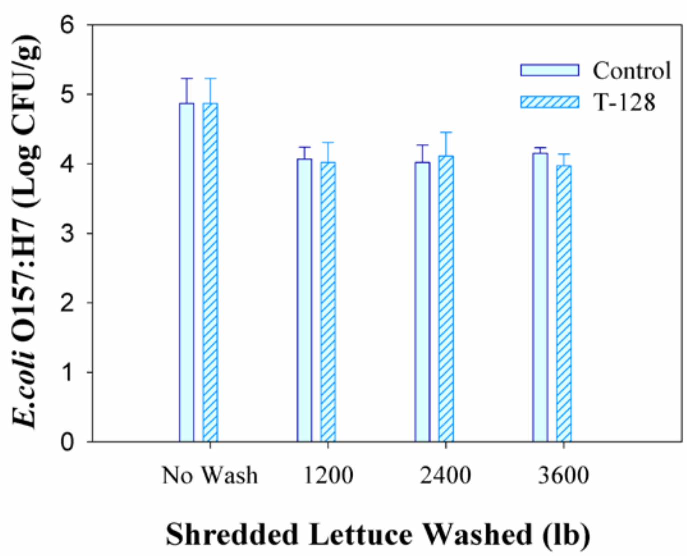

Figure 7. Pilot plant trial: The effect of T-128 on the recovery of *E.coli* O157:H7 on inoculated spinach leaves.

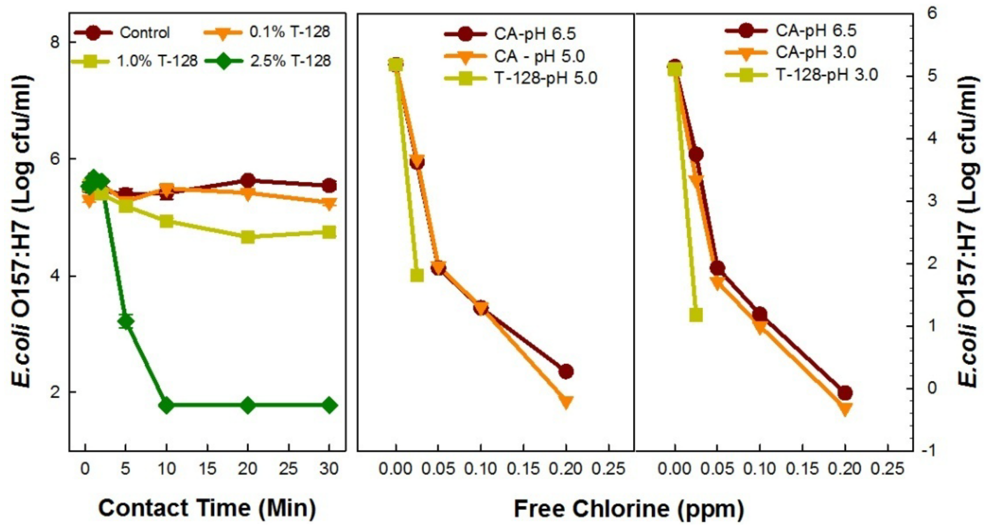

Figure 8. Left: Bactericidal activity of T-128. Cells were exposed to different concentrations of T-128 for various times before enumeration; Right: The effect of free chlorine concentration on *E. coli* O157:H7 inactivation as impacted by T-128 application. CA refers to citric acid.

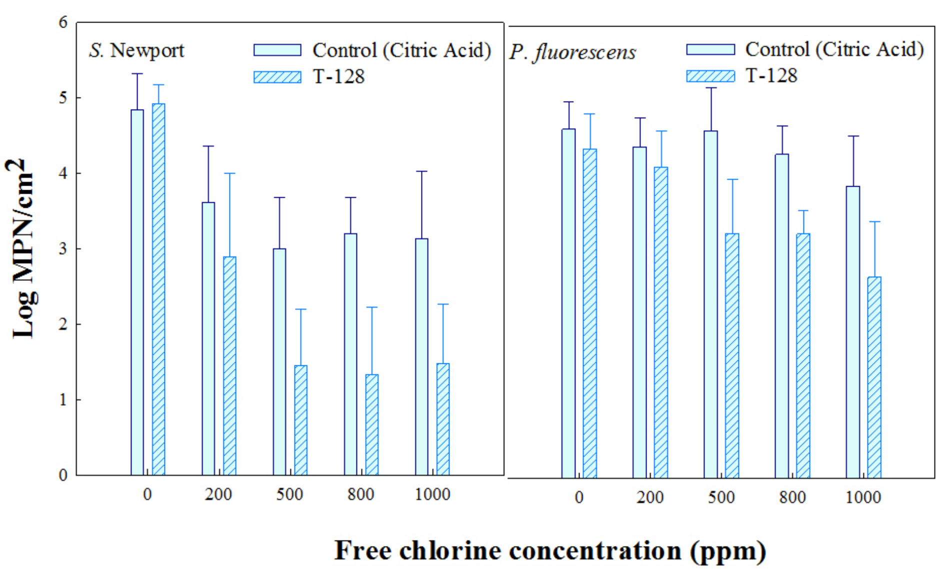

Figure 9. Bactericidal effect of T-128 compared to citric acid in chlorinated wash water at pH 5.0 on *Salmonella* Newport and *Pseudomonas fluorescens* in biofilms on cantaloupe rinds.

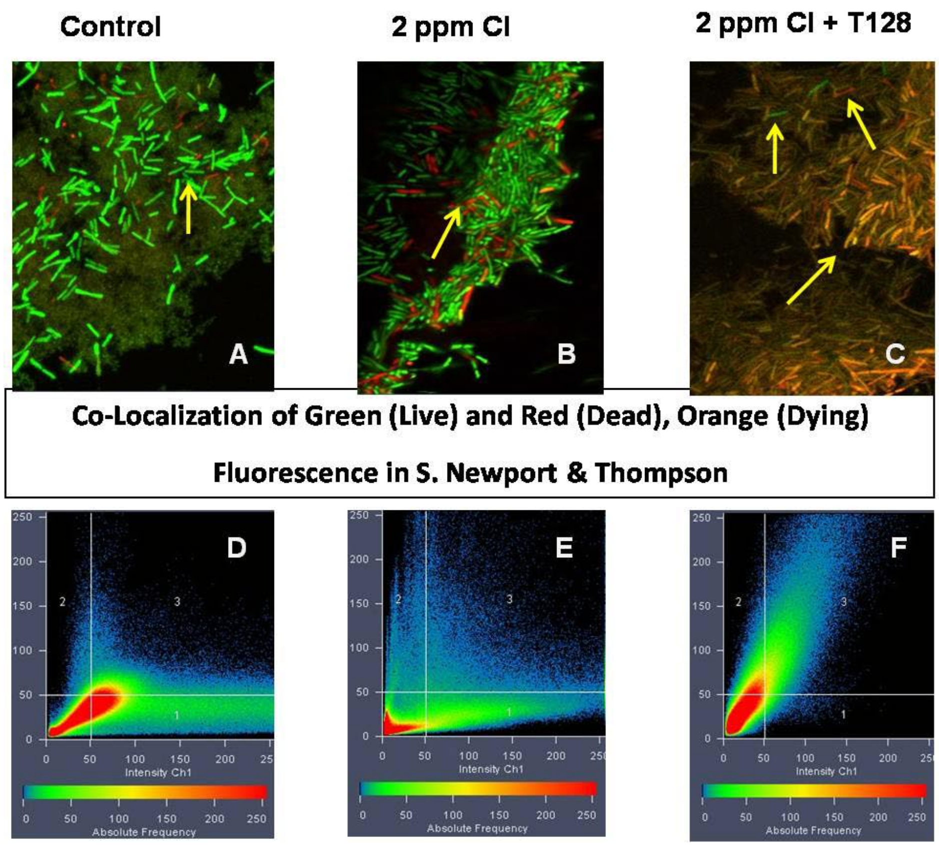

Figure 10. Confocal laser-scanning microscopy images of *Salmonella* Thompson and Newport survival in biofilms exposed to chlorine solutions at pH 5.0, with and without T-128. A*)* Untreated control biofilm, green = live cells; B) Chlorinated water (pH 5.0 citric acid) treated biofilm; red = dead cells; C) Chlorinated water with 0.1% T-128 (pH 5.0); orange, yellow, and red = membrane damaged and dead cells; D, E, and F) Co-localization intensity graphs show semi-quantitatively amounts of live (x-axis) vs. dead (y-axis) cells corresponding to A, B, and C frames above.

APPENDICES

Publications and Presentations (required)

Appendix 1. A peer-reviewed journal article titled "Chlorine stabilizer T-128 enhances efficacy of chlorine against cross-contamination by *E. coli* O157:H7 and *Salmonella* in fresh-cut lettuce processing" published on Journal of Food Science 2010. 76:218-224.

Appendix 2. An oral presentation titled "Improving Produce Safety by Stabilizing Chlorine in Washing Solutions with High Organic Loads", Center for Produce Safety Symposium, Orlando, FL, June, 2011.

Appendix 3. An oral presentation titled "Chlorine stabilizer T-128 enhances efficacy of chlorine against cross-contamination by *E. coli* O157:H7 and *Salmonella* in fresh-cut lettuce processing", Institute of Food Technologists (IFT) annual meeting, New Orleans, LA, June, 2011.

Appendix 4. An oral presentation titled "Effects of Novel Chlorine Stabilizer in Washing Solutions with High Organic Loads", Produce Marketing Association annual meeting, Orlando, FL, October, 2010.

Appendix 5. A poster presentation titled "Improving Produce Safety by Stabilizing Chlorine in Washing Solutions with High Organic Loads", Center for Produce Safety Symposium, Davis, CA, June, 2010.

Budget Summary (required)

USDA Team ($ 243,909)

The funds from CPS were received as four installment payments. The majority of the funds were used to support a postdoctoral research associate working on this project. The remaining funds were used for experiment materials and travel for food science conference attendance as detailed below:

- Salary for a Visiting Scientist/Postdoctoral Research Associate and other student interns.
- Routine lab supplies and consumables (chlorine, lettuce, multi-channel pipette, tips, media, micro-plates, culture broth, etc.).
- The majority of the lab supplies provided by the CPS grant were used to support the four week-long large pilot-plant trials conducted by a team of seven USDA researchers. This include purchasing essential supplies needed to set up a mobile lab in Salinas CA, and routine lab supplies large quantities of water and microbial tests per day. Specifically, items purchased include sterile filter stomacher bags, single and multi-channel pipetters and sterile tips, microbial media, COD digestion vials, general and selective media and media bottles, chlorine testing powders, EHEC serological confirmation test kits, timers, culture broth, petri plates, sterile disposable and general lab ware, etc.).
- Travel for PI Luo, and Co-PIs Nou and Millner to attend project related professional meeting including CPS, IFT and IAFP symposia (Orlando, FL; New Orleans, LA; and Milwaukee, WI).

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

Xiangwu Nou, Yaguang Luo, LaVonda Hollar, Yang Yang, Hao Feng, Patricia Millner, and Daniel Shelton

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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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), 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

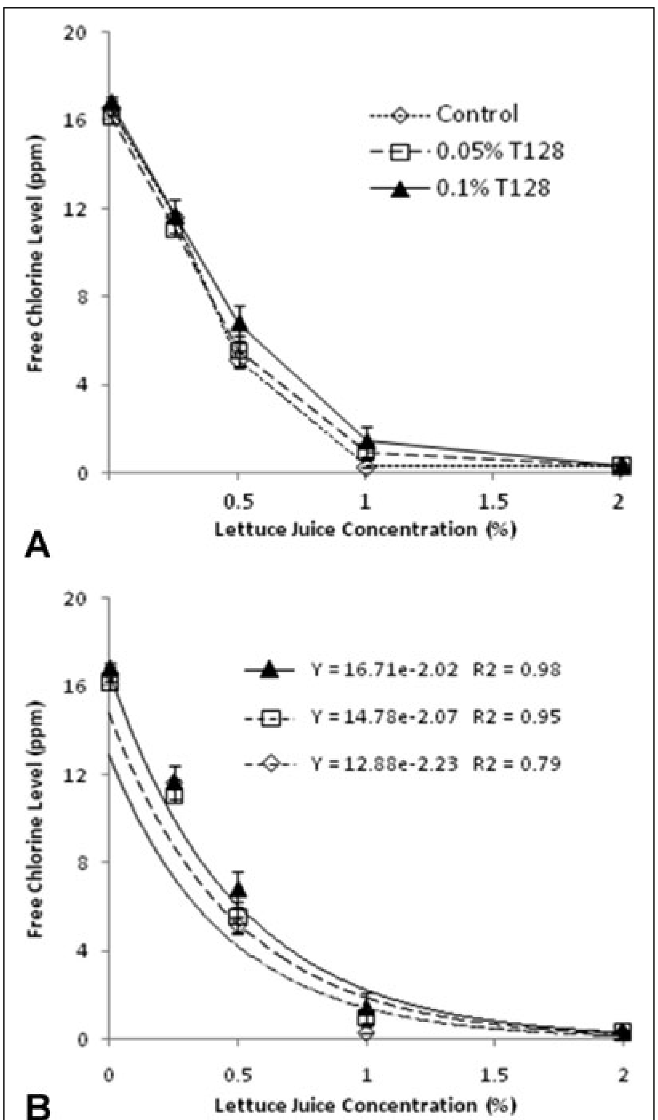

Figure 1–Effect of organic loads and T-128 (0.05% and 0.1%) on free chlorine level in wash solutions. Lettuce extract was used as the organic source. Wash solutions were exposed to the source of organic loads for 1 min before samples were taken for free chlorine measurement. (A) Data presented as line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

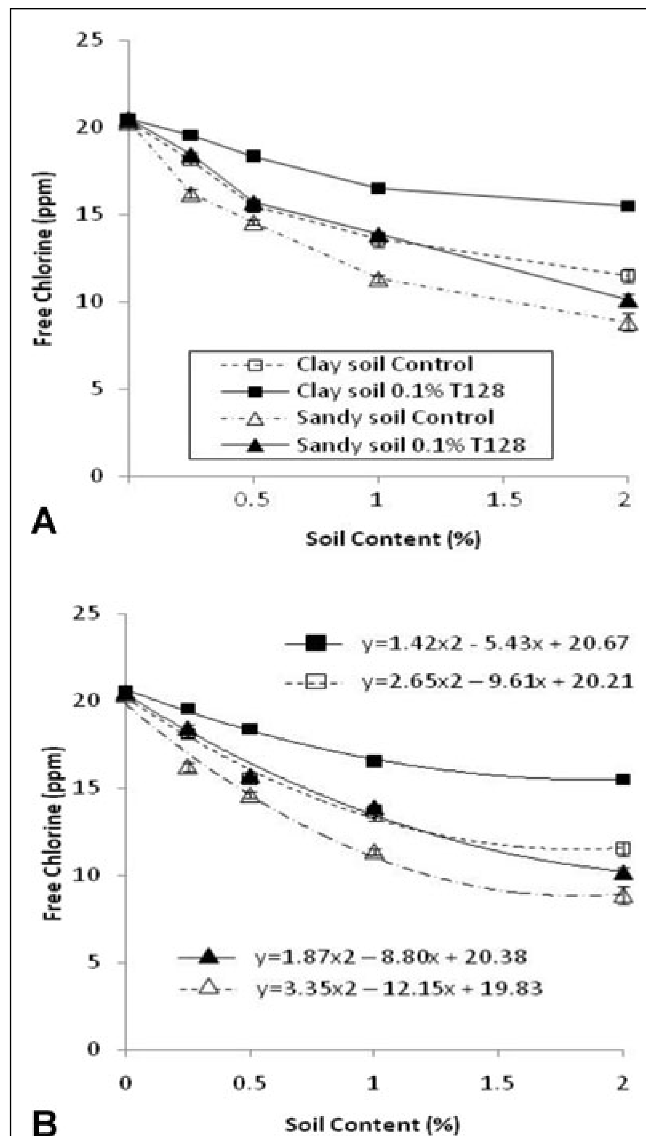

Figure 2–Effect of organic loads and T-128 (0.1%) on free chlorine level in wash solutions. Clay and sandy soils from lettuce production fields were used as the organic source. Wash solutions were exposed to the source of organic loads for 1 min before samples were taken for free chlorine measurement. (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.

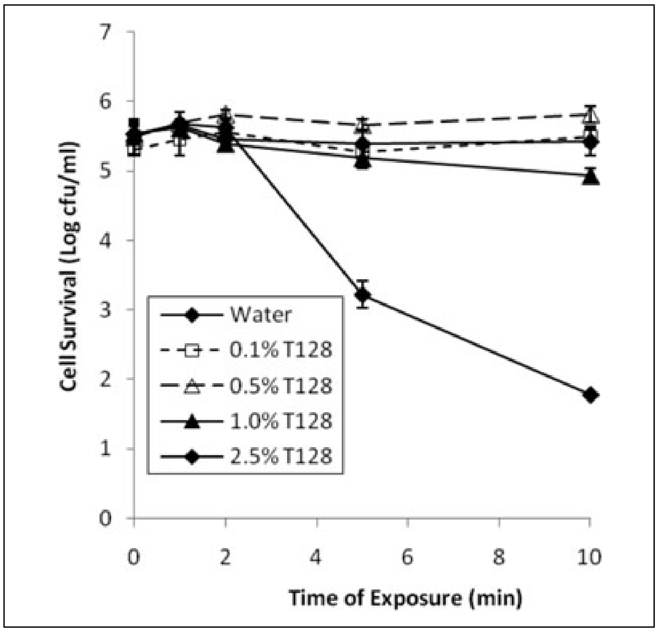

Figure 3–Bactericidal activity of T-128. *Escherichia coli* O157:H7 strain B6–914 (pGFP) was exposed to different concentrations of T-128 for indicated length of time before enumeration using an MPN procedure. Calculated detection limit used in this assay is 1.78 $\log_{10}$ CFU/mL.

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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Improving Produce Safety by Stabilizing Chlorine in Washing Solutions with High Organic Loads

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Introduction

Research Problem:

- Chlorine is depleted in wash solutions, leading to potential pathogen survival and cross-contamination.

Innovative Resolution:

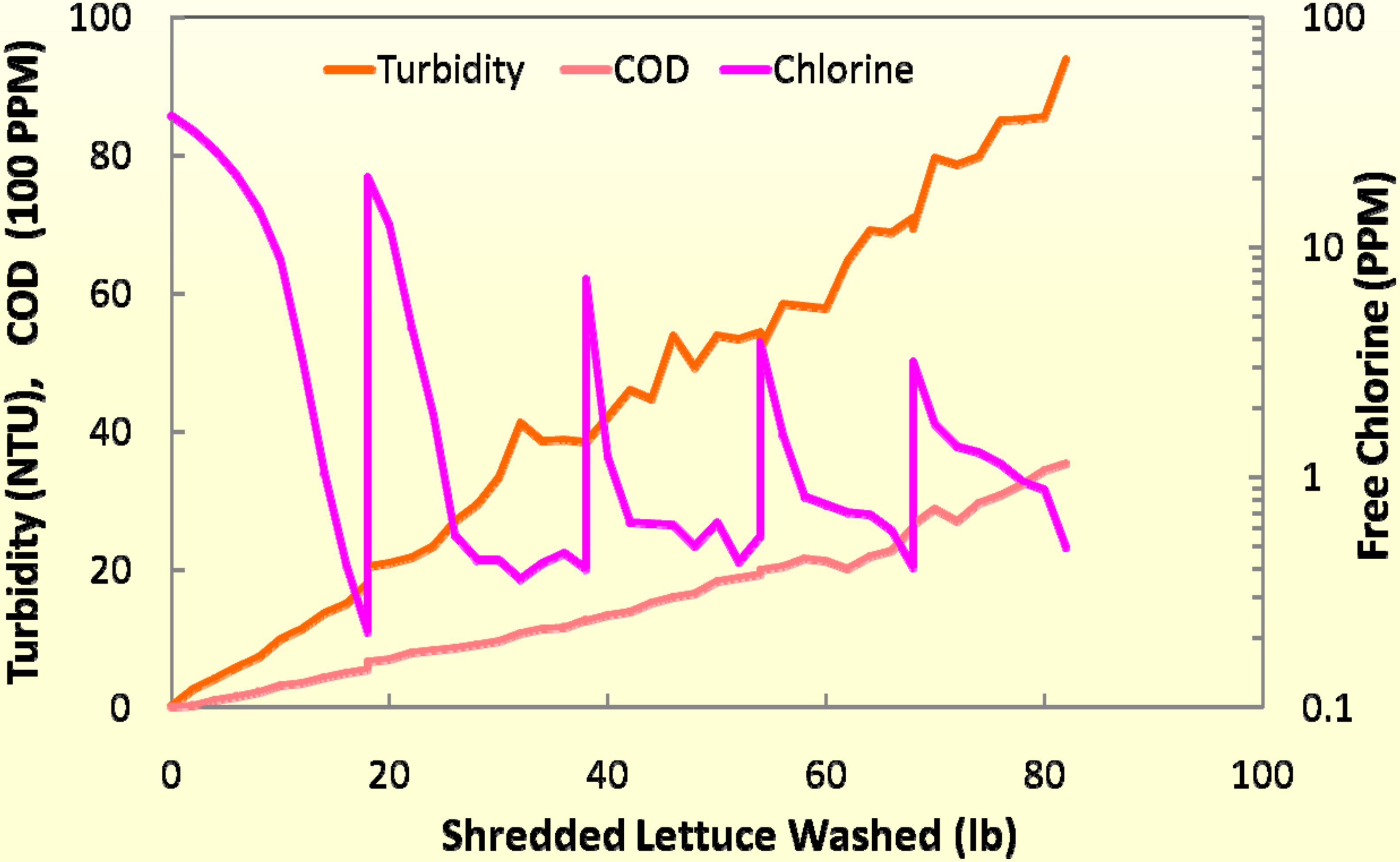
- Industry scientists formulated T-128.
- Automated pH control.

Objective:

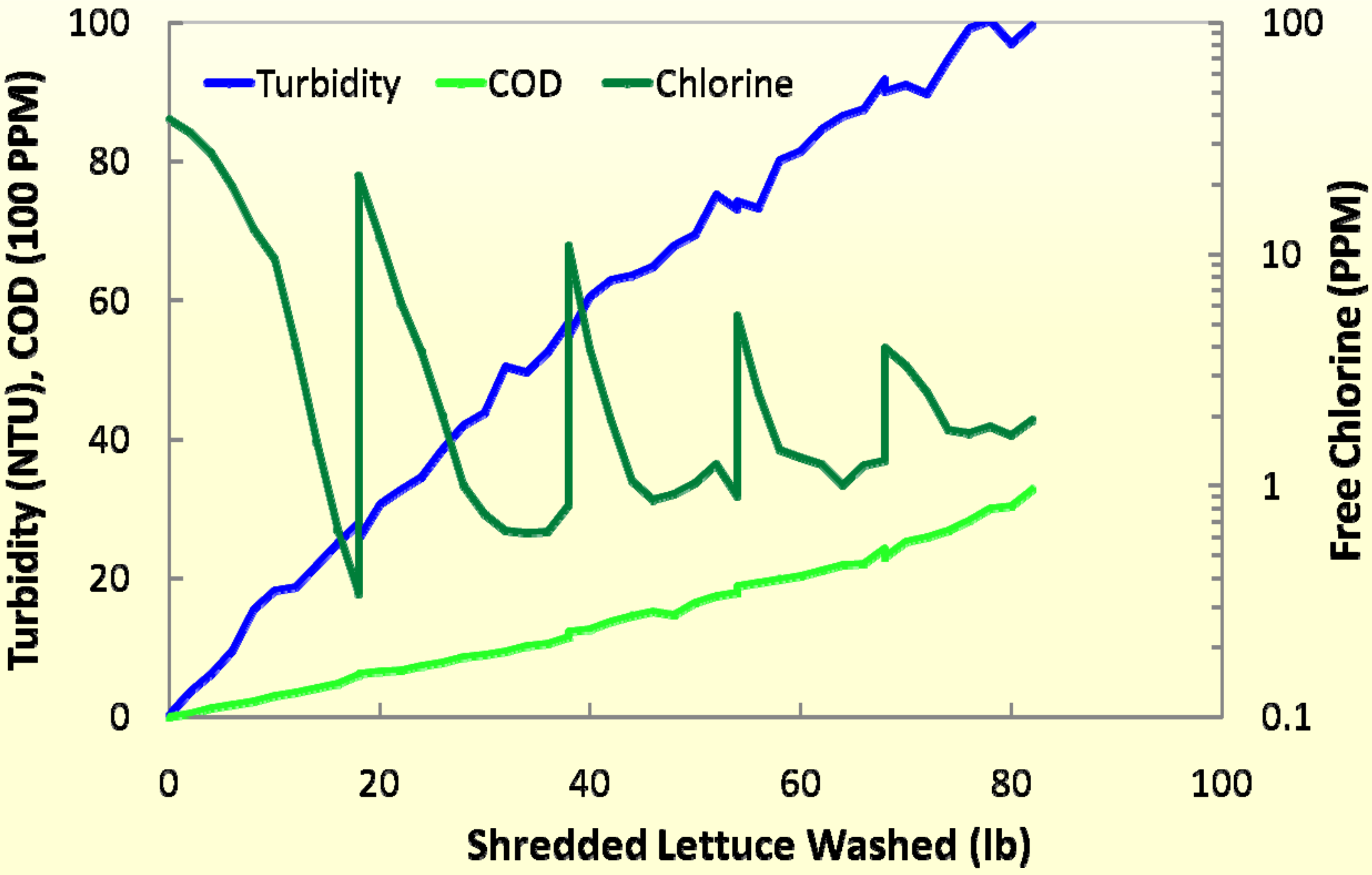
- Evaluate T-128 efficacy against pathogen survival and cross-contamination in both laboratory and pilot plant settings.

Laboratory Studies

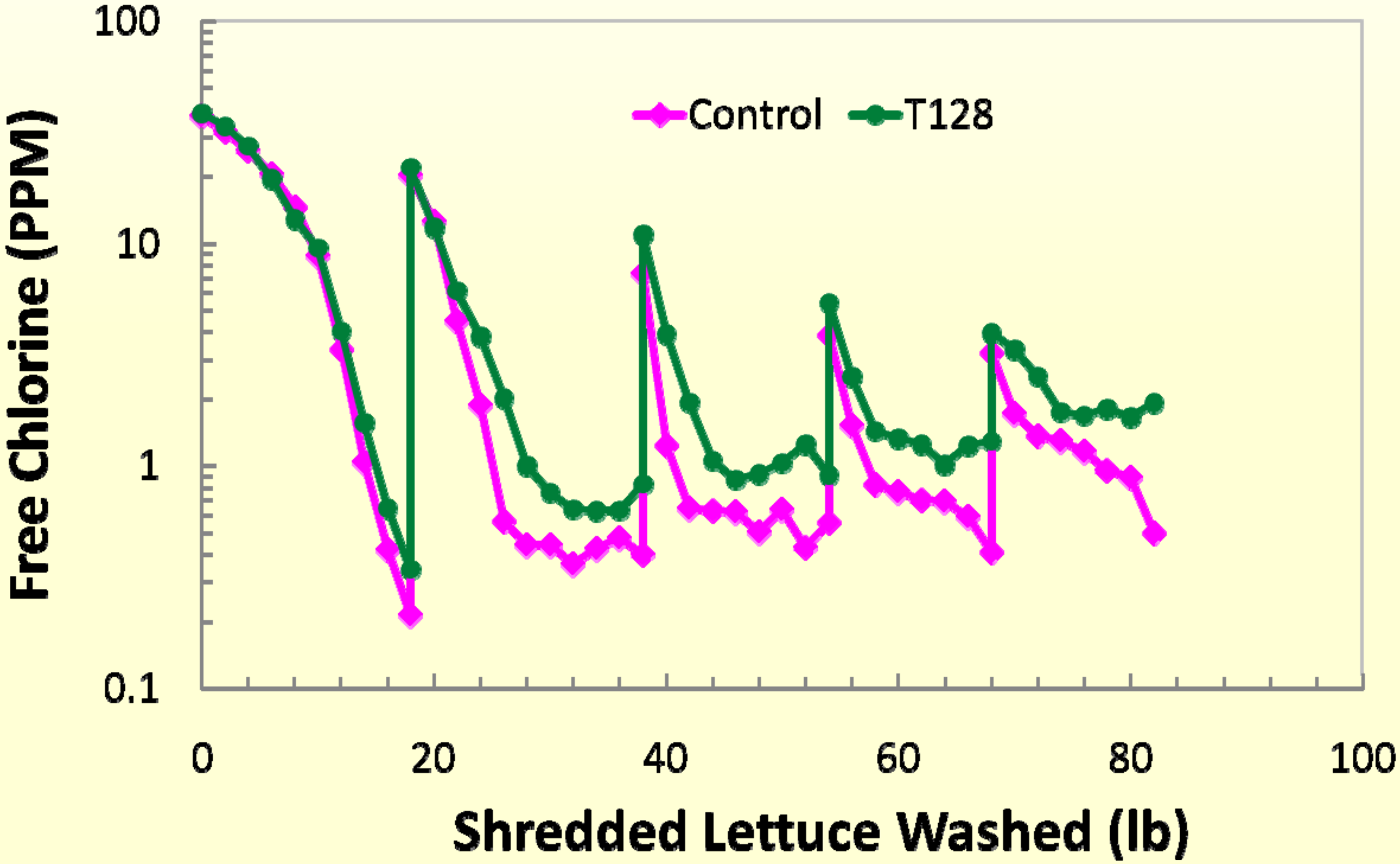
Changes in Water Quality during Lettuce Wash (Control)



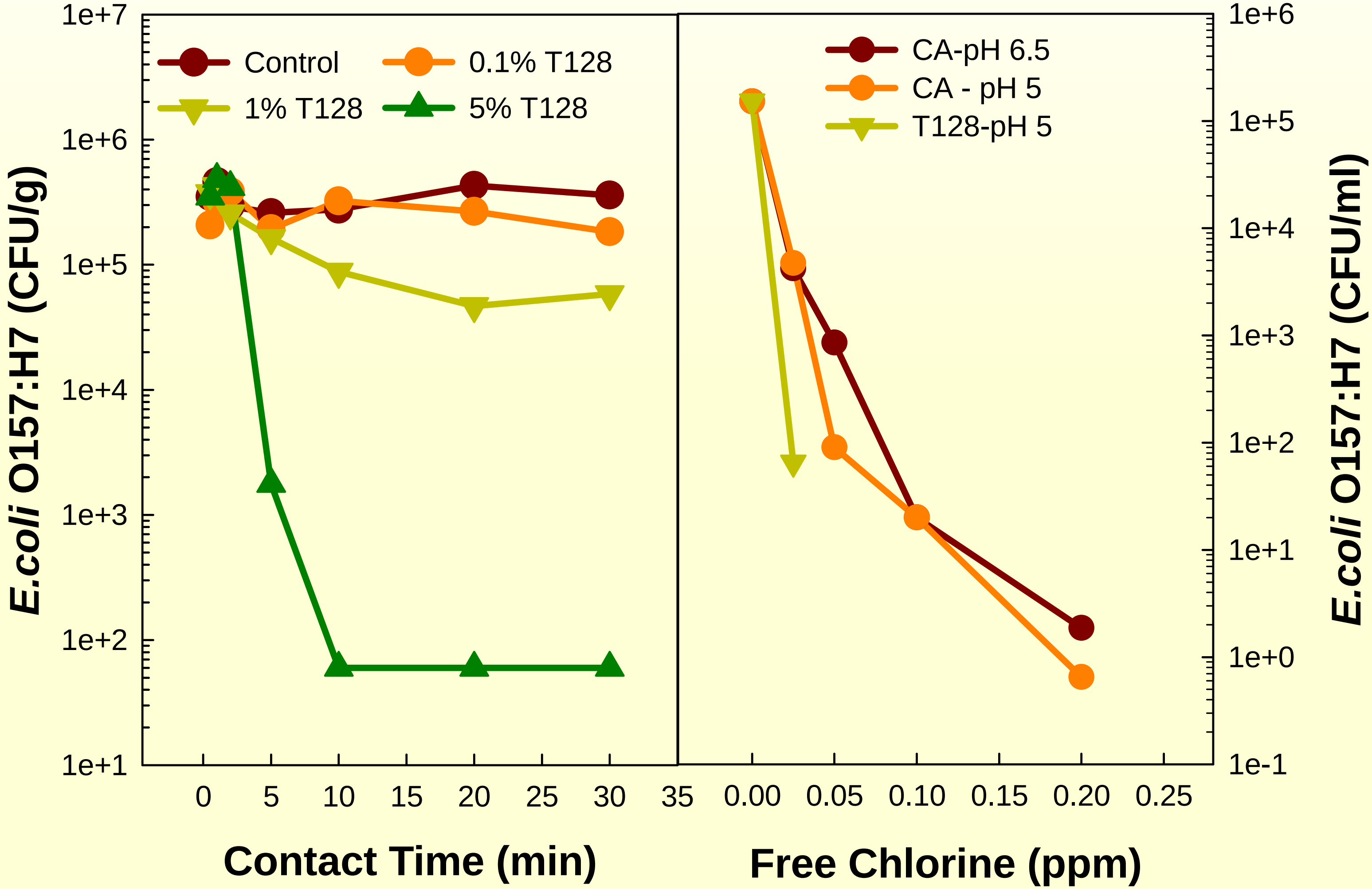
Changes in Water Quality during Lettuce Wash (T128)



T128 Retards Chlorine Loss Caused by Organic Materials

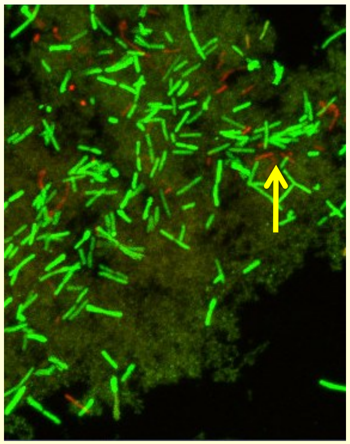


Synergistic Effect of T128 and Chlorine

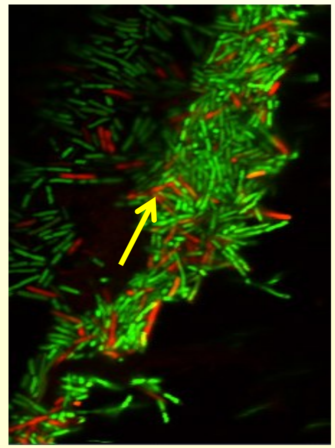


T128 Combined w/Chlorine Exhibits Improved Reduction on *Salmonella* Biofilm (Laboratory Study)

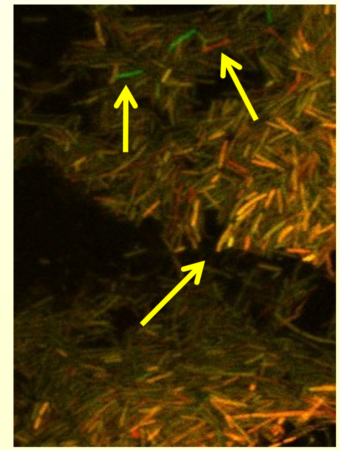
Control



2 ppm Cl

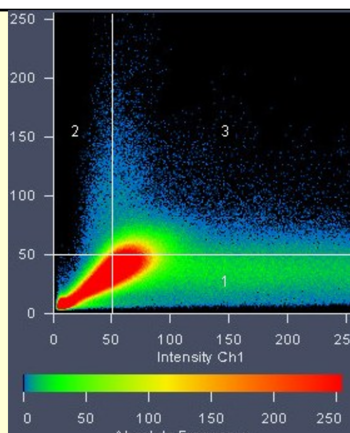


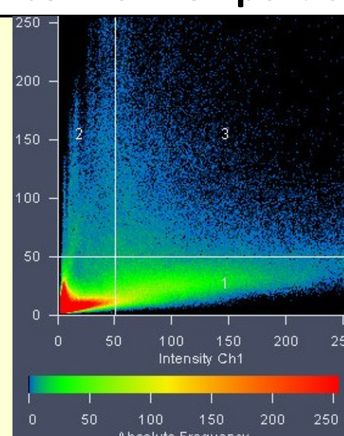
2 ppm Cl + T128

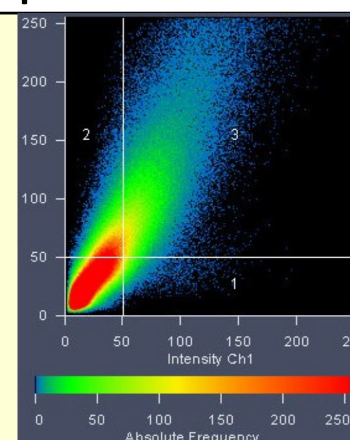


Co-Localization of Green (Live) and Red (Dead), Orange (Dying)

Fluorescence in S. Newport & Thompson



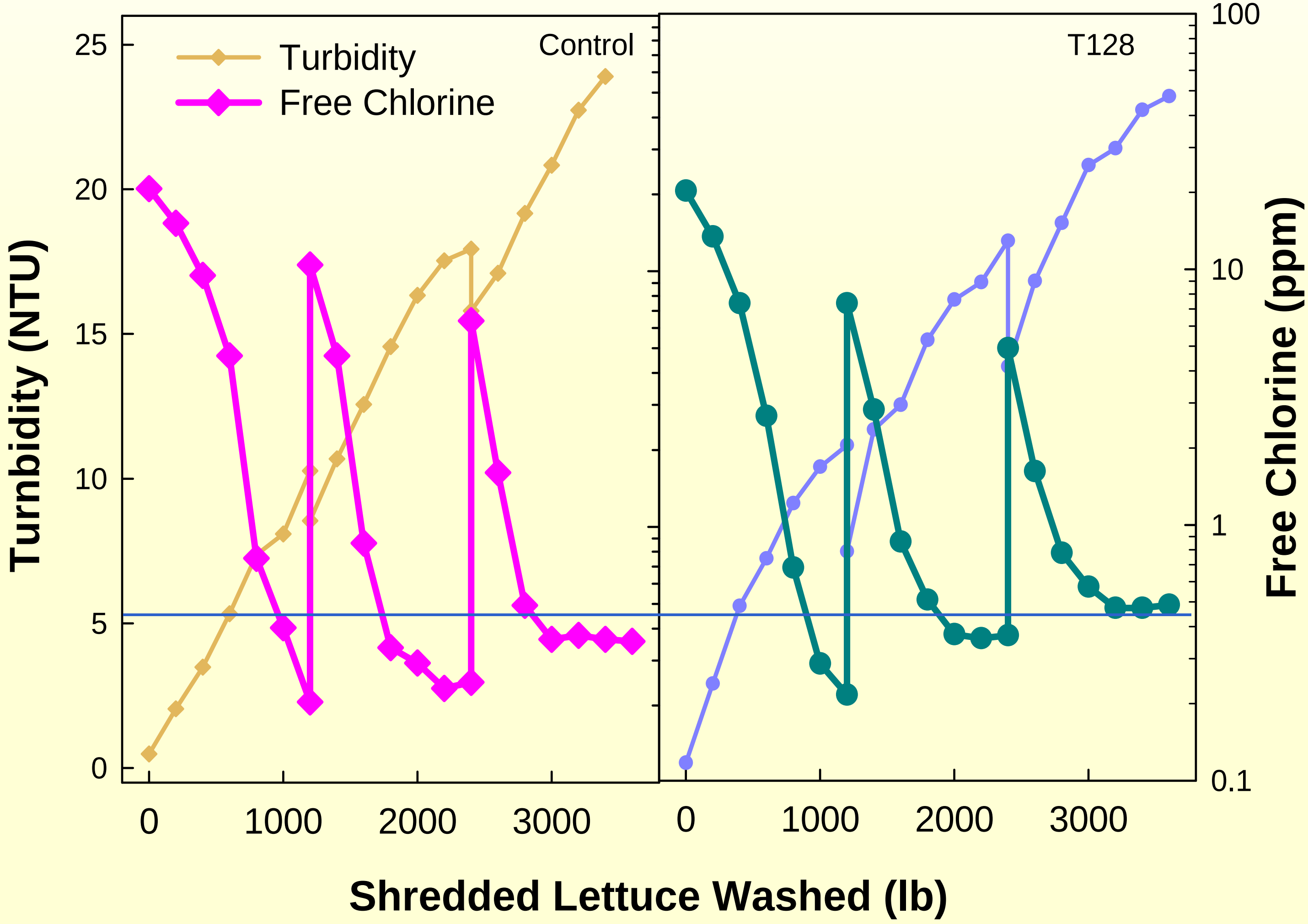




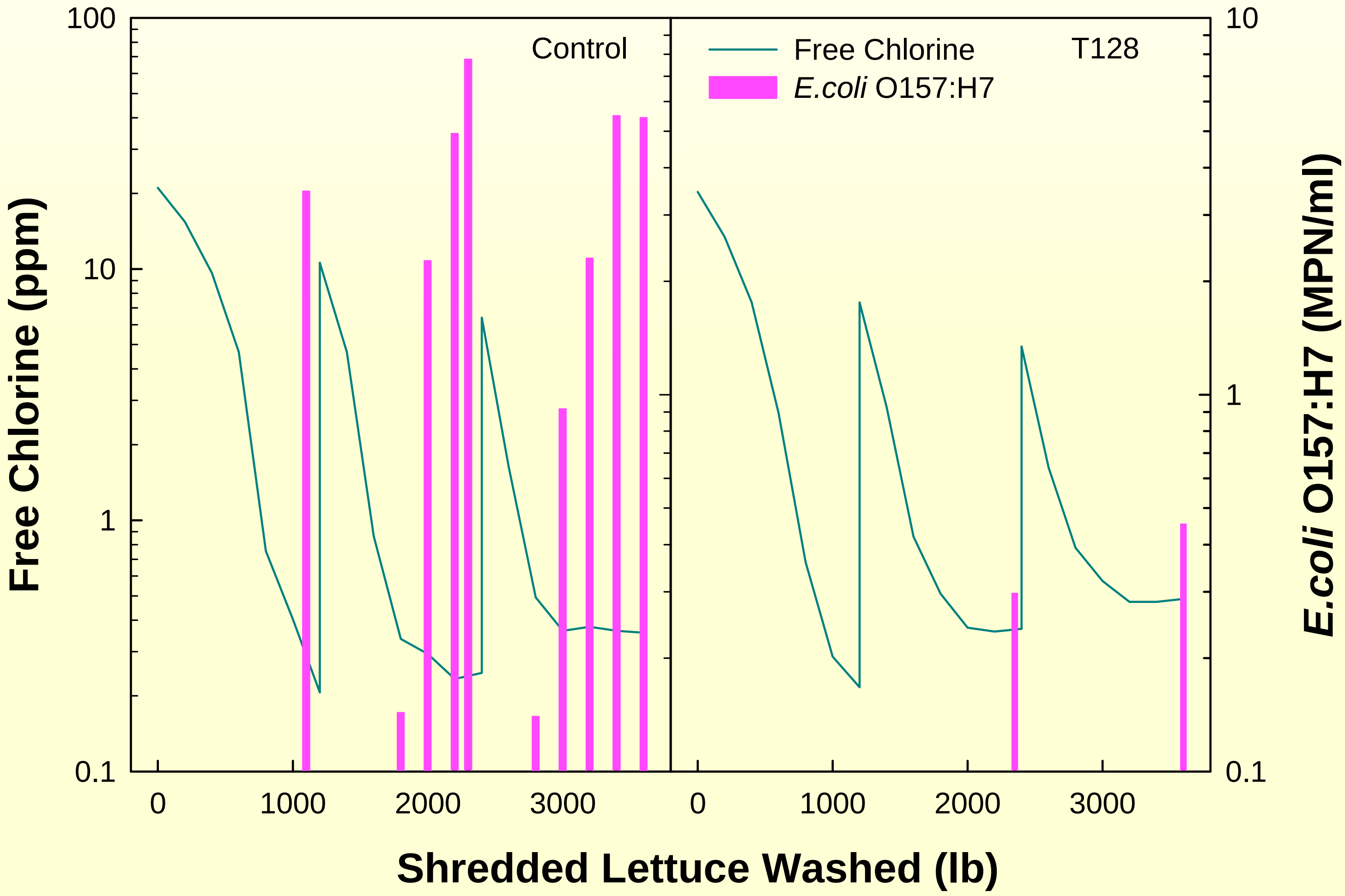
Pilot Plant Studies

- Clean iceberg lettuce shreds were discharged to the wash tank at 100 lb/min; artificially contaminated spinach leaves were discharged to the same wash tank at 0.25 lb/min, simulating sporadic contamination scenario.
- Chlorine was added before the run, and after every 1200 lb lettuce washed.
- Water and lettuce samples were collected from the primary tank every 2 minutes, and tested for *E.coli*.

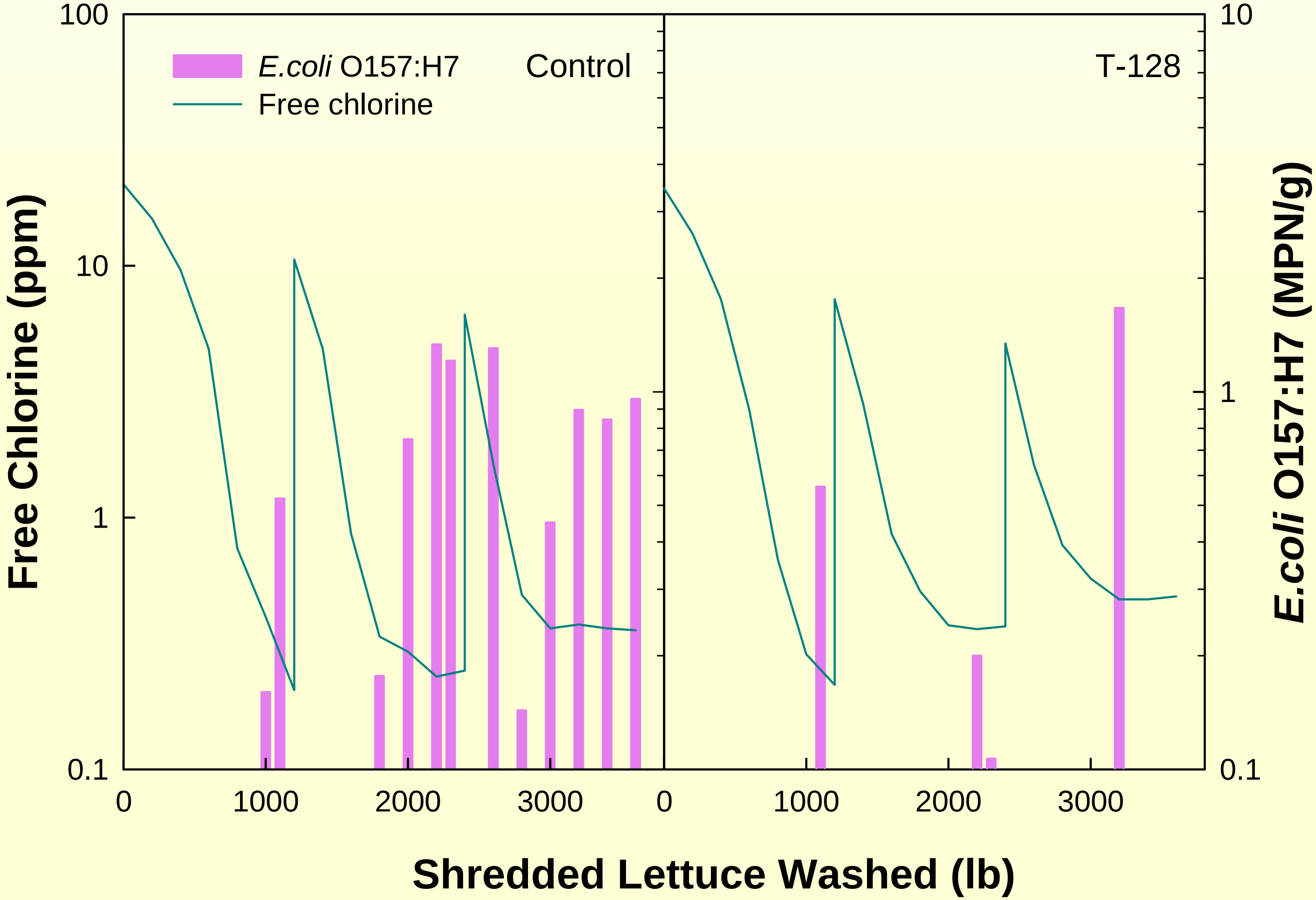
T128 Reduced Chlorine Loss by High Organic Loads



T128 Improved Chlorine's Effectiveness at Preventing Pathogen Survival in Wash Solution



T128 Improved Chlorine's Effectiveness at Preventing Pathogen Cross-contamination



Summary

Extensive laboratory and pilot plant studies demonstrate that T128 significantly reduces the potential for *E. coli* O157:H7 to survive in wash solution and spread during lettuce wash when chlorine approaches depletion due to high organic loads.

Improving Produce Safety by Stabilizing Chlorine in Washing Solutions with High Organic Loads

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New wash aid T-128 improves efficacy of chlorine against cross-contamination by bacterial pathogens in fresh-cut lettuce processing

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IFT 2011. New Orleans

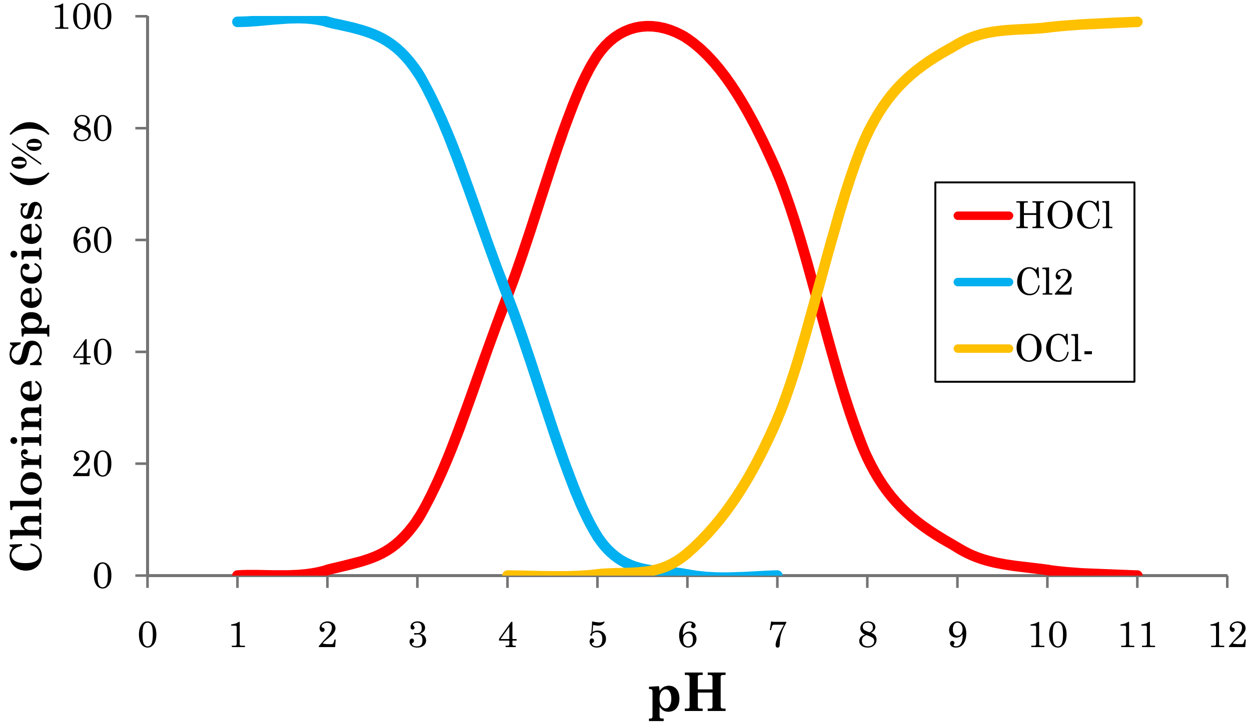
Background

- Chlorine is the most commonly used sanitizer for fresh cut produce.
- Easy production and use, low cost.
- Highly effective against bacteria in solution.

PROBLEMS

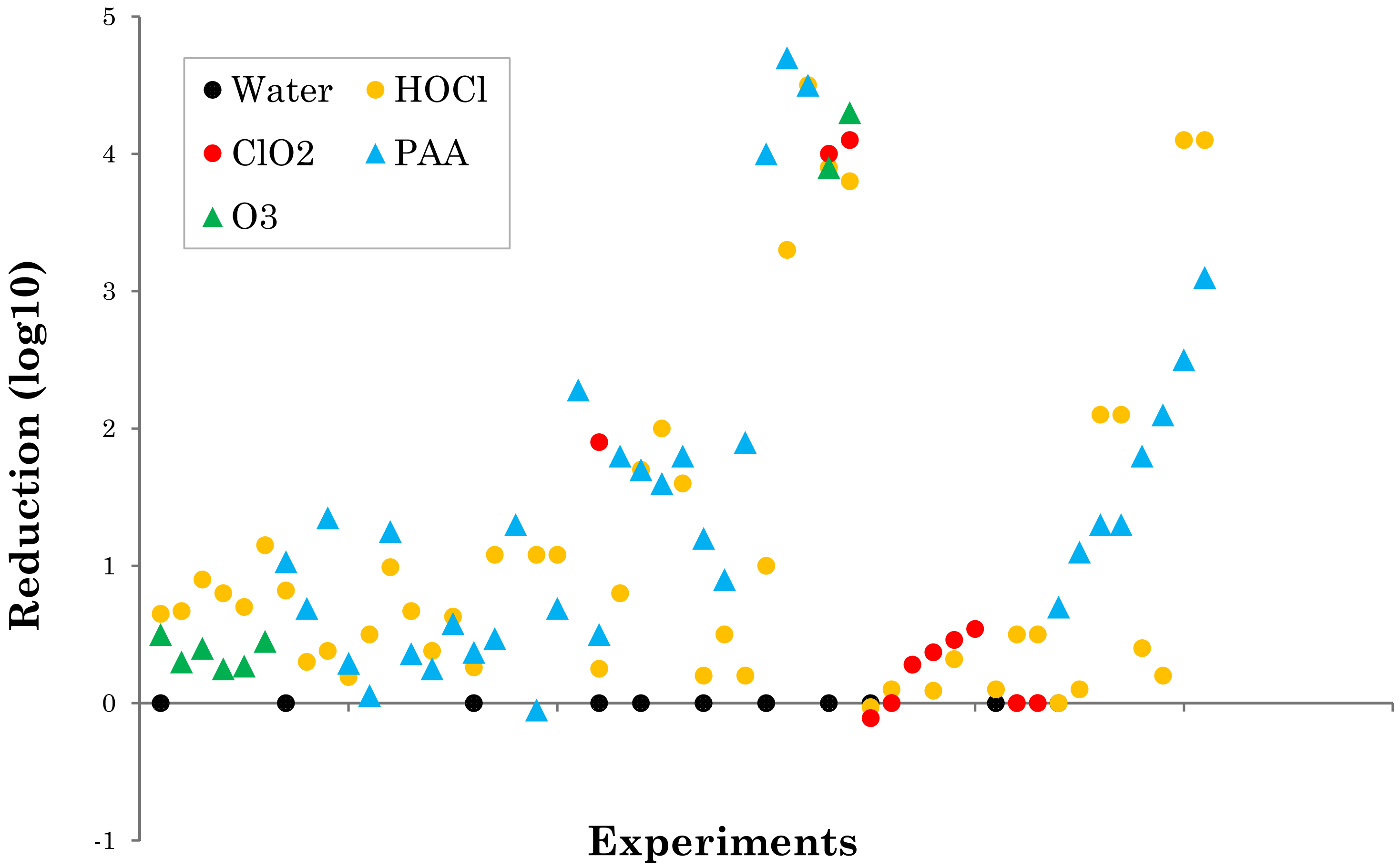
- Low efficacy against surface attached bacteria
- Easily depleted by organic matter
- Stringent pH requirement
- Chlorine off-gassing (Low pH)
- Potential harmful disinfection by-product
    - Trihalomethanes (THMs)
    - Haloacetic acids (HAAs)

Effect of pH on free chlorine



$Cl_2 + H2O \longleftrightarrow HOCl \longleftrightarrow OCl^- + Na^+$

Antimicrobial Efficacy of Selected Sanitizers on Fresh Produce (Compared to Water)



- Chlorine is highly effective for controlling cross contamination during produce processing, but it's efficacy for surface microbial reduction is not sufficient.

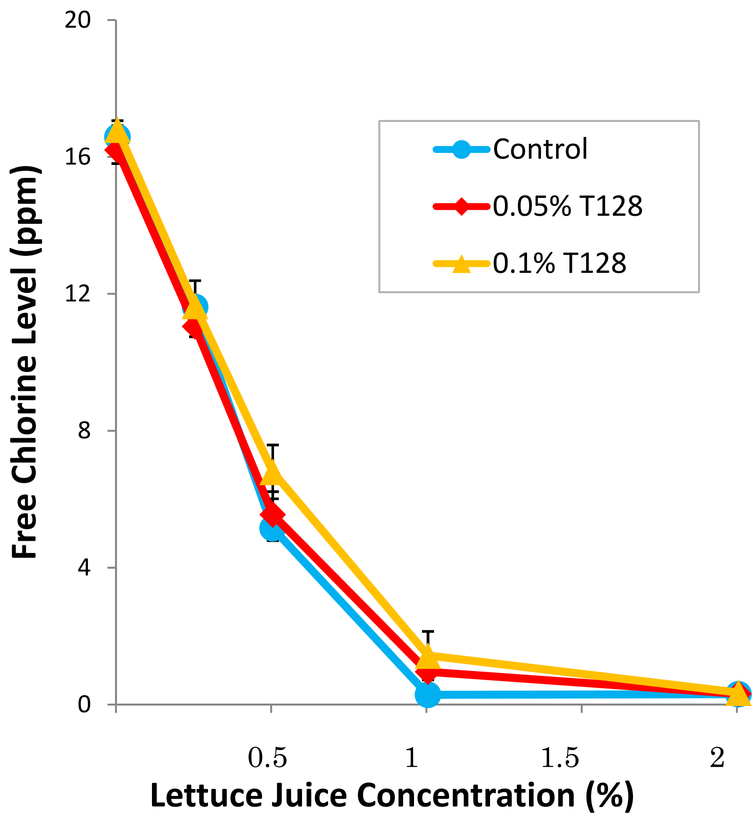
- Chlorine efficacy is influenced by many factors. (pH, organic load, produce type, exposure time, water source, agitation, inoculums)

- Other "alternative sanitizers" have not been consistently shown being more effective than chlorine.

What is T-128?

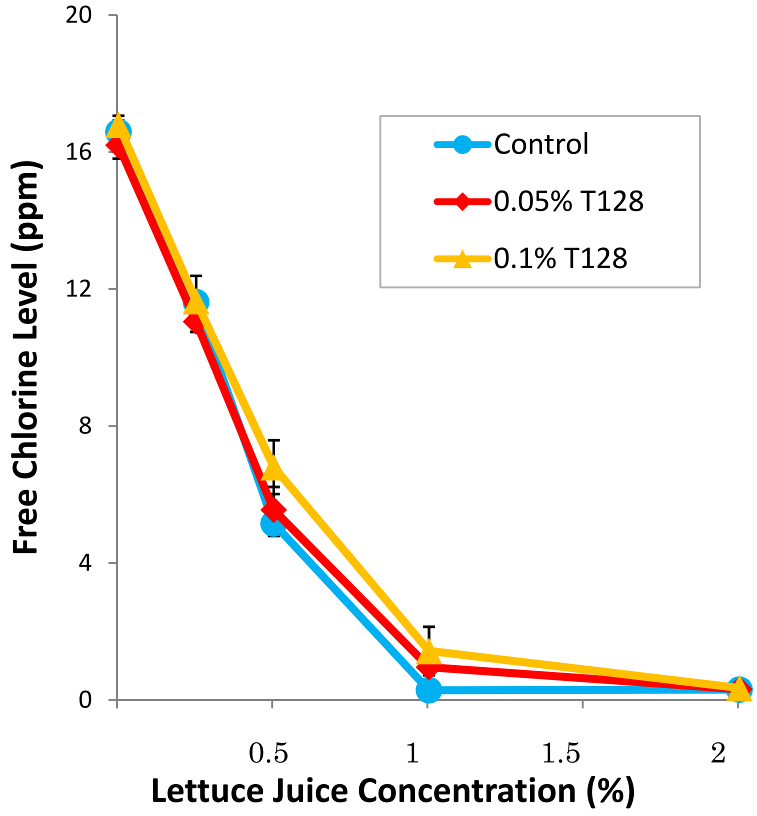
- Proprietary formula developed by industry scientist to use along with chlorine.
- Composed of GRAS chemicals including an inorganic acid and a diol.
- Optimal Operational pH 3-5.5
- Mechanism unknown. Stabilize or sequester diatomic chlorine?
- Wash aid. No additional label requirement

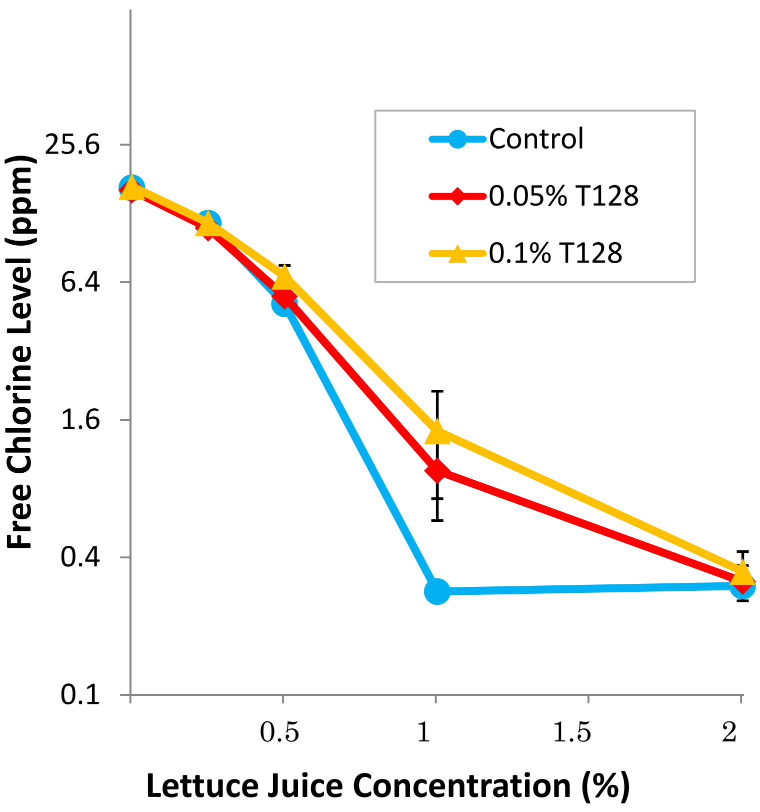
Effect of T128 on free chlorine in wash solution



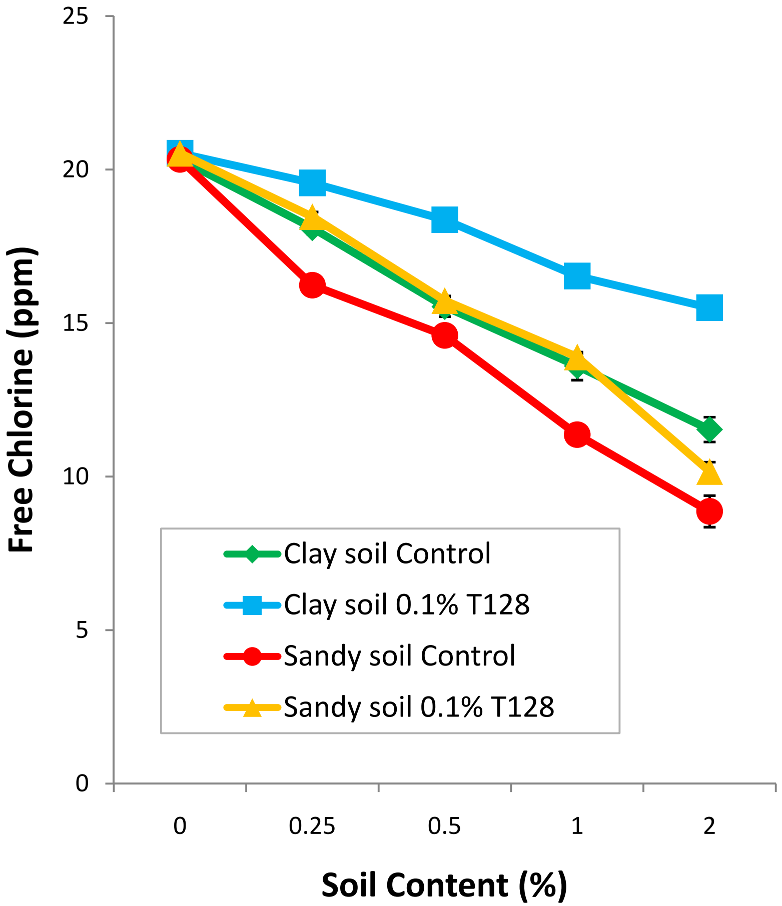
- Citric acid used to adjust solution pH to 6.5 (Control)
- T128 added to indicated concentration without further pH adjustment.
- pH and free chlorine monitored I min after mixing lettuce extract in wash solution

Effect of T128 on free chlorine in wash solution



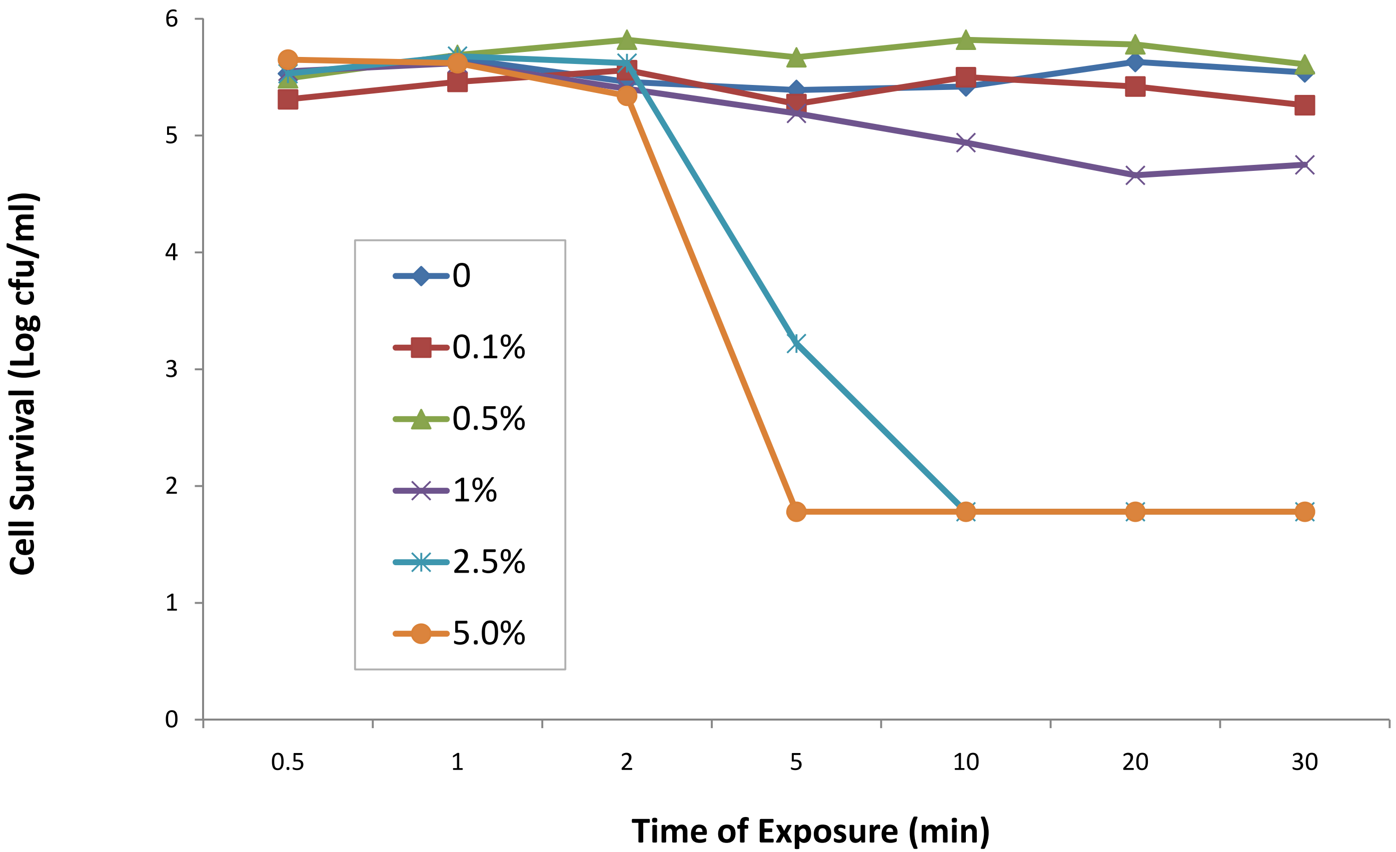


Effect of organic loads on free chlorine with/without T128



- T128 delayed free chlorine depletion in presence of organic material
  - Soil-moderate
  - Lettuce extract-less significant

Antimicrobial effect of T-128



Lettuce washing conditions

- Iceberg lettuce cut to ½" strips and weighed out 25g.
- Cut lettuce inoculated with bacterial strains at 10^5 cfu/g by placing 5ul droplets on surface. Kept at 4^oC for 20 hrs before treatment.
- Wash solutions prepared using distilled water and Na hypochlorite. Citric acid added to adjust pH to 6.5 (Control), or T128 added to 0.05 or 0.1%, without further adjusting pH.
- Lettuce extract added to water to indicated concentration for 1 min
- Inoculated lettuce washed in 1L solution for 1 min (1:40 W/V).
- Samples of lettuce and solution taken and residual chlorine neutralized. Surviving cells enumerated using an MPN procedure.
- Free chlorine levels monitored before and after lettuce washing.

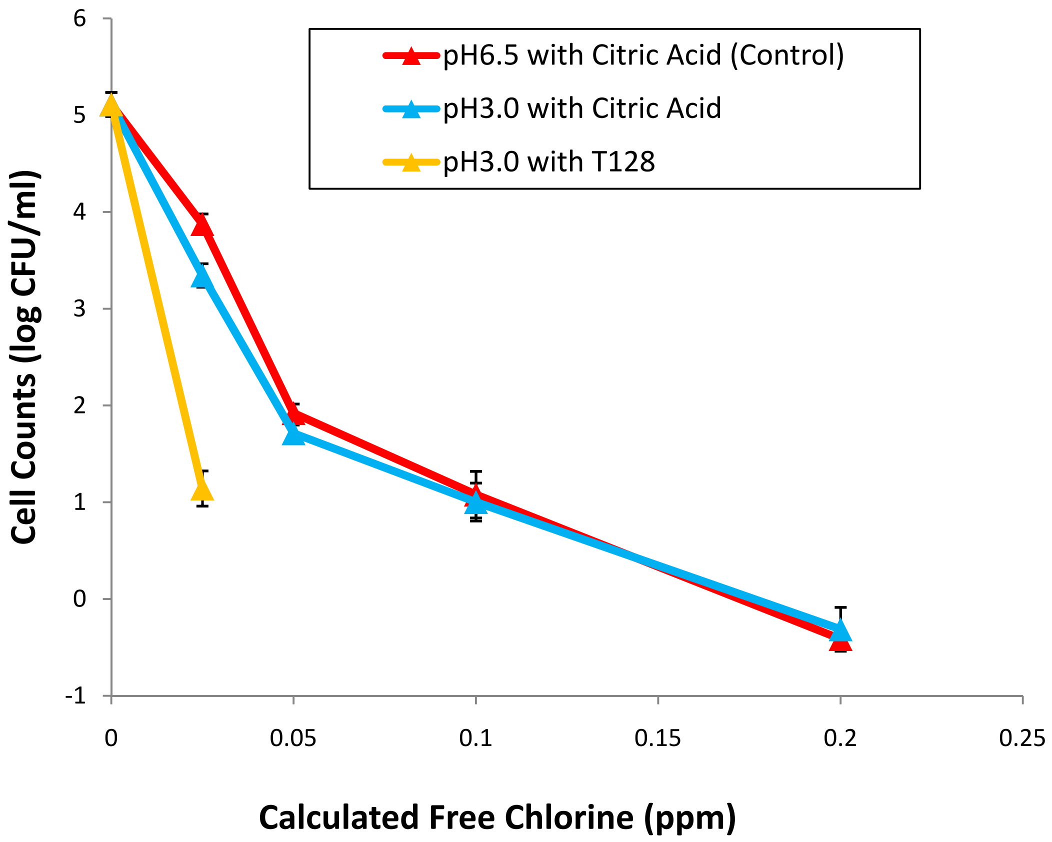
Bacterial Survival Following Chlorine Treatment

Wash Solution	Lettuce Extract (v/v %)	Bacteria Survival on Inoculated lettuce (log cfu/g)			Bacterial Survival in solution (log cfu/ml)*		
		Sal	O157:H7	O157:H7	Sal	O157:H7	O157:H7
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	--	--	--

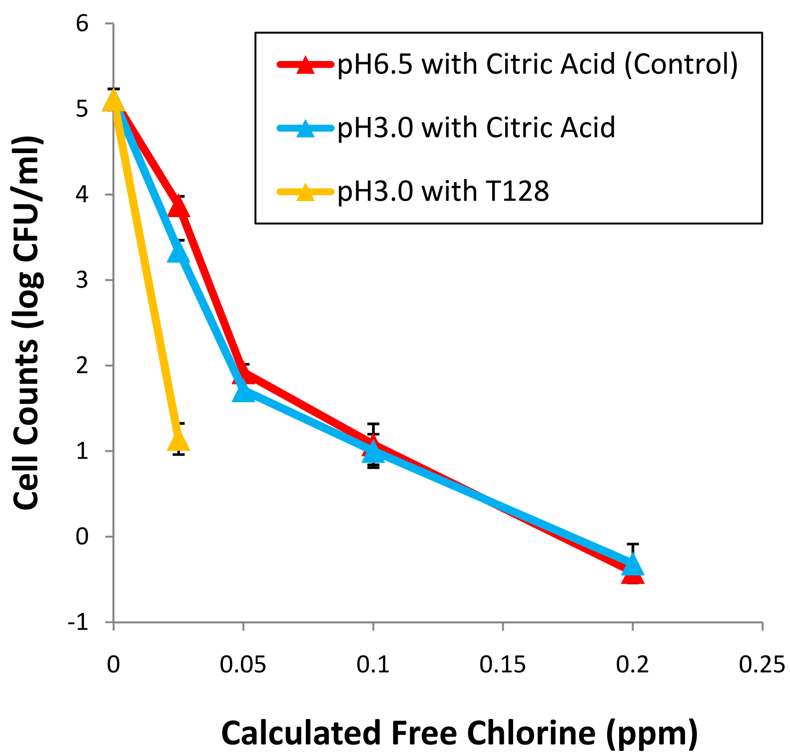
Cross Contamination

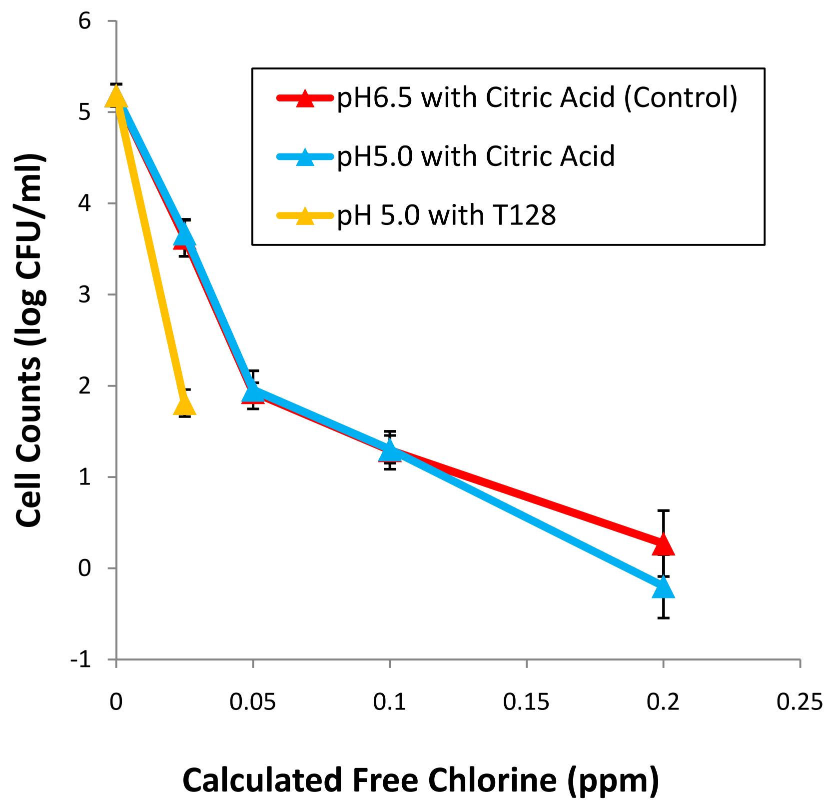
Wash Solution	Lettuce Extract (v/v %)	Bacterial Survival on Non-Inoculated Lettuce (MPN/g)*	
		E. coli O157:H7	Salmonella
Water	0	79.4 ± 2.0	63.1 ± 1.3
Chlorine water	0	--	< 0.16
	0.5	--	< 0.16
	1.0	10.0 ± 5.0	10.0 ± 4.0
Chlorine water + 0.05% T-128	0	< 0.16	--
	0.5	--	< 0.16
	1.0	0.2 ± 0.2	< 0.16
Chlorine water + 0.1% T-128	0	--	--
	0.5	< 0.31	--
	1.0	< 0.16	< 0.5

Sensitivity of E. coli O157:H7 to low concentration of free chlorine in water

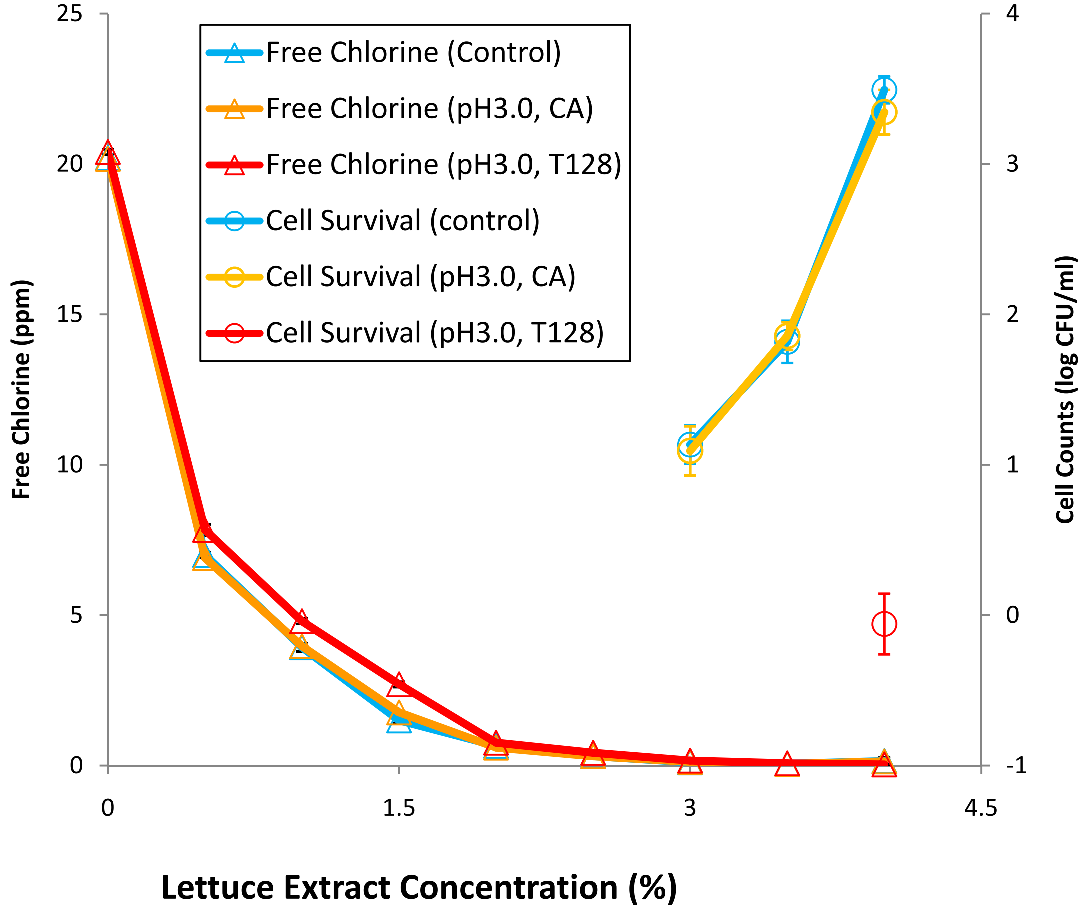


SENSITIVITY OF E. COLI O157:H7 TO LOW CONCENTRATION OF FREE CHLORINE IN WATER

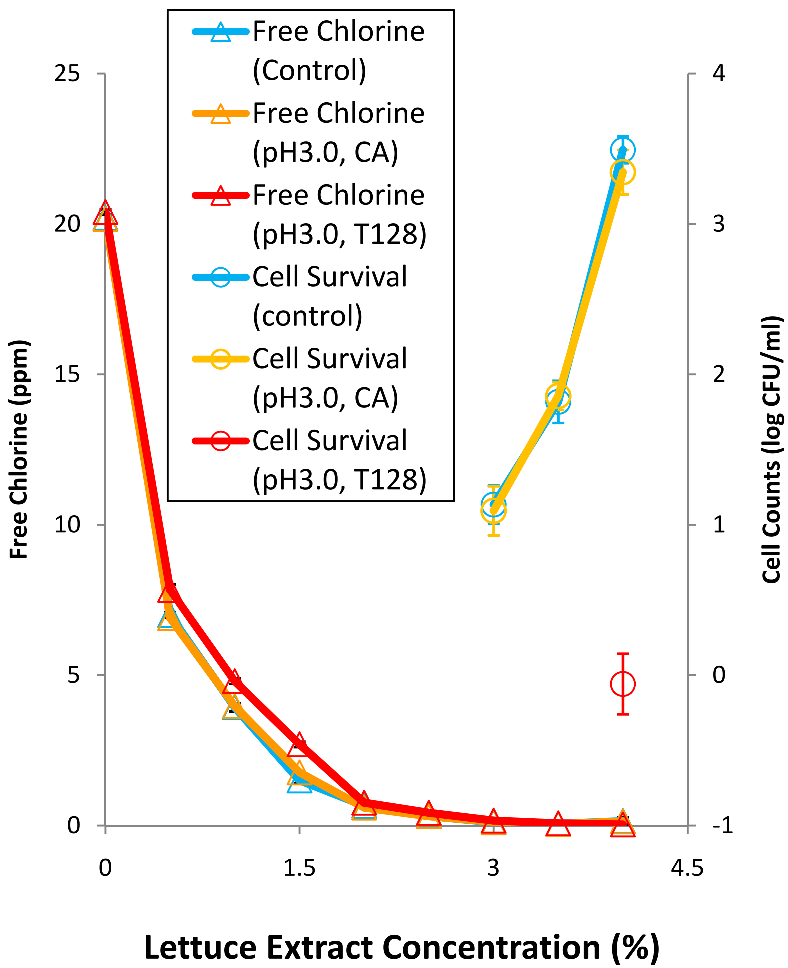


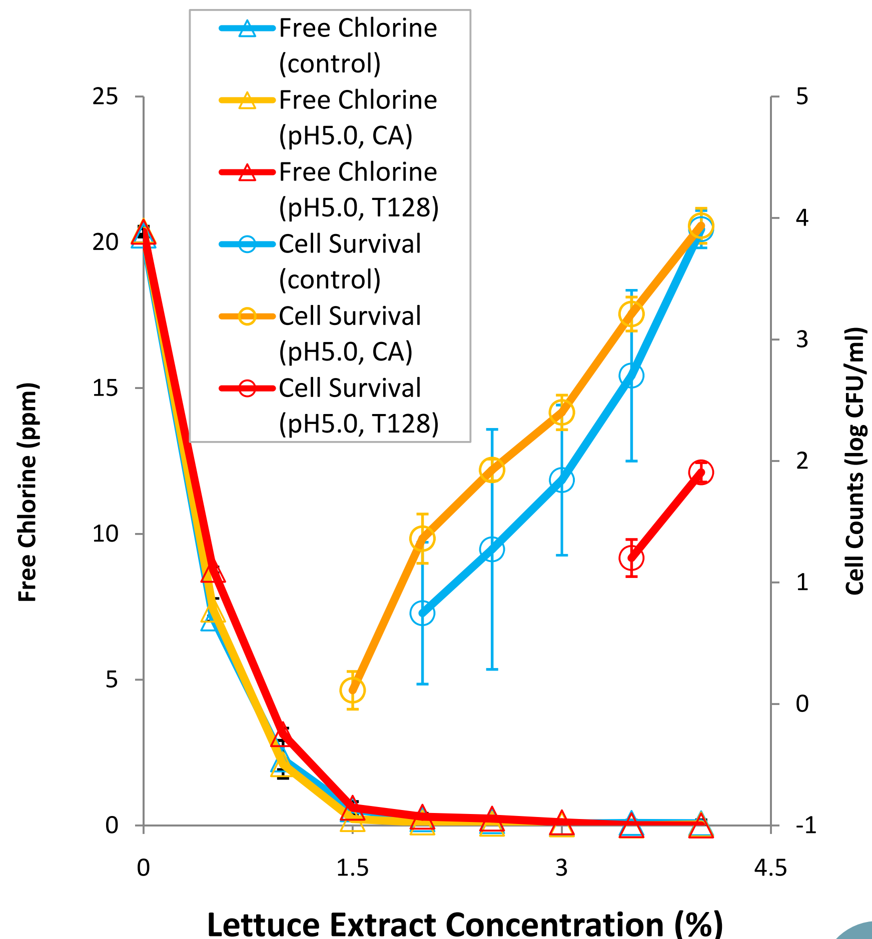


Survival of E. coli O157:H7 in chlorine solution

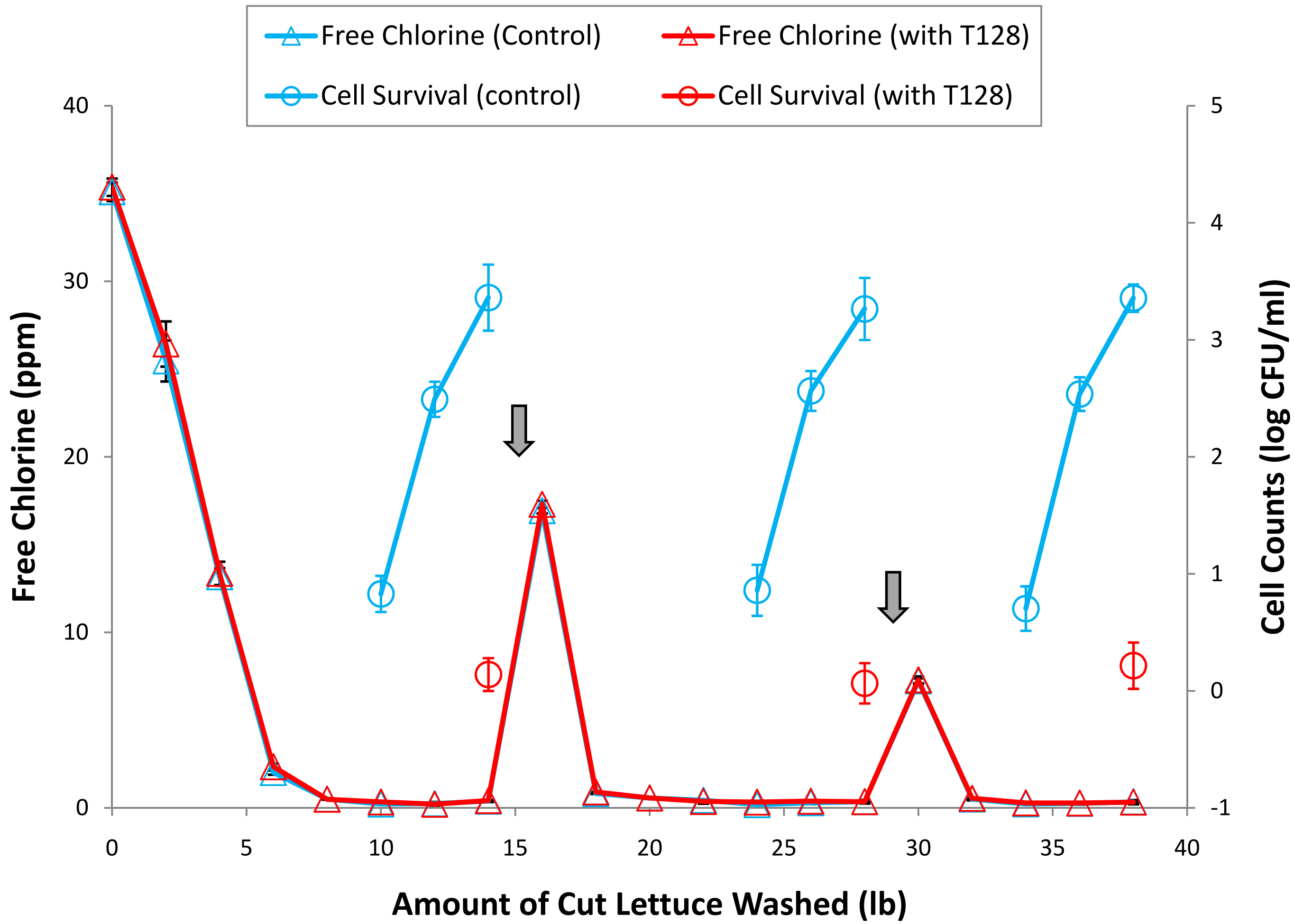


Survival of E. coli O157:H7 in chlorine solution





Effect of replenish chlorine



Effect of T128 on Produce Quality

Quality Parameter and Treatment	Storage Time (days)				
	0	4	7	11	15
O2 Partial Pressure (kPa)					
Control	1.5	1.24±1.01	0.50±0.19	1.36±0.19	7.715±0.04
T-128	1.5	0.09±0.02	0.35±0.05	0.39±0.02	9.03±0.02
CO2 Partial Pressure (kPa)					
Control	0.03	6.66±0.12	4.17±1.13	3.30±1.10	4.70±0.24
T-128	0.03	6.54±0.51	4.37±0.73	4.77±0.03	4.94±0.04
Visual Score (1-9 Point)					
Control	9	8.33±0.00	8.00±0.00	5.78±0.22	5.58±0.39
T-128	9	8.22±0.11	7.22±0.22	6.00±0.00	5.40±0.40
Relative EC (%)					
Control	10.7±0.3	6.3±0.3	4.6±0.1	2.2±0.1	3.5±0.1
T-128	10.4±0.9	4.7±0.2	3.2±0.2	3.0±0.3	3.7±0.1

Summary

- T128 reduced slightly to moderately the rate of free chlorine depletion in wash solution containing high organic load.
- T128 significantly reduced the survival of bacterial pathogens in wash solution containing high organic load, therefore the potential of cross contamination is significantly reduced.
- T128 application in wash solution did not negatively impact the produce quality parameters tested in this study.

Future Research

- Determine the effectiveness of T128 at pilot plant scale.
- Mechanism of T128 interacting with free chlorine. Why is the reduced the survival of E. coli and Salmonella without significant increase in free chlorine level?
- T128 application with sanitizers other than chlorine
- Formula optimization

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Thank you!

The PMA Fresh Summit 2010

Effects of Novel Chlorine Stabilizer in Washing Solutions with High Organic Loads

Patricia Millner

October 16, 2010

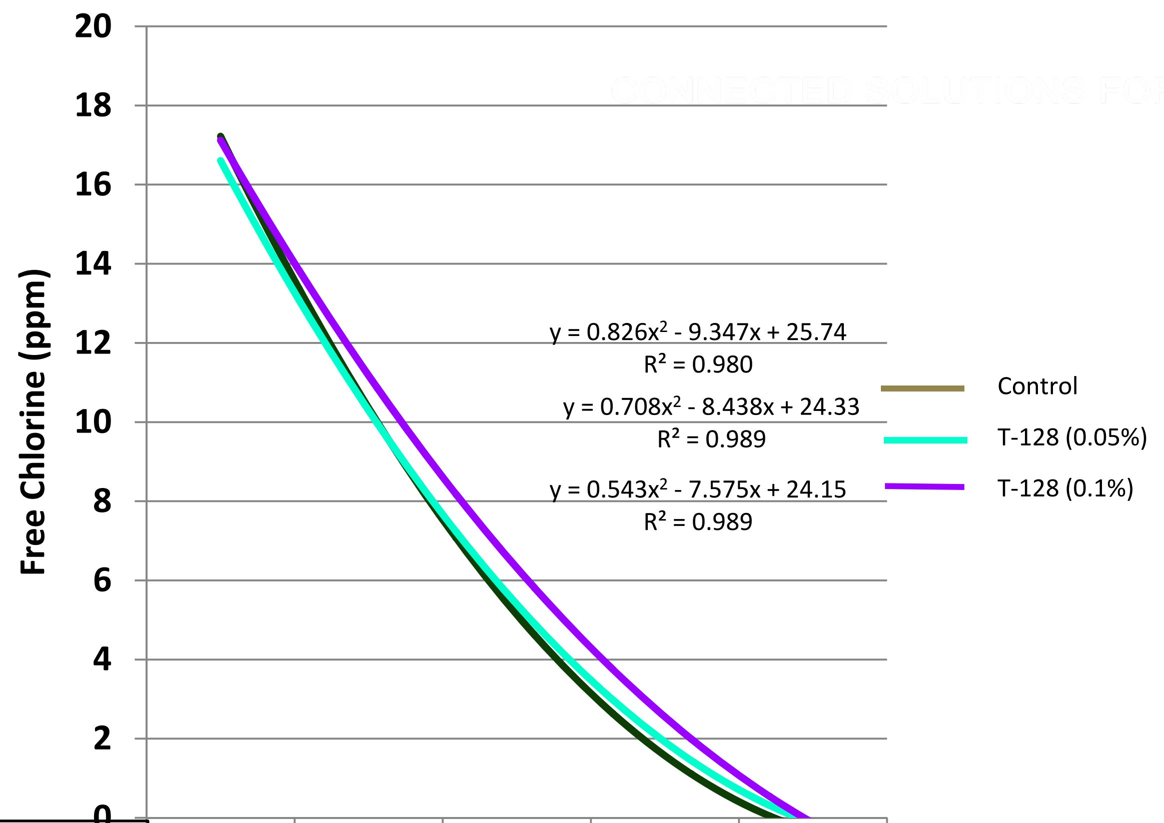
Chlorine Stabilization in Fresh Produce Wash Solutions

- **<u>PROBLEM</u>: Chlorine is depleted in wash solutions**
  - **- Surge of high organic loads, e.g., plant juice and tissue**
  - **- Foreign materials, e.g., soil and organic debris**

- **<u>INNOVATIVE RESOLUTION</u>: New Leaf Inc. formulated T-128 using GRAS food ingredients *US Patent Pending*.**
  - **T-128 substitutes for acid in adjusting pH to 3.5 – 5.5.**
  - **T-128 designed to slow depletion of free chlorine in surges of high organic loads during fresh-cut produce wash operations.**

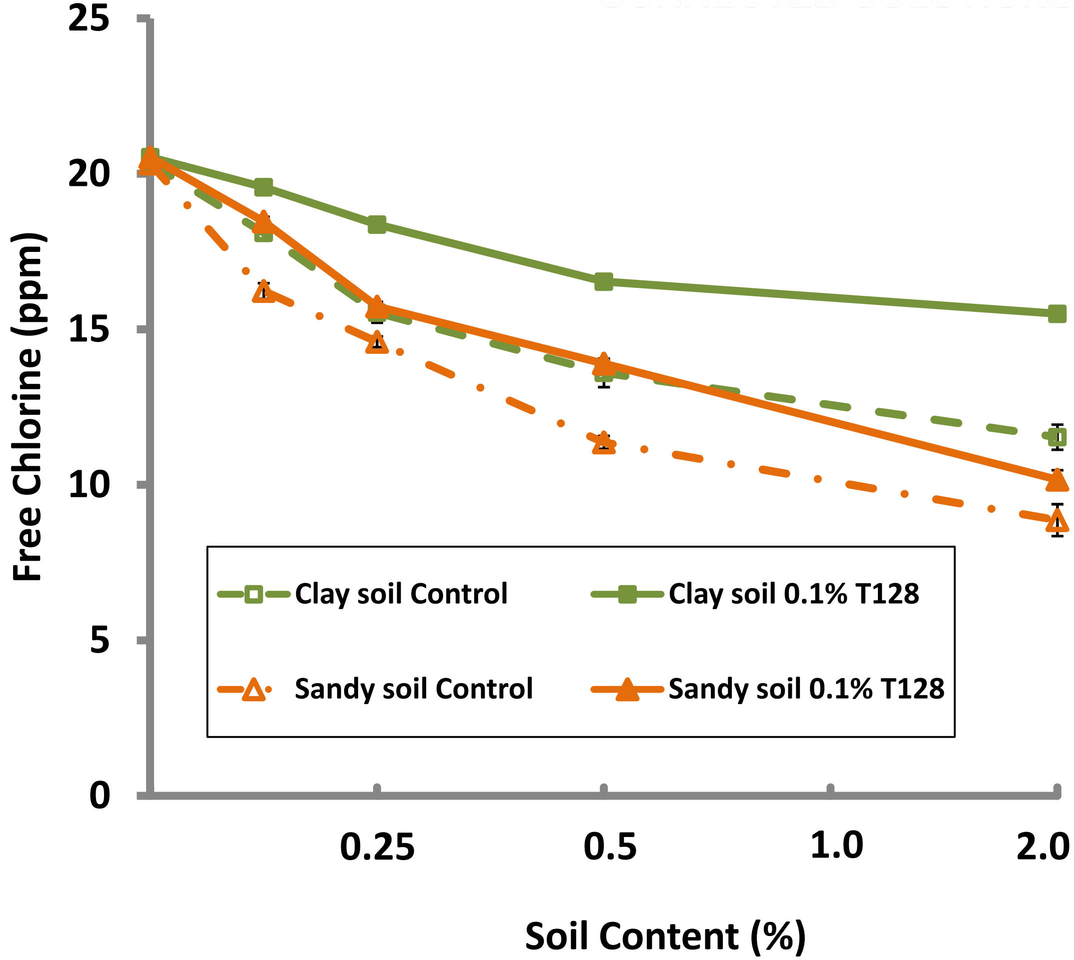
- **<u>RESEARCH</u>: Evaluate efficacy of T-128 on stabilizing chlorine in conditions typical of commercial fresh-cut produce washing and impacts on pathogen survival.**

Organic Load and T-128 Effect on Free Chlorine in Wash Solutions

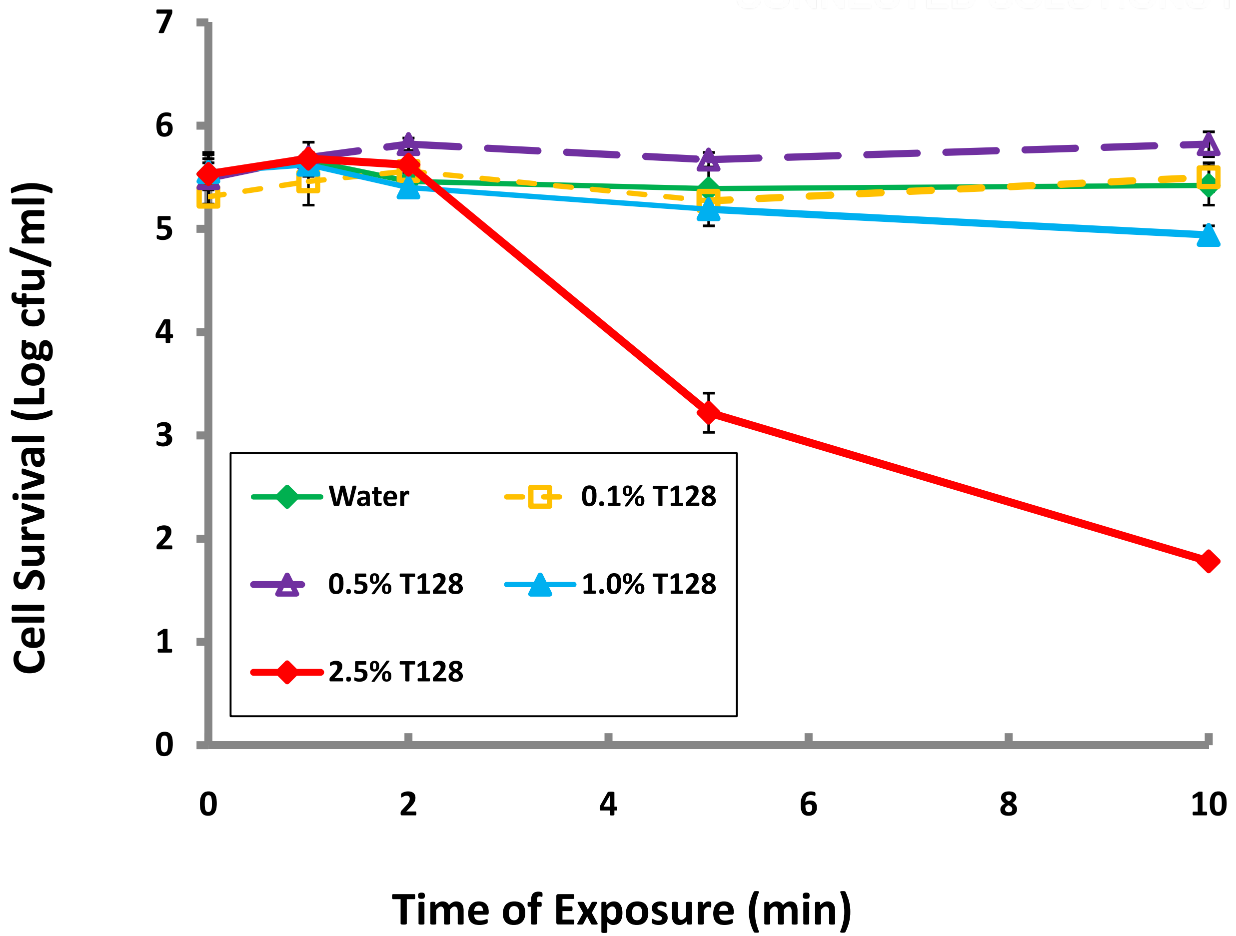


Lettuce Juice Content (%)	0.00	0.25	0.50	1.00	2.00
Control	16.5833	11.6167	5.15	0.2833	0.3
T-128 (0.05%)	16.2	11.05	5.55	0.9567	0.3133
T-128 (0.1%)	16.8	11.65	6.8	1.43	0.3467
COD (mg/L)	63.00	nd	325.00	532.00	942.00
Turbidity	0.43	nd	4.74	9.04	16.85
TDS (mg/L)	1.67	13.33	25.67	57.00	92.00

Effect of Soil and T-128 on Free Chlorine in Wash Solutions



Bactericidal activity of T-128 on *E. coli* O157:H7



Effect of T-128 & Chlorine on Pathogen Survival (Lab Scale)

Wash Solution	Lettuce Extract (v/v %)	Survival on Inoculated lettuce (log cfu/g)			Survival in solution (log cfu/ml)*		
		Salmonella	*Gfp E. coli* O157:H7	*E. coli* O157:H7	*Salmonella*	*Gfp E. coli* O157:H7	*E. coli* O157:H7
Chlorine Water (pH 6.5)	0	3.1	3.5	3.1	--	--	--
	0.25	3.2	3.0	2.6	--	--	--
	0.5	3.0	3.0	2.6	--	< 1.3	< 1.0
	1.0	3.5	3.5	3.0	1.2	1.8	1.6
	2.0	3.5	3.7	3.4	2.6	2.5	2.5
Chlorine Water + 0.05% T-128 (pH 3.1)	0	2.9	3.4	2.9	--	--	--
	0.25	3.2	3.4	2.7	--	--	--
	0.5	3.4	3.5	2.9	--	--	--
	1.0	3.7	3.8	3.4	--	--	--
	2.0	3.7	3.5	3.2	--	--	--
Chlorine Water + 0.1% T-128 (pH 2.8)	0	3.2	2.9	3.0	--	--	--
	0.25	3.5	3.6	3.2	--	--	--
	0.5	3.7	3.6	3.3	--	--	--
	1.0	3.5	3.4	3.2	--	--	--
	2.0	3.3	3.4	3.4	--	--	--

Reduced Cross-Contamination of Lettuce with T-128 (Lab Scale)

Wash Solution	Lettuce Extract (v/v %)	Pathogen Survival Non-Inoculated Lettuce (CFU/g)	
		E. coli O157:H7	*Salmonella* Typhimurium
Water (pH 6.5)	0	79.4 ± 2.0	63.1 ± 1.3
Chlorine water (pH 6.5)	0	non-detect	< 0.16
	0.5	non-detect	< 0.16
	1.0	10 ± 5.0	10 ± 4
Chlorine water + 0.05% T-128 (pH 3.1)	0	< 0.16	non-detect
	0.5	non-detect	< 0.16
	1.0	0.2 ± 0.2	< 0.16
Chlorine water + 0.1% T-128 (pH 2.8)	0	non-detect	non-detect
	0.5	< 0.31	non-detect
	1.0	< 0.16	< 0.5

Effect of T-128 on Lettuce Quality Attributes

Quality Attributes and Treatment		Storage Time (days)				
		0	4	7	11	15
O_2 Partial Pressure (kPa)						
pH 6.5	Control	1.00	0.23	0.50	1.36	7.72
pH 2.8	T-128 (0.1%)	1.00	0.09**	0.35	0.39**	9.03**
CO_2 Partial Pressure (kPa)						
pH 6.5	Control	0.03	6.66	4.17	3.30	4.70
pH 2.8	T-128 (0.1%)	0.03	6.54	4.37	4.77	4.94
Visual Score (1-9 Point)						
pH 6.5	Control	9.00	8.33	8.00	5.78	5.58
pH 2.8	T-128 (0.1%)	9.00	8.22	7.22	6.00	5.40
Relative EC (%)						
pH 6.5	Control	10.75	6.31	4.56	2.19	3.48
pH 2.8	T-128 (0.1%)	10.42	4.72**	3.22	2.98	3.67

Summary

- **<u>PROBLEM</u>: Chlorine depletion in wash solutions**
  - **High organic loads, e.g., plant juice and tissue**
  - **Foreign materials, e.g., soil and organic debris**

- **<u>PROMISING RESOLUTION</u>: New Leaf Inc. GRAS formula T-128, *US Patent Pending***

- **USDA-ARS LAB RESEARCH RESULTS:**
  - **T-128 is used to adjust pH of the chlorine solution.**
  - **T-128 significantly retards depletion of free chlorine especially in the presence of soil and to minor extent with 1.0 % (v/v) lettuce juice**
  - **T-128 reduces cross-contamination of lettuce by and survival of *E. coli* O157:H7 and *Salmonella* sv. Typhimurium in lab-scale trials.**
  - **T-128 exhibits bactericidal activity at concentrations/contact time of 2.5% or greater for 5 min or longer.**
  - **No adverse impacts on lettuce quality developed with T-128 (0.1%)**

Improving Produce Safety by Stabilizing Chlorine in Washing Solutions with High Organic Load

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Introduction

Chlorine is widely used by the fresh and fresh-cut produce industry to reduce microbial populations and prevent pathogens cross-contamination during produce washing. However, the organic materials released from cut produce react with chlorine and degrade its efficacy for pathogen inactivation. A chlorine stabilizer usable in produce wash systems with high organic load could greatly improve produce safety.

Objectives

- Evaluate effectiveness of T-128 to stabilize chlorine in the presence of a high organic load and foreign materials typical of commercial fresh-cut produce wash operation conditions.
- Determine the dynamic changes of wash water quality and free chlorine concentration during leafy green wash processing, and the consequential results on pathogen survival in the wash solution and the potential for cross-contamination, with or without T-128.

About T-128

WHAT - T-128 is a chemical mixture formulated by New Leaf Inc. containing GRAS food ingredients.

HOW – T-128 is substituted for citric acid to adjust pH to the desired level (3.5 – 5.5).

WHY – T-128 is intended to slow down the degradation of free chlorine in the presence of a high organic load during commercial fresh-cut produce wash operations.

Fig. 1. A pilot plant trial system with T-128 dosing equipment

Results

Fig. 2

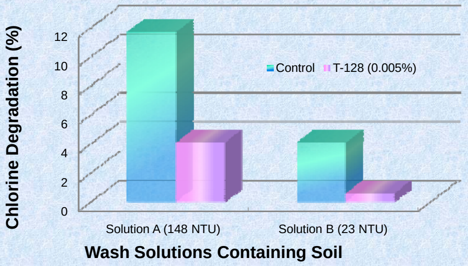
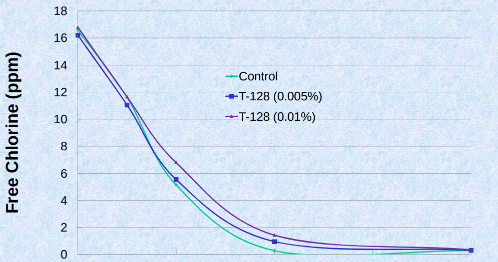


Figure 2 demonstrates that T-128 significantly reduced the soil generated degradation of free chlorine in the wash solution, compared to the control (chlorinated water without T-128).

Fig. 3



Juice (%)	0.00	0.50	1.00	1.50	2.00
COD (mg/L)	63	325	532		942
Turbidity	0.43	4.74	9.04		16.85

Figure 3 suggests that T-128 slightly reduced the loss of free chlorine caused by reaction with lettuce exudates. The organic load in the wash solution was measured in terms of percent lettuce juice, chemical oxygen demand (COD) and turbidity (NTU).

Table 1.

Treatment	Lettuce juice (%)	Free Chlorine (ppm)	*Salmonella* Typhimurium	*E. Coli* O157:H7
Control	0	16.2	ND	ND
	0.25	11.6	ND	ND
	0.5	4.75	ND	ND
	1.0	**0.26**	**1.30**	**1.70**
	2.0	**0.29**	**2.04**	**2.00**
T-128 (0.01%)	0	16.65	ND	ND
	0.25	12.3	ND	ND
	0.5	6.95	ND	ND
	1.0	2.2	ND	ND
	2.0	0.41	ND	ND

Data in Table 1 shows that residual free chlorine decreased rapidly with increase in organic load (lettuce juice). When juice concentrations reached 1-2 % (Fig. 3), free chlorine levels in the control dropped below 0.3 ppm, resulting in pathogen survival; however, when T-128 was added, pathogen survival was not detected (ND).

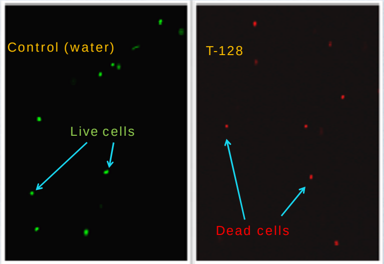


Fig. 4. Laser confocal images of live (green) and dead (red) *E. coli* cells in suspension. This picture demonstrates that T-128 itself has a weak bacteriocidal effect; at high concentration and extended reaction time T-128 can cause *E. coli* inactivation.

Summary

The addition of T-128 to the chlorinated wash solution significantly reduced the potential for pathogen survival and mitigated the risks of solution-mediated cross-contamination in presence of high organic load and soils.

Future Goals

- Determine efficacy of T-128 to reduce pathogen survival and transference in chlorinated wash water processing of herbs, tomatoes, and cantaloupes.
- Evaluate impact of T-128 on produce quality and shelf life.