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# Determination of Free Chlorine Concentrations Needed To Prevent *Escherichia coli* O157:H7 Cross-Contamination during Fresh-Cut Produce Wash†

YAGUANG LUO,[1]* XIANGWU NOU,[1] YANG YANG,[1] ISABEL ALEGRE,[2] ELLEN TURNER,[1] HAO FENG,[3] MARIBEL ABADIAS,[2] AND WILLIAM CONWAY[4]

[1]*U.S. Department of Agriculture, Agricultural Research Service, Environmental Microbial and Food Safety Laboratory, Beltsville, Maryland 20705, USA;* [2]*Centre UdL-IRTA, XaRTA-Postharvest, Rovira Roure, 191, 25198-Lleida, Catalonia, Spain;* [3]*Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA; and* [4]*U.S. Department of Agriculture, Agricultural Research Service, Food Quality Laboratory, Beltsville, Maryland 20705, USA*

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## ABSTRACT

This study was conducted to investigate the effect of free chlorine concentrations in wash water on *Escherichia coli* O157:H7 reduction, survival, and transference during washing of fresh-cut lettuce. The effectiveness of rewashing for inactivation of *E. coli* O157:H7 on newly cross-contaminated produce previously washed with solutions containing an insufficient amount of chlorine also was assessed. Results indicate that solutions containing a minimum of 0.5 mg/liter free chlorine were effective for inactivating *E. coli* O157:H7 in suspension to below the detection level. However, the presence of 1 mg/liter free chlorine in the wash solution before washing was insufficient to prevent *E. coli* O157:H7 survival and transfer during washing because the introduction of cut lettuce to the wash system quickly depleted the free chlorine. Although no *E. coli* O157:H7 was detected in the wash solution containing 5 mg/liter free chlorine before washing a mix of inoculated and uninoculated lettuce, low numbers of *E. coli* O157:H7 cells were detected on uninoculated lettuce in four of the seven experimental trials. When the prewash free chlorine concentration was increased to 10 mg/liter or greater, no *E. coli* O157:H7 transfer was detected. Furthermore, although rewashing newly cross-contaminated lettuce in 50 mg/liter free chlorine for 30 s significantly reduced ($P = 0.002$) the *E. coli* O157:H7 populations, it failed to eliminate *E. coli* O157:H7 on lettuce. This finding suggests that rewashing is not an effective way to correct for process failure, and maintaining a sufficient free chlorine concentration in the wash solution is critical for preventing pathogen cross-contamination.

Leafy green vegetables are a highly nutritious source of minerals, vitamins, and phytonutrients, which confer many health benefits including anticancer and antiaging properties; however, the risk for pathogen contamination in these vegetables also is high. In the period between 1996 and 2006, microbial contamination at the farm, packinghouse, or processor level contributed to 24 foodborne illness outbreaks associated with leafy greens, accounting for 34% of such specific produce-linked outbreaks *(14, 24)*. The nationwide spinach outbreak in 2006 caused three deaths, 205 confirmed infections, and 103 hospitalizations *(3, 5)*. Although a single production block in California most likely was responsible for all the contaminated spinach *(3, 16)*, the resulting widespread impact illustrates the potential for pathogen contamination to have devastating economic and social consequences.

In the absence of practical technologies that provide a kill step for eliminating all pathogens without significantly diminishing produce quality, a sanitizing wash to reduce pathogen populations is critically important in fresh-cut produce processing *(12, 29)*. However, washing also has the potential to cause pathogen cross-contamination, especially when water is reused and recirculated. Hence, 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 fresh and fresh-cut produce industry because of its minimal impact on the nutritional and aesthetic quality of the product, its established ability to kill pathogens in suspensions, and its low cost. Extensive study has revealed that hypochlorous acid is the most effective form of chlorine *(32)*. Maintaining a relatively consistent level of hypochlorous acid during commercial fresh-cut wash operations is a technical challenge in practice because of the quick reaction of this acid with organic materials in the produce wash solution *(12, 32)*. When freshly cut produce is introduced into the wash system, the sudden surge in organic matter from exudates can result in the rapid depletion of hypochlorous acid, creating a situation

* Author for correspondence. Tel: 301-504-6186; Fax: 301-504-5107; E-mail: yaguang.luo@ars.usda.gov.

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in which chlorine demand exceeds chlorine availability, which increases the potential for pathogen cross-contamination. Decreasing pH to 6.5 or below can significantly increase the proportion of hypochlorous acid present in the chlorine solution; however, formation of toxic chlorine gas also increases *(4, 30)*. Although free chlorine levels in the wash water can be increased to some degree by replenishing chlorine-generating chemicals, repeated addition of chlorine to wash water that is high in organic load also results in the increased formation of toxic chlorine byproducts and generation of chlorine gas. The accumulation of noxious chlorine gas *(12, 30)* in the production facility (often enclosed) may necessitate the evacuation of the employees from the entire processing plant.

For all of these reasons, the free chlorine concentrations in most commercial systems often fluctuate in the single digit range, although 200 mg/liter is allowed by the U.S. Food and Drug Administration. When free chlorine concentrations are this low, the introduction of additional loads of cut produce into the wash system could easily cause the temporary depletion of free chlorine, and thus products may be washed in water with insufficient chlorine to prevent pathogen survival. When this happens, pathogens from contaminated lettuce could cause widespread cross-contamination.

In an attempt to mitigate pathogen cross-contamination of produce washed in water with inadequate free chlorine, many fresh-cut produce companies have prescribed rewash as the corrective action in their hazard analysis and critical control point (HACCP) program *(15)*. This approach is partially based on hypotheses from the 1990s that (i) chlorine wash is a terminal kill step for fresh-cut produce, and (ii) freshly contaminated pathogens may not have had time to attach to the lettuce, and thus the subsequent wash could completely remove or inactivate these pathogens. In light of recent findings that chlorine is not a kill step on produce and that it achieves no more than a 1- to 2-log reduction of pathogens *(1, 2, 8, 18)*, questions have arisen as to whether HACCP plans should be amended to advise disposing of rather than rewashing the products that had been washed in water with free chlorine concentrations below the critical limit. Questions also remain as to the minimum chlorine concentration required to prevent pathogen survival in fresh-cut produce wash solutions and thus to prevent solution-mediated cross-contamination. Experiments reported here were conducted to address these issues.

## MATERIALS AND METHODS

***Escherichia coli* O157:H7 strains.** Attenuated laboratory strains and produce-associated outbreak strains of *E. coli* O157:H7 were used in this study. CDC B6914/pGFP (AmpR) *(11)*, CDC F6460-Nal *(22, 23)*, and ATCC 700728 *(13)* (attenuated) were obtained from the collections of the Environmental Microbial and Food Safety Laboratory (Beltsville, MD), and lettuce-associated outbreak strain RM4407 *(26)* was kindly provided by Dr. R. Mandrell (U.S. Department of Agriculture, Agricultural Research Service, Western Regional Research Center, Albany, CA). CDC B6914/pGFP was used for all produce wash–related experiments. Individual *E. coli* O157:H7 strains were grown overnight in tryptic soy broth (TSB; BD, Franklin Lakes, NJ) at 37°C. Cells were harvested by centrifugation for 5 min at 6,038 × *g* (5°C) and washed once in sterile phosphate buffered saline (PBS). Precipitated cells were resuspended in an equal volume of PBS and subsequently further diluted as needed for inoculation.

**Lettuce inoculation.** Romaine lettuce (*Lactuca sativa* L.) was obtained from a produce wholesale market (Jessup, MD) and stored at 1°C for 24 h before the inoculation process. The lettuce heads were trimmed to remove damaged and outer leaves. The inoculum containing *E. coli* O157:H7 at approximately $10^6$ CFU/ml was randomly deposited on adaxial and abaxial sides of lettuce leaves (~10 g) in 20 droplets of 5 μl each, with a final targeted *E. coli* O157:H7 inoculum level of approximately $10^4$ CFU/g of lettuce. Inoculated lettuce leaves were left in a biological safety hood for 60 min to allow the droplets to evaporate and/or to adsorb to the leaf surfaces and then were placed in plastic bags (unsealed to allow for gas exchange) and stored at 5°C for 20 h to allow *E. coli* O157:H7 attachment.

**Wash solution preparation and produce washing.** Wash solutions containing targeted free chlorine concentrations (0 to 100 mg/liter) were prepared using 6% sodium hypochlorite, and pH was adjusted to 6.5 with citric acid. The actual free chlorine concentrations before and after treatment were determined with a chlorine photometer (CP-15, HF Scientific Inc., Ft. Myers, FL). Water quality parameters, including total dissolved solids (TDS), turbidity, and pH, were measured with a conductivity meter (model 135A, Orion Research Inc., Beverly City, MA), a turbidity meter (Aquafast, Thermo Orion, Beverly, MA), and a digital pH meter (Oakton Instruments, Vernon Hills, IL), respectively, before and after each wash.

To evaluate the effect of chlorine concentration on *E. coli* O157:H7 reduction, wash solutions containing free chlorine of 0, 1, 5, 25, and 100 mg/liter were prepared as described above. The inoculated lettuce (150 g; $10^4$ CFU/g *E. coli* O157:H7) was manually shredded into 6.4-mm-wide strips and washed in 3 liters of chlorine solution (produce to solution ratio of 1:20; wash water temperature of 22°C) for 30 s with manual agitation. Washed lettuce was drained and dewatered in a manually actuated spin dryer (Good Grips, OXO, Elmira, NY), and duplicate 25-g samples were removed for microbial analyses. To evaluate the transference of *E. coli* O157:H7 during washing, inoculated lettuce was shredded into 6.4-mm-wide strips, and the uninoculated lettuce was cut into squares (25.4 by 25.4 mm) with a lettuce cutter (KutLett SKK2, Silver King, Minneapolis, MN). Care was taken to ensure that the inoculated and uninoculated lettuce leaves had recognizably different shapes. Samples of inoculated lettuce strips (30 g each) were submerged in 3 liters of wash solution, and immediately after, 120 g of uninoculated lettuce squares were submerged in the same solution. After 30 s of washing and follow-up dewatering, the inoculated lettuce strips and uninoculated lettuce squares were carefully sorted, and duplicate samples of each were taken for microbial analyses. To assess the effectiveness of rewashing on the removal and/or inactivation of *E. coli* O157:H7 from freshly contaminated lettuce, uninoculated lettuce squares that were washed with inoculated lettuce in water only (0 mg/liter chlorine) were collected after sorting and rewashed within 2 h in 50 mg/liter free chlorine solution for 30 s. Samples before and after rewashing were enumerated for *E. coli* O157:H7. In separate experiments, *E. coli* O157:H7 cells of various strains were directly inoculated into the wash solution to determine the minimal chlorine levels for pathogen inactivation in solution. After 30 s

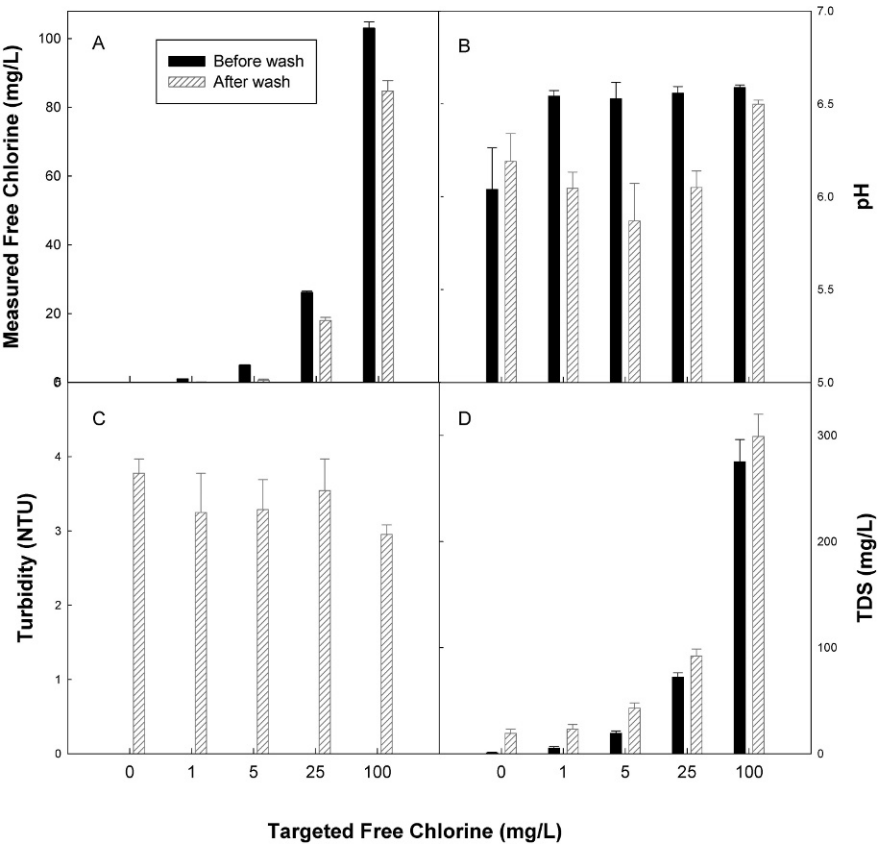


FIGURE 1. *Changes in (A) free chlorine, (B) pH, (C) turbidity, and (D) total dissolved solids (TDS) in wash water during romaine lettuce wash. Data presented are the means of five replicate experiments; vertical lines represent standard errors.*

of exposure, residual chlorine in the solution was neutralized with sodium thiosulfate *(17)* immediately after treatment, before samples were taken for bacterial enumeration.

**Enumeration of *E. coli* O157:H7.** Cells of *E. coli* O157:H7 that survived chlorine treatments in solution and on lettuce were enumerated using a most-probable-number (MPN) procedure as previously described *(25)* with minor modification. To enumerate cells in solution, eight aliquots (1 ml) of the solution were mixed with equal volumes of 2× TSB supplemented with sodium pyruvate (0.1%, wt/vol) and 10-fold serially diluted in 8 by 12 deep-well microplates (2.5 ml per well). To enumerate *E. coli* O157:H7 (strain CDC B6914/pGFP) cells on lettuce, each sample (25 g) was placed in a filter stomacher bag with 125 ml of TSB supplemented with ampicillin (100 μg/liter). The samples were macerated with a stomacher blender (Stomacher 400, Seward, London, UK) at 230 rpm for 2 min. Five to eight aliquots (3 ml each) of the filtrate were added to a 8 by 6 deep-well microplate (6.0 ml per well) and 10-fold serially diluted with TSB-ampicillin. The microplates were covered with breathable film and incubated overnight at 37°C. The turbidity of each well after incubation was recorded, and 3-μl droplets of the cultures were arrayed on sorbitol MacConkey agar plates supplemented with 50 μg/liter cefixime and 2.5 mg/liter potassium tellurite (ctSMAC; Invitrogen, Carlsbad, CA). Appropriate antibiotics were also included in the ctSMAC plates to select for the target strains, i.e., ampicillin (100 μg/liter) for strain CDC B6914/pGFP and nalidixic acid (25 μg/liter) for strain CDC F6460-Nal. The growth of *E. coli* O157:H7 strain CDC B6914/pGFP also was indicated by the presence of green fluorescence. Serological testing with a rapid RIM *E. coli* O157:H7 latex agglutination assay (Remel Inc., Lenexa, KS) was used periodically for further confirmation of characteristic *E. coli* O157:H7 colonies. An MPN calculator (VB-6 version) was used to compute the MPN of cells for each sample *(6)*. Detection limits were defined as the MPN when a single undiluted well was determined to be positive for *E. coli*. Detection limits of 0.12 to 0.36 MPN/ml or 0.36 MPN/g were achieved in different experiments because numbers of wells per dilution and volumes of each liquid aliquot differed.

**Experimental design and statistical analysis.** The experiment was conducted based on a factorial design with three to seven replications. For data presented in Figure 1, the difference in the before and after washing measurement for the free chlorine, pH, TDS, and turbidity variables were analyzed as one-factor linear models using Proc Mixed (SAS Institute Inc., Cary, NC) with targeted free chlorine as the main factor. For studies related to microbial reduction, survival, and transference (Figs. 2 and 3 and Tables 1 through 3), *E. coli* O157:H7 populations were subjected to a log transformation before statistical analysis. For treatments with nondetectable *E. coli* O157:H7 counts, the value of the detection limit was used. For all studies, assumptions of normality and variance homogeneity of the linear model were checked, and the variance grouping technique was used to correct for variance heterogeneity. When effects were significant, means were compared using Sidak adjusted *P* values to maintain the experimentwise error at $\leq 0.05$.

## RESULTS AND DISCUSSION

**Inactivation of *E. coli* O157:H7 in the wash solution.** Survival of *E. coli* O157:H7 in the wash solution is highly dependent on the free chlorine concentration in the solution. All four tested strains of *E. coli* O157:H7 decreased significantly in numbers after exposure to as little as 0.125 mg/liter free chlorine for 30 s (Table 1). *E. coli* O157:H7 survival further decreased with increases in free chlorine concentration. No surviving *E. coli* O157:H7 cells

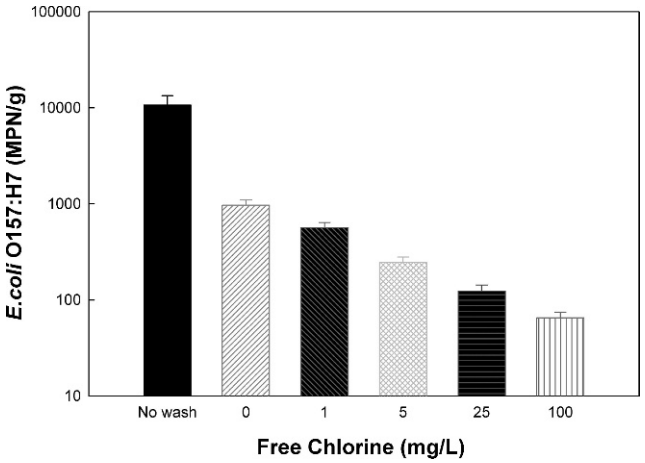


FIGURE 2. E. coli *O157:H7 populations recovered from inoculated fresh-cut romaine lettuce after washing in solutions containing 0 to 100 mg/liter free chlorine. Data presented are the means of five replicate experiments with duplicate samples per replication; vertical lines represent standard errors.*

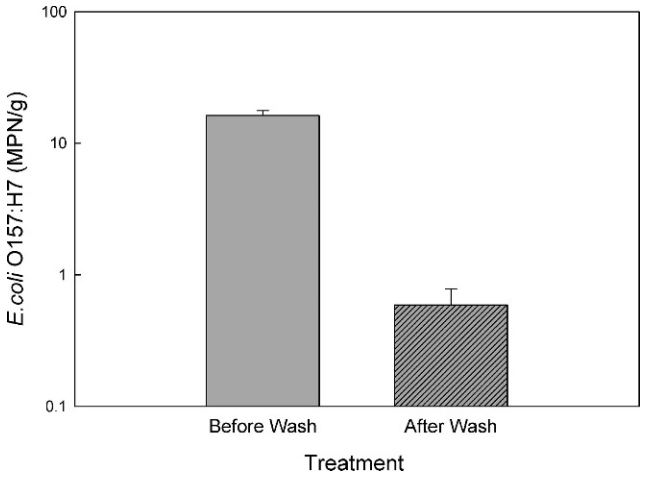


FIGURE 3. E. coli *O157:H7 populations recovered from cross-contaminated lettuce rewashed in 50 mg/liter free chlorine solution for 30 s. Data presented are the means of three replicate experiments; vertical lines represent standard errors.*

were found when the free chlorine concentration was increased to 0.5 mg/liter or above. Although all four strains tested were susceptible to low concentrations of chlorine in solution, the strain F6460 had a significantly higher survival rate and strain RM4407 had a significantly lower survival rate than the rest of the strains after exposure to 0.125 or 0.25 mg/liter free chlorine. Similar results were obtained with *Salmonella* (data not shown) and by Rice et al. *(28)* and Zhao et al. *(33)*. However, when a chlorine-resistant *E. coli* strain was used, a higher concentration of chlorine was needed to inactivate it *(9)*. *E. coli* O157:H7 inactivation results presented in Table 1 were obtained after 30 s of exposure, which was intended to mimic the wash solution contact time commonly used by fresh-cut produce processors. The *E. coli* O157:H7 inactivation rate may increase with increased exposure time *(28)*.

**Changes in water quality and free chlorine concentrations during lettuce washing.** When cut produce is introduced to a wash solution, free chlorine concentrations and water quality change immediately. Figure 1A shows the decline in free chlorine concentrations from 1 and 5 mg/liter before washing to 0.06 and 1.7 mg/liter, respectively, after washing. Solutions with initial free chlorine concentrations of 25 and 100 mg/liter retained copious free chlorine (16 and 88 mg/liter, respectively) in the wash solution despite a significant decline in free chlorine after washing. The measured free chlorine is the residual chlorine after satisfying the chlorine demand of organic materials. When the chlorine concentration is low, almost all chlorine present in the wash solution is consumed in the reaction with organic materials, leaving no or minimal residual free chlorine in the wash solution. Consequently, chlorine concentration decreases dramatically. In contrast, when chlorine concentration is high, the loss of chlorine through its reaction with the organic material has much less impact on the overall chlorine concentration because there is still plenty of free chlorine remaining in the wash solution.

Washing of cut lettuce in the chlorine solution also affected the solution pH. In general, romaine lettuce juice pH ranged from 6.1 to 6.3 (average of 6.2). Depending on the initial pH of the wash solution, washing of lettuce gradually changed the solution pH toward that of the lettuce juice pH (Fig. 1B).

Turbidity is an indicator of water quality in terms of soil, debris, and organic material in the wash solution. Before introduction of the lettuce, the wash solution was clear and the turbidity reading was low, ranging from 0.2 to 0.5 NTU. After washing, turbidity readings increased significantly ($P < 0.01$), although the difference among chlorine treatments was small because turbidity is affected by the lettuce exudates rather than the chlorine treatment (Fig. 1C).

TDS is a measure of the combined content of all inorganic and organic substances contained in a liquid. Unlike turbidity, TDS readings were significantly influenced ($P < 0.01$) by chlorine concentration. Increasing chlorine concentration in the wash water resulted in proportional increases in TDS readings. Washing lettuce as described resulted in an additional increase of 18 to 24 mg/liter in TDS, regardless of the initial chlorine concentration (Fig. 1D). This increase was most likely due to soil and debris rinsed from the lettuce surface and the release of lettuce exudates into the solution.

**Pathogen reduction during washing of fresh-cut lettuce.** *E. coli* O157:H7 populations on inoculated lettuce averaged 4.0 log MPN/g before washing (Fig. 2). Washing in water alone reduced *E. coli* O157:H7 populations by an average of 1.0 log MPN/g. Washing with solutions containing free chlorine at 1, 5, 25, and 100 mg/liter resulted in an additional 0.2, 0.6, 0.9, and 1.2 log MPN/g reduction in *E. coli* O157:H7, respectively, compared with water wash (0 mg/liter chlorine). Although the treatment difference is small, especially that between the 0 and 1 mg/liter free chlorine treatments, all treatment differences were significant ($P = 0.001$). This finding differs from those of

TABLE 1. *Survival of various strains of* E. coli *O157:H7 in chlorine solutions*

| Strain | *E. coli* O157:H7 recovered (MPN/ml) from solution with target free chlorine concn of: | | | | | |
|---|---|---|---|---|---|---|
| | 0 mg/liter | 0.125 mg/liter | 0.25 mg/liter | 0.5 mg/liter | 1.0 mg/liter | 2.0 mg/liter |
| B6914 | >210,000 | 70.08 | 2.15 | ND[a] | ND | ND |
| F6460 | >210,000 | 210 | 0.45 | ND | ND | ND |
| ATCC700728 | >210,000 | 70.13 | 0.48 | ND | ND | ND |
| RM4407 | >210,000 | 4.08 | 0.17 | ND | ND | ND |

[a] ND, not detected at a detection limit of 0.12 MPN/ml.

most authors, who often reported that chlorine concentration had no effect on pathogen reduction *(7, 27)*. However, concentrations tested in most of those studies were high, with few experimental repetitions and large sample variations. In the present study, romaine lettuce was quantitatively inoculated, and the experiment was repeated five times. Efforts were made to minimize sample variations and increase experiment precision. These factors contributed to the high statistical power of the analysis and therefore the high sensitivity for detecting small treatment differences. The use of the eight-tube (well) MPN method in this study further increased its sensitivity for detecting small treatment differences in comparison to the traditional plating method used in other studies. Nevertheless, although all treatment differences were significant, the differences were small.

**Recovery of *E. coli* O157:H7 in the solution after washing inoculated lettuce.** When free chlorine concentration in the wash solution was 1 mg/liter or less, the introduction of lettuce into the wash system instantly depleted the free chlorine and allowed survival of *E. coli* O157:H7 in the wash solution (Table 2 and Fig. 1). When free chlorine was present at 5 mg/liter or above before washing, the introduction of lettuce into the wash system did not deplete the free chlorine, and *E. coli* O157:H7 was not detected in the wash water (below the detection limit of 0.36 MPN/ml). This finding is in agreement with data presented in Table 1 for the effect of chlorine concentration in solution on the survival of various strains of *E. coli* O157:H7.

**Transference of *E. coli* O157:H7 from inoculated to uninoculated lettuce during washing.** Pathogens may be transferred from contaminated to clean lettuce when the wash solution is low in free chlorine. *E. coli* O157:H7 was detected on uninoculated lettuce after it was washed with inoculated lettuce in solutions with a prewash free chlorine concentration of 0 to 1 mg/liter (Table 2). *E. coli* O157:H7 transference was not detected when the washing solution had 25 mg/liter or greater free chlorine before washing. *E. coli* O157:H7 transference from inoculated to clean lettuce was found in two of the four trials when the lettuce slices were washed in a solution containing an initial free chlorine concentration of 5 mg/liter, although *E. coli* O157:H7 did not survive in the wash solution (Table 2). This result suggests that although the average postwash concentration of free chlorine was 1.7 mg/liter for the 5-mg/liter chlorine treatment, microenvironments in which free chlorine is temporarily depleted might exist in the solution, allowing survival of *E. coli* O157:H7 and consequent cross-contamination of lettuce. *E. coli* O157:H7 also may have been transferred by direct contact between clean and inoculated samples in the wash solution. A third possibility is that more time is needed for the lower chlorine concentration to kill bacteria in solution. During that time, some bacteria can be transferred to lettuce leaves and attach, becoming inaccessible to the chlorine. However, when the initial free chlorine concentration was 25 mg/liter or higher, there was no transference of *E. coli* O157:H7 from contaminated to clean lettuce. This finding is important because the HACCP program used by the produce industry often considers the chlorinated wash as the critical control point with the control limit set at no less than 1 mg/liter free chlorine wash for 30 s at a pH of less than 7.0 *(15)*.

To further investigate the effect of chlorine wash solutions on prevention of pathogen transfer, three addi-

TABLE 2. *Recovery of* E. coli *O157:H7 from wash solution and uninoculated lettuce after washing a mix of inoculated and uninoculated lettuce in 0- to 100-mg/liter solutions of free chlorine*

| Sample | Trial | *E. coli* O157:H7 recovered (MPN/ml) after a prewash free chlorine concn of: | | | | |
|---|---|---|---|---|---|---|
| | | 0 mg/liter | 1 mg/liter | 5 mg/liter | 25 mg/liter | 100 mg/liter |
| Wash solution | I | 4.3 | 0.4 | ND[a] | ND | ND |
| | II | 7.7 | 7.3 | ND | ND | ND |
| | III | 5.9 | ND | ND | ND | ND |
| | IV | 4.6 | 1.1 | ND | ND | ND |
| Uninoculated lettuce | I | 0.645 | 0.4 | ND | ND | ND |
| | II | 14.6 | 2.1 | ND | ND | ND |
| | III | 16.85 | 1.2 | 0.65 | ND | ND |
| | IV | 17.35 | 1.15 | 0.36 | ND | ND |

[a] ND, not detected at a detection limit of 0.36 MPN/ml in solution or 0.36 MPN/g on lettuce.

TABLE 3. *Recovery of* E. coli *O157:H7 from wash solution and uninoculated lettuce after washing a mix of inoculated and uninoculated lettuce in 0- to 25-mg/liter solutions of free chlorine*

| Sample | Trial | *E. coli* O157:H7 recovered (MPN/ml) after a prewash free chlorine concn of: | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 mg/liter | 1 mg/liter | 2 mg/liter | 5 mg/liter | 10 mg/liter | 15 mg/liter | 25 mg/liter |
| Wash solution | I | 8.15 | 1.4 | 0.4 | ND[a] | ND | ND | ND |
| | II | 5.95 | 1.15 | 0.65 | ND | ND | ND | ND |
| | III | 18.5 | 1.65 | 1.145 | ND | ND | ND | ND |
| Uninoculated lettuce | I | 18.5 | 2.1 | 2.3 | 0.65 | ND | ND | ND |
| | II | 11.9 | 2.1 | 1.75 | ND | ND | ND | ND |
| | III | 24.5 | 3.1 | 1.95 | 0.60 | ND | ND | ND |

[a] ND, not detected at a detection limit of 0.36 MPN/ml in solution or 0.36 MPN/g on lettuce.

tional experimental trials were conducted with free chlorine treatment concentrations set at much closer intervals, i.e., 0, 1, 2, 5, 10, 15, and 25 mg/liter. The prewash solution containing 1 to 2 mg/liter free chlorine significantly reduced *E. coli* O157:H7 survival in the wash solution compared with water alone ($P = 0.005$), although the *E. coli* O157:H7 was not completely eliminated (Table 3). When the free chlorine concentration reached 5 mg/liter or above, *E. coli* O157:H7 survival was not observed in the wash solution. *E. coli* O15:H7 recovery from uninoculated lettuce was dependant on free chlorine concentration. Compared with the water-only wash, the presence of 1 and 2 mg/liter free chlorine in solution significantly reduced ($P = 0.0003$) but did not eliminate the transference of *E. coli* O157:H7 from inoculated to uninoculated lettuce. Similar to the findings in the earlier trials (Table 2), when the free chlorine concentration in the wash solution was 5 mg/liter before washing, *E. coli* O157:H7 was detected on uninoculated lettuce even though no *E. coli* O157:H7 was found in the same wash solution. When the free chlorine concentration in the wash solution was further increased to 10 mg/liter or above, no *E. coli* O157:H7 was found on uninoculated lettuce. This finding indicates that a higher chlorine concentration is needed to prevent produce cross-contamination than is needed to prevent pathogen survival in the wash solution. Overall, under the testing conditions, 5 mg/liter free chlorine was needed to prevent pathogen survival in solution, and 10 mg/liter free chlorine was necessary to prevent pathogen transference from contaminated to uncontaminated lettuce during washing of this fresh-cut lettuce.

The change in free chlorine concentration observed after washing was affected by organic loads in the wash system, which is affected by raw product quality and fresh-cut product cutting and washing procedures. For example, the cut size and shape can significantly affect the release of lettuce latex and thus the organic load in the wash system; the ratio of product to wash solution also would affect the ability to maintain free chlorine concentrations *(21)*. In our experiment, because of the need for pathogen containment, the lettuce was cut manually with a knife rather than with a commercial cutter such as those used in industry. For this reason, the tissue damage encountered during our experiment might be less than that occurring in commercial operations. As such, the changes in free chlorine concentration and other water quality parameters presented in our study might be less dramatic than those in commercial settings.

**Effect of rewash on inactivation of *E. coli* O157:H7.** Rewashing lettuce in chlorinated solutions has been described as the corrective action in model HACCP plans and is widely practiced by industry to address the potential food safety concerns associated with cut produce that has been washed in solutions with insufficient chlorine concentration *(15)*. However, the effectiveness of this practice for ensuring food safety has not been validated. In a recent study, when lettuce was contaminated during prewash, a subsequent wash did not produce a significant reduction of the pathogen population *(20)*. In our study, rewashing with 50 mg/liter free chlorine for 30 s significantly reduced ($P = 0.002$) the *E. coli* O157:H7 population on freshly contaminated lettuce; however, small numbers of *E. coli* O157:H7 cells remained on lettuce samples in all three experimental trials. This finding suggests that contrary to the widely held belief, rewashing is not an effective means of correcting a failure in process control such as cross-contamination due to insufficient sanitizer. Although freshly contaminated *E. coli* O157:H7 may have only a short time to adhere and attach to lettuce, cut edges and crevasses on lettuce could allow *E. coli* O157:H7 to attach and thereby become inaccessible to the chlorine in the wash *(10, 19, 31)*.

In conclusion, our results indicate that the tested strains of *E. coli* O157:H7 were inactivated by a 30-s exposure to solutions containing free chlorine concentrations of 0.5 mg/liter or above when pure cultures at $>2 \times 10^4$ MPN/ml were introduced into the solution. When lettuce shreds inoculated with *E. coli* O157:H7 were introduced into the solution, a postwash residual free chlorine concentration of more than 0.5 mg/liter was needed to prevent *E. coli* O157:H7 survival in the solution and solution-mediated cross-contamination. However, a prewash free chlorine concentration of 10 mg/liter or greater was needed to prevent both solution-mediated and direct contact–mediated pathogen transference from contaminated to uncontaminated lettuce. Rewashing was not effective for eliminating *E. coli* O157:H7 on newly cross-contaminated lettuce. Maintaining a sufficient free chlorine concentration is critical for preventing pathogen cross-contamination during washing operations for fresh-cut produce.

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