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Association between bacterial survival and free chlorine concentration during commercial fresh-cut produce wash operation

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Determining the minimal effective free chlorine (FC) concentration for preventing pathogen survival and cross-contamination during produce washing is critical for developing science- and risk-based food safety practices. The correlation between dynamic FC concentrations and bacterial survival was investigated during commercial washing of chopped Romaine lettuce, shredded Iceberg lettuce, and diced cabbage as pathogen inoculation study during commercial operation is not feasible. Wash water was sampled every 30 min and assayed for organic loading, FC, and total aerobic mesophilic bacteria after chlorine neutralization. Water turbidity, chemical oxygen demand, and total dissolved solids increased significantly over time, with more rapid increases in diced cabbage water. Combined chlorine increased consistently while FC fluctuated in response to rates of chlorine dosing, product loading, and water replenishment. Total bacterial survival showed a strong correlation with real-time FC concentration. Under approximately 10 mg/L, increasing FC significantly reduced the frequency and population of surviving bacteria detected. Increasing FC further resulted in the reduction of the aerobic plate count to below the detection limit (50 CFU/100 mL), except for a few sporadic positive samples with low cell counts. This study confirms that maintaining at least 10 mg/L FC in wash water strongly reduced the likelihood of bacterial survival and thus potential cross contamination of washed produce.

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1. Introduction

Fresh fruits and vegetables are nutrient-rich foods with high levels of minerals, vitamins, and phytochemicals. 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 (Lynch et al., 2009; Callejon et al., 2015; Arnade et al., 2015; Jung et al., 2014; Painter et al., 2013). Produce can be contaminated with pathogens during primary production and is often consumed raw without a ‘kill step’ such as cooking (Bartz et al., 2017). No effective or practical disinfection technologies are currently available to eliminate pathogens without significantly degrading produce quality, and post-harvest pathogen cross-contamination can significantly increase the size of foodborne illness outbreaks (Gil et al., 2009).

Washing is a critical process in preparing for fresh-cut “ready-to-eat” food product and is often the only step that could remove foreign materials and tissue exudates, and inactivate pathogens (Gil et al., 2009). Most fresh-cut produce washing is conducted by immersing produce in tanks or flumes of wash water, which is recirculated and reused due to the need for cost reduction and water conservation (Gil et al., 2009). During this process, pathogens dislodged from contaminated produce can survive in wash water, and spread to other clean produce that are washed simultaneously or subsequently in the same process water, causing pathogen cross-contamination of a large quantity of produce, if uncontrolled (Holvoet et al., 2012; López-Gálvez et al., 2009, 2010; Pérez-Rodríguez et al., 2014). Thus, the presence of sanitizers is critical to prevent pathogen survival and cross-contamination in fresh-cut produce washing operations (Luo et al., 2011; Tomas-Callejas et al., 2012).

Among available sanitizers, chlorine is most widely used in the fresh and fresh-cut produce industry due to its low cost, ease of use,

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and its effectiveness against vegetative bacteria and some enteric viruses (Gombas et al., 2017; Van Haute et al., 2013; 2015; 2017). Traditionally, a free-chlorine concentration of 1 mg/L has been considered as the “Control Limit” and “rewash” as the “Corrective Action” in the hazard analysis and critical control points (HACCP) programs (Hurst, 2002; IFPA, 2001). Earlier research from Luo et al. (2011) demonstrated that this minimum FC concentration is inadequate to prevent pathogen survival and cross-contamination, and that rewashing is ineffective as a corrective action once produce has become contaminated. Luo et al. (2011) also reported that no cross-contamination was found when the FC concentration was at least 10 mg/L. Shen (2014) evaluated the effect of residual FC (i.e., measured free and available chlorine concentration after chlorine reaction with organic load) on pathogen cross-contamination and reported no pathogen cross-contamination during a decline in residual FC from 40.8 to 9.4 mg/L, but detectable cross-contamination after the FC declined to 4.6 mg/L and below. Gómez-López et al. (2014) used a different approach to evaluate pathogen survival during the dynamic changes in FC concentration affected by adding *Escherichia coli* O157:H7 inoculated spinach juice and continuously replenishing chlorine. When a free available chlorine concentration of 5 mg/L was maintained, no pathogens were detected in the wash water during the entire 1-h testing period. The reasons for these reported different results are unclear. While testing conditions in these reports were certainly different, future research will also need to determine if chlorine depletion cycles vs periodic replenishment vs. continuous replenishment have any impact on pathogen survival at the same level of free chlorine concentration.

Maintaining a stable FC concentration during fresh-cut produce washing is challenging, although residual FC is relatively stable in pure water (Suslow, 1997; Luo, 2007; Luo et al., 2011, 2012). Cut produce release copious amount of organic materials that quickly react with and deplete FC (Luo, 2007; Luo et al., 2012). Although the FC concentration can be restored to some extent through frequent addition of sodium hypochlorite, increased rate of addition is required as the accumulation of organic materials progresses over time. When the wash water's organic load gets too high, not only does the repeated chlorine addition become ineffective to restore FC concentration, the formation of hazardous chlorine byproducts, including trihalomethanes also causes concerns (Connell, 1996). The formation of hazardous chlorine off-gas if overdosing occurs together with excessively low pH may necessitate the temporary closure of a processing plant and the evacuation of employees (Connell, 1996). Furthermore, system water volume to product ratio and how/where chlorine is added to the water all impact the propensity to "gas-off". Thus, it is critical to determine the minimal FC residual that effectively prevents pathogen cross-contamination and also is feasible for commercial implementation.

In recognizing the food safety needs and practical challenges of maintaining sufficient sanitizer concentration during commercial fresh-cut produce wash operations, a working group consisting of technical experts from the produce industry, US government researchers, and scientists from academic fields has recently developed a guidance document entitled “Guidelines to validate control of cross-contamination during washing of fresh-cut leafy vegetables” (Gombas et al., 2017). One critical research need identified by the authors is specific scientific data obtained under commercial operating conditions to determine the minimal FC concentration required to prevent pathogen cross-contamination and the assessment of industry's process capability to maintain such FC concentration. Given that inoculating produce with human pathogens or even their toxin-free surrogates during commercial operations is not feasible due to the inherent food safety risks, assessment of the inactivation/survival of indigenous bacteria in wash water as impacted by real time FC concentration in a commercial setting may provide valuable insight.

In this paper, we report the first study to investigate the dynamic changes in organic load, pH and FC concentration, and the relationship between bacterial survival and the real time FC concentration, during commercial fresh-cut produce wash operations while processing major fresh-cut commodities including chopped Romaine lettuce, shredded Iceberg lettuce, and diced cabbage.

2. Materials and methods

2.1. Fresh-cut produce washing system

The experiment was conducted in collaboration with a medium-sized US fresh-cut produce processing facility during their routine commercial production. All tests were coordinated between the processor and researchers to ensure that all testing conditions were as consistent as possible with minimal disruption to the regular production flow. The wash system consists of a sequential double flume configuration, with wash water from individual flumes recirculating through a catch tank with a 1 mm screen for large debris removal and free chlorine replenishment (Fig. 1). The primary flume (flume A) has a capacity of 9000 L and the secondary (flume B) a capacity of 7100 L. The flume is filled with pre-chilled water (4 °C) before production starts with additional chilled water added to both flumes to compensate for processing water loss. A portion of the wash water in flume A is replaced periodically to avoid the excessive accumulation of organic load during operation. The FC and pH are adjusted with sodium hypochlorite and a phosphoric acid-based acidulant, respectively to approximate targeted values (Luo et al., 2012; Nou and Luo, 2010; Shen et al., 2012). The pH and FC level in the flumes were monitored and maintained using an Automated Analytic Platform™ (SmartWashSolutions Inc, Salinas, CA, USA).

2.2. Materials and processing

Romaine lettuce (*Lactuca sativa* var. *longifolia*), Iceberg lettuce (*Lactuca sativa* var. *capitata*), and cabbage (*Brassica oleracea* var. *capitata*) were harvested, stored at 5 °C and used within two days of harvesting. Fresh Romaine lettuce was trimmed onsite, and cut into 25 × 25 mm pieces (chop) using a belt-fed slicer (TranSlicer® 2510 Cutter, Urschel Laboratories, Inc., Chesterton, IN, USA) and introduced at an average rate of 1560 kg/hr into flume A. Iceberg lettuce was pre-cored in the field, cut into 6 mm strips (shred) using TranSlicer® 2510 Cutter (Urschel Laboratories) and introduced at an average rate of 3660 kg/hr into flume A. Cabbage was trimmed and cored onsite and cut into 5 × 5 mm pieces (dice) using a DiversaCut (Urschel Laboratories) and introduced at an average rate of 2240 kg/hr into flume A. Cut vegetables were sequentially washed in flumes A and B, each with a residence time of about 30 s. Three independent runs of the washing operation were conducted for each type of produce.

2.3. Sample collection

For each independent run, Wash water samples were collected at two locations in each flume (Fig. 1) in 30-min intervals for up to approximately 2.5 h production schedule of the selected products. FC and total chlorine were determined immediately upon sample collection, and the other water quality parameters were tested in an auxiliary laboratory at the facility. Parallel samples were taken using sterile containers for microbiological analyses. For Romaine lettuce, the microbial data from the first trial was not included due to issues encountered in setting up the mobile microbial lab. So, only 2 repetitions of the microbial data were used for chopped

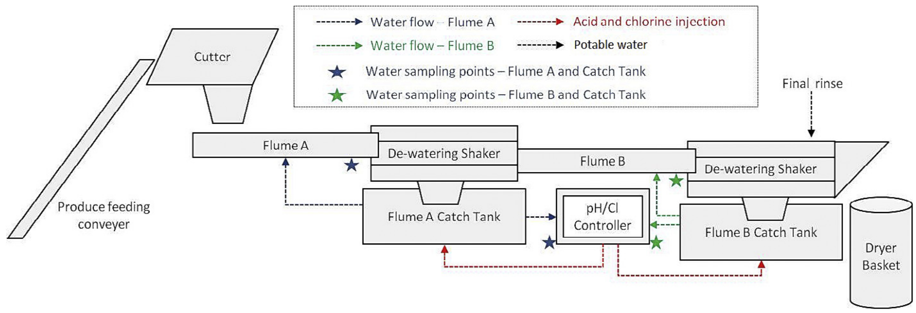

Fig. 1. Schematic illustration showing the commercial flume wash system and sampling points used in this study.

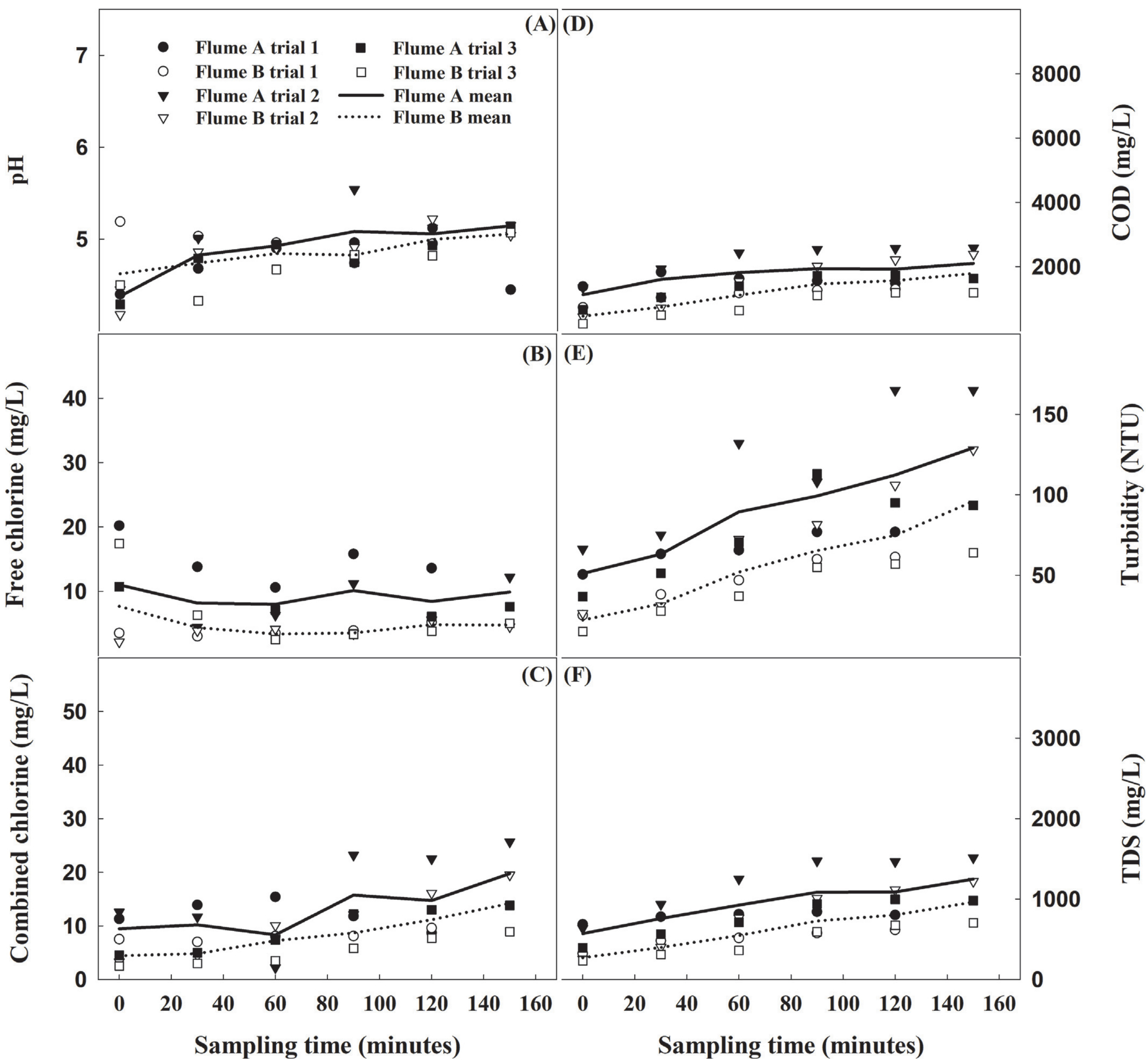

Fig. 2. Changes in pH, chemical oxygen demand (COD), turbidity, total dissolved solids (TDS), free chlorine (FC), and combined chlorine (CC) over time in wash water for processing chopped Romaine lettuce.

Romaine lettuce while 3 repetitions each were used for shredded Iceberg lettuce and diced cabbage.

2.4. Physicochemical analyses

The pH was determined with a digital pH meter (Oakton Instruments, Vernon Hills, IL, USA). FC and total chlorine concentrations were measured using the N,N-diethyl-*p*-phenylenediamine (DPD) method (Eaton and Franson, 2005) with a Chlorine Photometer (HF Scientific Inc., FT. Myers, FL, USA). Combined chlorine (CC) was calculated by subtracting the measured FC from the total chlorine. Turbidity was measured using a turbidimeter (Orion AQ4500, Thermo Scientific, Singapore). Total dissolved solid (TDS) was determined with a TDS meter (135A, Thermo Orion, Germany) while chemical oxygen demand (COD) was analyzed following a reactor digestion method (Zhou et al., 2014).

2.5. Microbiological analyses

Water samples for microbial analysis were filtered through a Whirl-Pak bag with 0.3 mm perforated polyethylene separator (Nasco, Fort Atkinson, WI, USA) and transferred into a sterile 100 ml bottle prefilled with sodium thiosulfate (final concentration 1 mg/mL) to neutralize residual chlorine. The water sample was serially diluted as necessary and plated on Aerobic Count Plate Petrifilm (3M, Maplewood, MN, USA) in duplicate, to determine aerobic plate count (APC). Petrifilms were incubated at 35 °C for 36 h, before enumerating colonies using a petrifilm counter (3M). The limit of detection (LOD) for analyses was 50 CFU/100 mL.

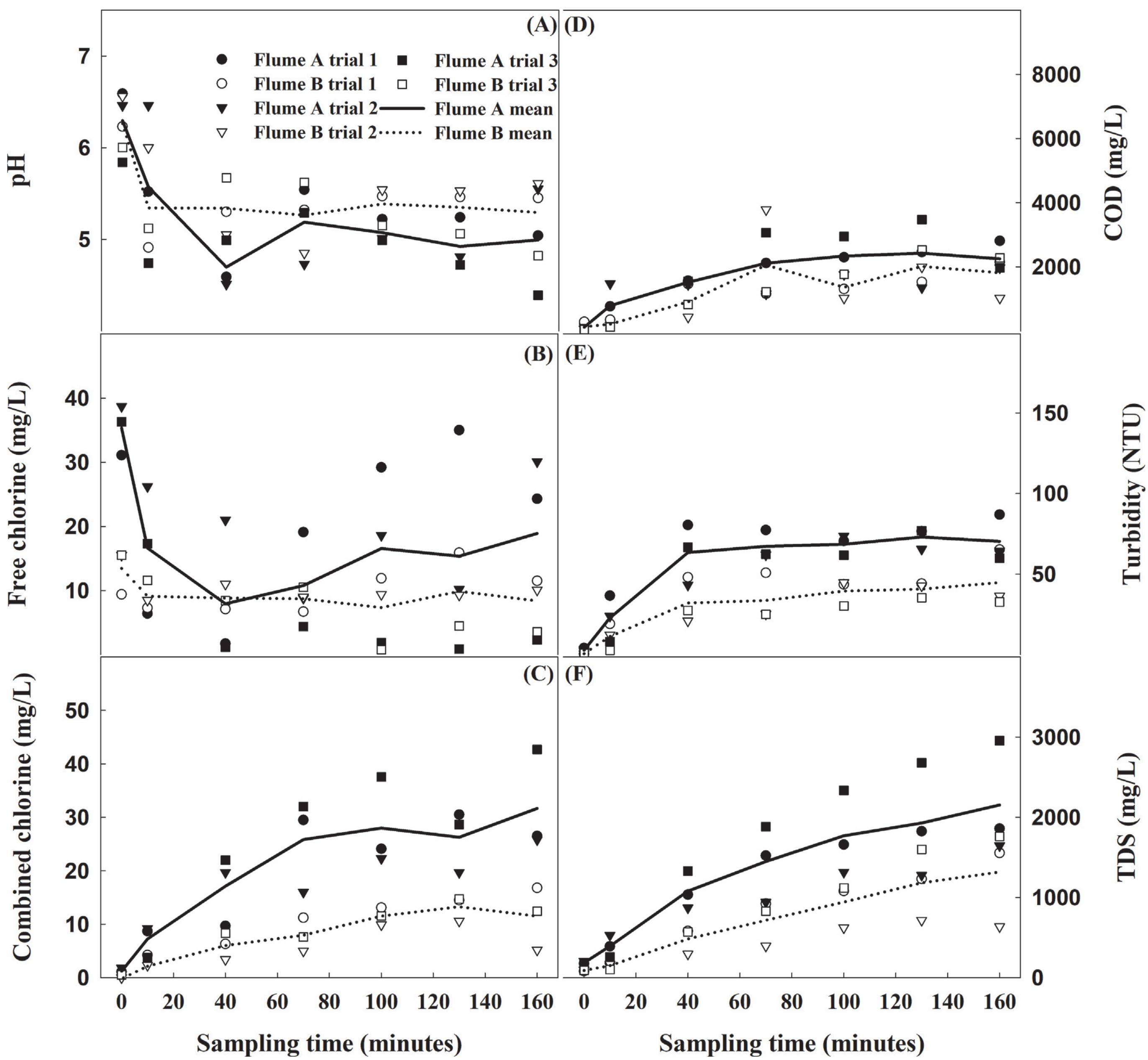

Fig. 3. Changes in pH, chemical oxygen demand (COD), turbidity, total dissolved solids (TDS), free chlorine (FC), and combined chlorine (CC) over time in wash water for processing shredded Iceberg lettuce.

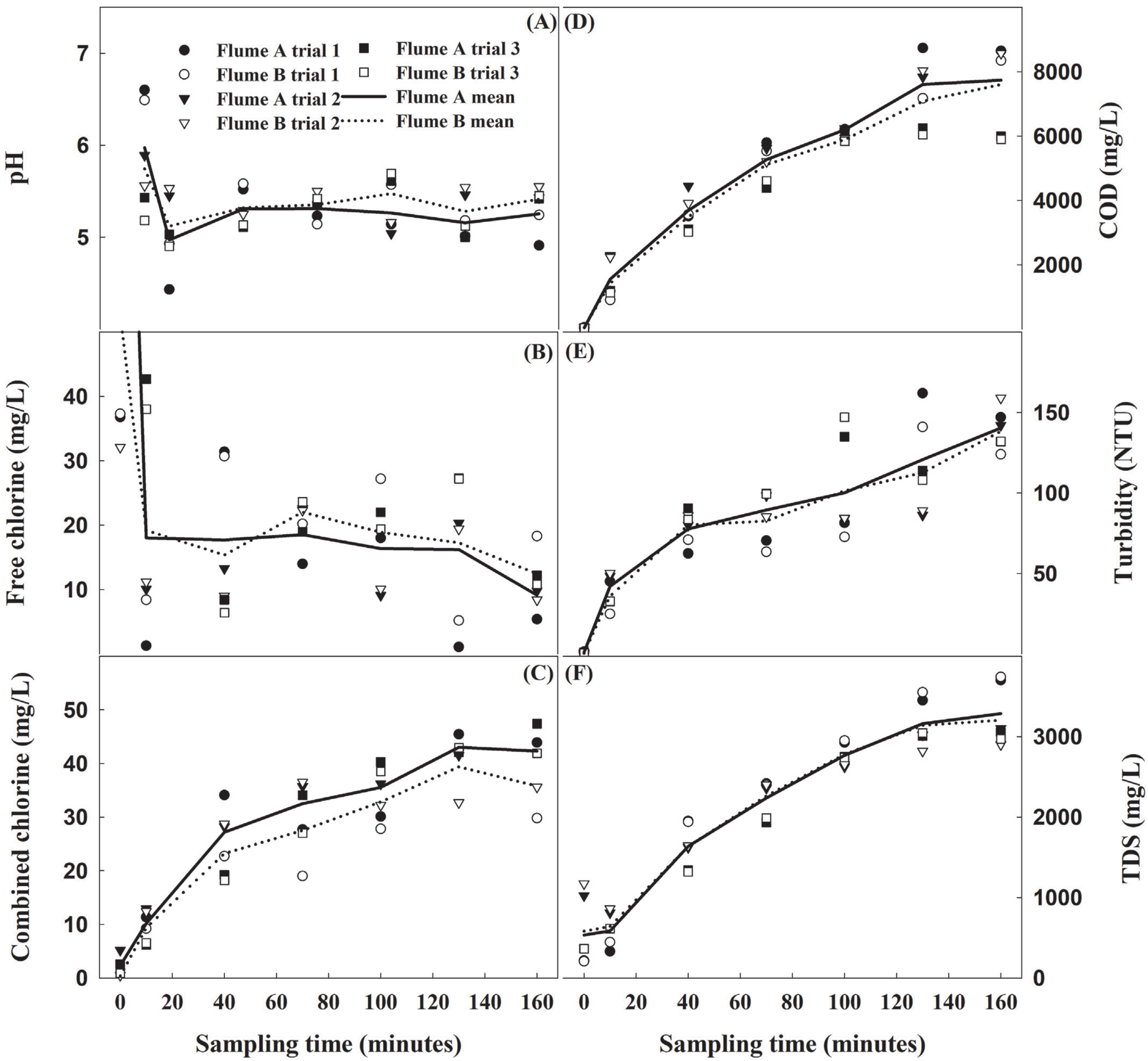

Fig. 4. Changes in pH, chemical oxygen demand (COD), turbidity, total dissolved solids (TDS), free chlorine (FC), and combined chlorine (CC) over time in wash water for processing diced cabbage.

2.6. Statistics

Statistical analyses were performed with SPSS Statistics 22 (IBM, Armonk, NY, USA). The Shapiro-Wilk test was used to assess the normality of the data, whereas Levene's test was used to check for equal variance among groups and to compare the variance of FC residual as a function of product type, processing time etc. When relevant, Levene's test was followed by pairwise comparison based on the Bonferroni correction. Differences in the magnitude of variables among groups of another variable were checked with ANOVA (with Tukey post-hoc test when relevant) or the non-parametric alternative Welch test (with Games-Howell post-hoc test when relevant). For statistics related to FC behavior, FC values at time = 0 min were not included due to the lack of the product at this stage. The cumulative % of wash water samples above a given FC residual "x" that were positive for aerobic mesophilic bacteria (AMB) was calculated as following:

$$cumulative\ \%\ AMB\ (at\ FC > x) = \frac{(Npos - Npos(FC \le xmg/L))}{N} \times 100\ \% \quad (1)$$

With:

Npos = total number of AMB positive wash water samples,
Npos (FC ≤ x mg/L) = total number of AMB positive wash water samples at FC below or equal to x mg/L,
N = total number of wash water samples (AMB positive + AMB negative).

3. Results

3.1. Changes in physicochemical properties of wash water during processing

Figs. 2–4 show the physicochemical parameters (COD, TDS, turbidity, FC, CC and pH) of the wash waters that have been examined in this study for chopped Romaine lettuce, shredded Iceberg lettuce, and diced cabbage, respectively. Significant differences were found among the wash waters from different products for all of the measured physicochemical parameters (Table 1). Exudates and other matter from the washed vegetables accumulated in the wash water over time during produce washing operations, as evidenced by the increase in COD, turbidity, and TDS. Although there were variations among different batches of products, the overall trend remained similar within each product (with the exception of chopped Romaine lettuce). However, significant differences were found among different products, processing time (cumulative quantity of products being washed), and between flumes A and B (with the exception of diced cabbage) as shown in Table 2. Turbidity, COD and TDS were significantly higher in flume A than in flume B for Romaine and Iceberg lettuce (Figs. 2–4; Table 2). Turbidity was lower in Iceberg lettuce wash water than in the others. COD, TDS, and the CC (formed due to reaction of FC with organics) were significantly higher in the wash water of diced cabbage than those of shredded Iceberg and chopped Romaine lettuce.

Table 1
Physicochemical parameters of wash water for different leafy vegetables

	p-value	Romaine	Iceberg	Cabbage
FC[a] (mg/L)	10^{-6}	6.5[b] ± 3.7[C]	11.5 ± 8.9[B]	16.8 ± 10.0[A,c]
CC (mg/L)	10^{-6}	10.4 ± 6.0[B]	13.5 ± 11.2[B]	25.8 ± 14.9[A]
COD (mg/L)	10^{-8}	1446 ± 640[B]	1434 ± 1002[B]	4484 ± 2811[A]
Turbidity (NTU)	10^{-6}	71.6 ± 37.6[A]	40.8 ± 26.4[B]	80.2 ± 47.5[A]
TDS (mg/L)	10^{-8}	763 ± 350[B]	988 ± 745[B]	2032 ± 1092[A]
pH	10^{-9}	4.9 ± 0.3[B]	5.4 ± 0.6[A]	5.4 ± 0.4[A]

[a] FC, CC, COD, and TDS refer to free chlorine, combined chlorine, chemical oxygen demand, and total dissolved solids, respectively.
[b] Means and standard deviations are taken from all collected measurements including flumes, all time points, and repetitions in production runs.
[c] Different letters within a row indicate significant differences at the 0.05 level.

The changes in pH, and FC were impacted by both the wash water conditioning (replenishment of chlorine and acid) and the washing processes. The pH was relatively stable over time, maintained at approximately 5.0 for all three products tested (Figs. 2–4). These values of pH were well below the upper limit of 6.5 for maximizing the concentration of hypochlorous acid, the form of chlorine with the highest efficacy against microorganisms (White, 2010). FC fluctuated considerably (Figs. 2–4), with larger fluctuations in FC and more rapid increase in CC observed in flume A than in flume B (Table 2). Overall in both flumes, the highest fluctuation in FC occurred when washing diced cabbage, followed by shredded Iceberg, and the lowest fluctuation occurred when washing chopped Romaine.

3.2. Microbial survival in wash water

A total of 216 water samples were collected and analyzed: 48 samples of Romaine lettuce wash water, 84 samples of Iceberg lettuce wash water, and 84 samples of cabbage wash water. Aerobic bacteria were detected sporadically throughout the production process with no direct correlation with the amount of produce washed in the flumes, nor with organic load. However, when the APCs of samples from the different wash waters were pooled, and graphed as a function of FC, a clear trend was observed. A decline in the frequency and population density of aerobic bacteria was associated with an increase in FC concentration (Fig. 5). The majority of AMB positive samples were found with FC below 10 mg/L, especially below 7.5 mg/L, with increasing FC concentration resulting in a sharp decline in AMB positives (Fig. 5a). At FC residuals above 10 mg/L very few positive samples were found. This trend was observed for the wash waters of all three studied crops. Above 20 mg/L no surviving Aerobic bacteria were detected. Fig. 5b, which displays the distribution of microbial population density relative to FC concentration, shows a considerable variation in microbial counts below 10 mg/L FC. Below 5 mg/L FC the geometric mean of APC was above 200 CFU/100 mL, whereas above 5 mg/L FC the mean APC dropped below 100 CFU/100 mL, and to 67 CFU/100 mL in the range of 7.5–10 mg/L. Above 10 mg/L FC the mean APC was essentially equal to the LOD (50 CFU/100 mL). APCs for wash water exposed to 0–10 mg/L FC generally followed the decreasing order: Romaine > Iceberg > cabbage (data not shown).

Table 2
The influence of operation time, flume and batch, shown as p-values, on measured physicochemical parameters of the leafy vegetable wash waters

		Romaine	Iceberg	Cabbage
Operation time	FC[a]	0.973	0.918	0.054
	CC	0.036	<0.001	<0.001
	pH	0.019	<0.001	0.323
	COD	0.022	<0.001	<0.001
	Turbidity	0.003	<0.001	<0.001
	TDS	0.007	<0.001	<0.001
Flume	FC	0.001	0.061	0.648
	CC	0.024	<0.001	0.451
	pH	0.777	0.221	0.574
	COD	0.007	0.163	0.818
	Turbidity	0.007	0.003	0.850
	TDS	0.004	0.010	0.988
Batch	FC	0.217	0.013	0.134
	CC	0.046	0.492	0.917
	pH	0.311	0.318	0.218
	COD	0.02	0.728	0.568
	Turbidity	0.04	0.258	0.845
	TDS	0.036	0.114	0.774

[a] FC, CC, COD, and TDS refer to free chlorine, combined chlorine, chemical oxygen demand, and total dissolved solids, respectively.

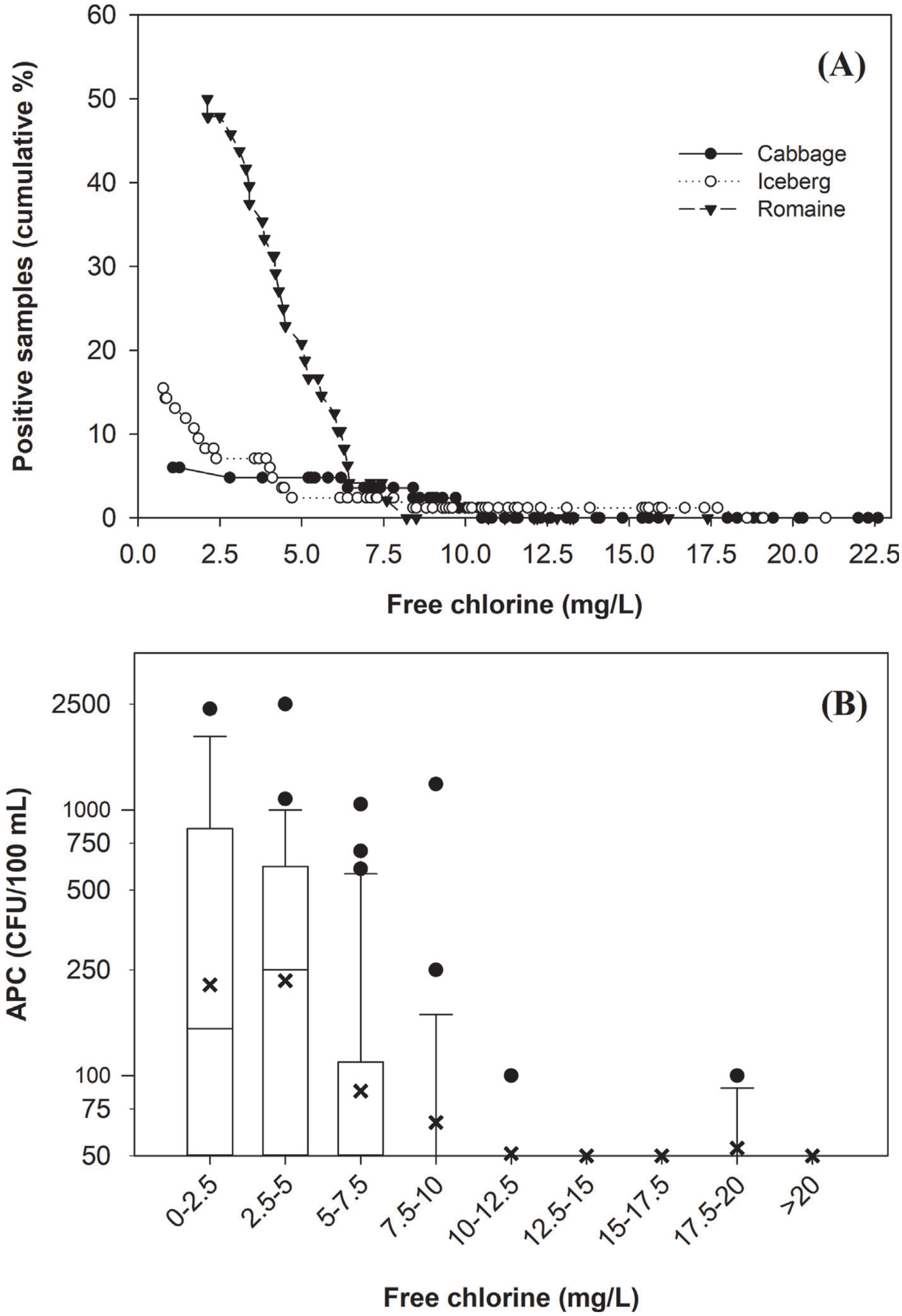

Fig. 5. Correlation of APC and FC in wash water. All data for APC in wash water samples of the three crops were aggregated and plotted. Panel A: Cumulative % of APC positive wash water samples above a given residual FC level; Panel B: Box and whisker plot of APC counts as a function of FC; showing 25th, 50th and 75th percentile outliers (•) and the geometric mean (×).

4. Discussion

Identification and validation of the critical FC concentration required to prevent pathogen cross-contamination has been a major task facing the produce industry and the US regulatory agency. The recently published guidelines for validation of fresh-cut produce wash water sanitation controls identified the determination of the minimal FC concentration for preventing pathogen cross-contamination as high priority for research (Gombas et al., 2017). Previous studies have shown that FC concentrations ranging from 5 to 25 ppm prevented pathogen cross-contamination, with most data pointing towards 10 ppm FC as the minimal FC required (Luo et al., 2011; Shen, 2014; Zhang et al., 2015; Gómez-López et al., 2014; Tomas-Callejas et al., 2012). However, due to the inherent risk of using a pathogen or non-pathogenic surrogate inoculation without benefit of a terminal kill step, all of these studies were conducted in the laboratory setting. The relationship between FC and bacterial survival during commercial processing, and the industry's process capability of maintaining minimal FC are unknown. In this study, we provided data demonstrating that 1) maintaining approximately 10 mg/L FC in wash water contributes significantly to the reduced survival of culturable AMB in the process water for fresh-cut vegetables under commercial operating conditions; and 2) maintaining 10 ppm FC during commercial wash operation is challenging, but feasible.

We observed sporadic low FC incidents concurrent with sudden surges of cut products being discharged into the wash system which exceeded the temporal response capacity of the wash system for chlorine dosing and water replenishment. Therefore, improved process control and optimized washing operations are critical for maintaining a stable FC concentration above the minimal requirement for preventing survival of AMBs and cross-contamination.

The main measured difference among the vegetable wash waters was the dissolved organic and particulate matter content, measured as COD (and also indirectly as TDS) and turbidity, respectively. In commercial processing of fresh-cut vegetables, the sources of COD are plant exudates, soil and other debris. For similar COD concentrations, the wash water for chopped Romaine lettuce had a higher turbidity than that of shredded Iceberg lettuce. It has been observed previously that wash water from different leafy vegetables has different COD to turbidity ratios (Selma et al., 2008; Van Haute et al., 2013), which is likely attributable to differences in tissue structure and composition among different leafy vegetables. In this study, the differences in COD, turbidity, and TDS observed may be attributable to a number of factors, including the wash throughput, cut size/shape, and inherent properties of the produce (e.g., higher solid content for cabbage compared to lettuce).

Conflicting reports exist in literature regarding the correlation of microbial inactivation to FC concentration and to organic load (COD). In a laboratory setting with FC concentration, organic load, and exposure time precisely controlled, Zhou et al. (2015) showed that the inactivation of *Salmonella* and *E. coli* O157:H7 was strongly correlated to FC concentration, irrespective of organic load. Similar results were also reported by Shen et al. (2013) and Van Haute et al. (2013). On the other hand, Gómez-López et al. (2014) found that during a continuous influx of COD (from fresh-cut spinach) a higher FC residual was necessary (5 mg/L FC) to inactivate *E. coli* O157:H7 than in a slower influx of COD (3 mg/L FC). In this study, a correlation between wash water COD and APC was not observed.

In fresh-cut vegetable wash water, FC concentration rapidly declines due to its reaction (primarily electrophilic substitution, and to lesser degree oxidation) with organic matter in the water. During this process, organic chloramines are formed as byproducts, which have a substantially decreased antimicrobial efficacy compared to FC (Donnermair and Blatchley, 2003; Amiri et al., 2010). The consumption of FC necessitates frequent dosing with additional chlorine to maintain a target FC residual. As increasing amounts of reactive organic matter are released into the water, the rate of FC depletion increases, as does the amount of additional sodium hypochlorite needed to maintain the targeted FC level. These observations collectively explain the increased variability in FC residual associated with the increased influx of organic matter (e.g., when washing diced cabbage as compared to chopped Romaine lettuce, as shown in Figs. 2 and 4 respectively).

The microflora of fresh leafy vegetables typically comprises primarily gram-negative bacteria (*Enterobacteriaceae, Pseudomonadaceae*), and secondarily gram-positive bacteria, e.g., lactic acid bacteria, and *Bacillus* spp. (Williams et al., 2013; Lima et al., 2013). Vegetative bacteria generally are susceptible to chlorine (LeChevallier et al., 1988; Lee et al., 2010; Van Haute et al., 2013; Zhang et al., 2015), but bacterial spores, e.g., *Bacillus* spp. which are frequently isolated from fresh produce (Elhariry, 2011), are highly resistant (Young and Setlow, 2003). This could suggest that the sporadic detection of low AMB in wash water with >10 mg/L FC was due to the survival of bacterial spores. Overall, bacterial survival, as detectable AMBs, was associated mainly with FC residual concentrations below 10 mg/L. This highlights the critical importance of maintaining at least 10 mg/L FC to prevent bacterial survival and thus cross-contamination in the fresh-cut produce wash water. It is worth noting that human pathogens may not behave exactly as AMB in their response to FC, and food safety practices are to prevent the survival and cross-contamination of human pathogens, rather than of non-pathogenic indigenous bacteria. However, in the absence of pathogen data during commercial fresh-cut operation, the authors hope that information presented in this study will shed a light on the relationship between FC and bacterial survival in real time.

5. Conclusions

This is the first report of the strong correlation between the survival of aerobic bacteria and the measured real time FC residual concentration during commercial fresh-cut produce wash operations. Three major fresh-cut products, with varying cut sizes and organic loading were tested. Overall, organic load increased gradually over time as more products were washed in the same flume water. Product type and throughput significantly impacted the rate of increase in organic load. Surviving AMB in the wash water, which closely correlated with FC concentration, was detected throughout the processing of all three products, irrespective of the organic load in the wash water. The vast majority of the instances of bacteria survival occurred when the FC concentration was below approximately 10 mg/L, especially when the FC was less than 5 mg/L. Increase in FC concentration resulted in a sharp decline in the frequency and population of detected bacteria. These results underscore the importance of maintaining sufficient free chlorine concentration to prevent bacterial survival and the associated water-mediated bacterial cross-contamination.

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References

Amiri, F., Mesquita, M.M.F., Andrews, S.A., 2010. Disinfection effectiveness of organic chloramines, investigating the effect of pH. Water Res. 44, 845–853.
Arnade, C., Kuchler, F., Calvin, L., 2015. The changing role of consumers and suppliers in a food safety event: the 2006 foodborne illness outbreak linked to spinach. Appl. Econ. 48, 1–13.
Bartz, F., Lickness, J.S., Heredia, N., de Aceituno, A.F.K., Newman, K.L., Hodge, D.W., Jaykus, L., García, S., Leon, J.S., 2017. Contamination of fresh produce by microbial indicators on farms and in packing facilities: elucidation of environmental routes. Appl. Env. Microbiol. 83, 11 10 e02984–16.
Callejon, R.M., Rodriguez-Naranjo, M.I., Ubeda, C., Hornedo-Ortega, R., GarciaParrilla, M.C., Troncoso, A.M., 2015. Reported foodborne outbreaks due to fresh produce in the United States and European Union: trends and causes. Foodborne Pathog. Dis. 12, 32–38.
Connell, G., 1996. The Chlorination/Chloramination Handbook. American Water Works Association, Denver, CO.
Donnermair, M.M., Blatchley 3rd, E.R., 2003. Disinfection efficacy of organic chloramines. Water Res. 37, 1557–1570.
Eaton, A.D., Franson, M., 2005. Standard Methods for Examination of Water and Wastewater, 21th Ed. APHA, AWWA, Washington.
Elhariry, H.M., 2011. Attachment strength and biofilm forming ability of *Bacillus cereus* on green-leafy vegetables: cabbage and lettuce. Food Microbiol. 28, 1266–1274.
Gil, M.I., Selma, M.V., Lopez-Galvez, F., Allende, A., 2009. Fresh-cut product sanitation and wash water disinfection: problems and solutions. Int. J. Food Microbiol. 134, 37–45.
Gombas, D., Luo, Y., Brennan, J., Shergill, G., Petran, R., Walsh, R., Hau, H., Khurana, K., Zomorodi, B., Rosen, J., Varley, R., Deng, K., 2017. Guidelines to

validate control of cross-contamination during washing of fresh-cut leafy vegetables. J. Food Prot. 80, 312–330.
Gómez-López, V.M., Lannoo, A.S., Gil, M.I., Allende, A., 2014. Minimum free chlorine residual level required for the inactivation of *Escherichia coli* O157:H7 and trihalomethane generation during dynamic washing of fresh-cut spinach. Food Control 42, 132–138.
Holvoet, K., Jacxsens, L., Sampers, I., Uyttendaele, M., 2012. Insight into the prevalence and distribution of microbial contamination to evaluate water management in the fresh produce processing industry. J. Food Prot. 75, 671–681.
Hurst, W.C., 2002. Safety aspects of fresh-cut fruits and vegetables. In: Fresh-cut Fruits and Vegetables: Science, Technology, and Market. CRC Press, Boca Raton, FL, pp. 45–90.
Jung, Y., Jang, H., Matthews, K.R., 2014. Effect of the food production chain from farm practices to vegetable processing on outbreak incidence. Microb. Biotechnol. 7, 517–527.
International Fresh-cut Produce Association, 2001. HACCP for the fresh-cut industry. In: Food safety guidelines for the fresh-cut produce industry. IFPA, Alexandria, VA, pp. 56–57.
LeChevallier, M.W., Cawthon, C.D., Lee, R.G., 1988. Inactivation of biofilm bacteria. Appl. Environ. Microbiol. 54, 2492–2499.
Lee, E.S., Yoon, T.H., Lee, M.Y., Han, S.H., Ka, J.O., 2010. Inactivation of environmental mycobacteria by free chlorine and UV. Water Res. 44, 1329–1334.
Lima, P.M., São José, J.F.B., Andrade, N.J., Pires, A.C.S., Ferreira, S.O., 2013. Interaction between natural microbiota and physicochemical characteristics of lettuce surfaces can influence the attachment of *Salmonella enteritidis*. Food Control 30, 157–161.
López-Gálvez, F., Allende, A., Selma, M.V., Gil, M.I., 2009. Prevention of *Escherichia coli* cross-contamination by different commercial sanitizers during washing of fresh-cut lettuce. Intl. J. Food Microbiol. 133, 167–171.
López-Gálvez, F., Gil, M.I., Truchado, P., Selma, M.V., Allende, A., 2010. Cross-contamination of fresh-cut lettuce after a short-term exposure during prewashing cannot be controlled after subsequent washing with chlorine dioxide or sodium hypochlorite. Food Microbiol. 27, 199–204.
Luo, Y., 2007. Fresh-cut produce wash water reuse affects water quality and packaged product quality and microbial growth in Romaine lettuce. Hortsci 42, 1413–1419.
Luo, Y., Nou, X., Yang, Y., Alegre, I., Turner, E.R., Feng, H., Abadias, M., Conway, W., 2011. Determination of free chlorine concentrations needed to prevent *Escherichia coli* O157: H7 cross-contamination during fresh-cut produce wash. J. Food Prot. 74, 352–358.
Luo, Y., Nou, X., Millner, P., Zhou, B., Shen, C., Yang, Y., Wu, Y., Wang, Q., Feng, H., Shelton, D., 2012. A pilot plant scale evaluation of a new process aid for enhancing chlorine efficacy against pathogen survival and cross-contamination during produce wash. Intl. J. Food Microbiol. 158, 133–139.
Lynch, M.F., Tauxe, R.V., Hedberg, C.W., 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiol. Infect. 137, 307–315.
Nou, X., Luo, Y., 2010. Whole-leaf wash improves chlorine efficacy for microbial reduction and prevents pathogen cross-contamination during fresh-cut lettuce processing. J. Food Sci. 75, M283–M290.
Painter, J.A., Hoekstra, R.M., Ayers, T., Tauxe, R.V., Braden, C.R., Angulo, F.J., Griffin, P.M., 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998-2008. Emerg. Infect. Dis. 19, 407–415.
Pérez-Rodríguez, F., Saiz-Abajo, M.J., Garcia-Gimeno, R.M., Moreno, A., Gonzalez, D., Vitas, A.I., 2014. Quantitative assessment of the *Salmonella* distribution on fresh-cut leafy vegetables due to cross-contamination occurred in an industrial process simulated at laboratory scale. Int. J. Food Microbiol. 184, 86–91.
Selma, M.V., Allende, A., Lopez-Galvez, F., Conesa, M.A., Gil, M.I., 2008. Heterogeneous photocatalytic disinfection of wash waters from the fresh-cut vegetable industry. J. Food Prot. 71, 286–292.
Shen, C., 2014. Evaluation of chlorine efficacy against *Escherichia coli* O157:H7 survival and cross-contamination during continuous produce washing process with water quality change. Intl. J. Food Sci. Nutr. Diet. 89–93.
Shen, C., Luo, Y., Nou, X., Bauchan, G., Zhou, B., Wang, Q., Millner, P., 2012. Enhanced inactivation of Salmonella and Pseudomonas biofilms on stainless steel by use of T-128, a fresh produce washing aid, in chlorinated wash solutions. Appl. Env. Microbiol. 78, 6789–6798.
Shen, C., Luo, Y., Nou, X., Wang, Q., Millner, P., 2013. Dynamic effects of free chlorine concentration, organic load, and exposure time on the inactivation of *Salmonella, Escherichia coli* O157:H7, and Non-O157 shiga-toxin-producing *E. coli*. J. Food Prot. 76, 386–393.
Suslow, T., 1997. Postharvest Chlorination. Basic Properties and Key Points for Effective Disinfection. University of California, Division of Agriculture and Natural Resources. Publication No. 8003, 8 pp. http://anrcatalog.ucdavis.edu/pdf/8003.pdf. Accessed June 23, 2016.
Tomas-Callejas, A.F., Lopez-Galvez, F., Sbodio, A., Artes, F., Artes-Hernandez, A., Suslow, T.V., 2012. Chlorine dioxide and chlorine effectiveness to prevent Escherichia coli O157:H7 and Salmonella cross-contamination on fresh-cut red chard. Food Control 23, 325–333.
Van Haute, S., Sampers, I., Holvoet, K., Uyttendaele, M., 2013. Physicochemical quality and chemical safety of chlorine as a reconditioning agent and wash water disinfectant for fresh-cut Lettuce washing. Appl. Environ. Microbiol. 79, 2850–2861.
Van Haute, S., Sampers, I., Jacxsens, L., Uyttendaele, M., 2015. Selection criteria for water disinfection techniques in agricultural practices. Crit. Rev. Food Sci. Nutr. 55, 1529–1551.
Van Haute, S., Tryland, I., Escudero, C., Vanneste, M., Sampers, I., 2017. Chlorine dioxide as water disinfectant during fresh-cut iceberg lettuce washing: disinfectant demand, disinfection efficiency, and chlorite formation. LWT - Food Sci. Technol. 75, 301–304.
White, G.C., 2010. White's Handbook of Chlorination and Alternative Disinfectants, fifth ed. John Wiley & Sons, Inc, Hoboken, New Jersey, ISBN 978-0-470-18098-3.
Williams, T.R., Moyne, A.L., Harris, L.J., Marco, M.L., 2013. Season, irrigation, leaf age, and *Escherichia coli* inoculation influence the bacterial diversity in the lettuce phyllosphere. PLoS One 8 (7), e68642.
Young, S.B., Setlow, P., 2003. Mechanisms of killing of *Bacillus subtilis* spores by hypochlorite and chlorine dioxide. J. Appl. Microbiol. 95, 54–67.
Zhang, B., Luo, Y., Zhou, B., Wang, Q., Millner, P.D., 2015. A novel microfluidic mixer-based approach for determining inactivation kinetics of *Escherichia coli* O157:H7 in chlorine solutions. Food Microbiol. 49, 152–160.
Zhou, B., Luo, Y., Nou, X., Millner, P.D., 2014. Development of an algorithm for feed-forward chlorine dosing of lettuce wash operations and correlation of chlorine profile with *Escherichia coli* O157:H7 inactivation. J. Food Prot. 77, 558–566.
Zhou, B., Luo, Y., Nou, X., Lyu, S., Wang, Q., 2015. Inactivation dynamics of *Salmonella* enterica, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in wash water during simulated chlorine depletion and replenishment processes. Food Microbiol. 50, 88–96.