10-2019

Modeling of Free Chlorine Consumption and Escherichia coli O157:H7 Cross-Contamination During Fresh-Cut Produce Wash Cycles

Mohammadreza Dehghan Abnavi
Cleveland State University

Ali Alradaan
Cleveland State University

Daniel Munther
Cleveland State University, d.munther@csuohio.edu

Chandrasekhar R. Kothapalli
Cleveland State University, c.kothapalli@csuohio.edu

Partha Srinivasan
Cleveland State University, p.srinivasan35@csuohio.edu

Publisher's Statement

This is the accepted version of the following article: Abnavi, M.D., Alradaan, A., Munther, D., Kothapalli, C.R. and Srinivasan, P. (2019), Modeling of Free Chlorine Consumption and Escherichia coli O157:H7 Cross-Contamination During Fresh-Cut Produce Wash Cycles. Journal of Food Science, 84: 2736-2744. doi:10.1111/1750-3841.14774, which has been published in final form at https://onlinelibrary.wiley.com/doi/abs/10.1111/1750-3841.14774

Modeling of Free Chlorine Consumption and *Escherichia coli O157:H7* Cross-Contamination During Fresh-Cut Produce Wash Cycles

Mohammadreza Dehghan Abnavi, Ali Alradaan, Daniel Munther, Chandrasekhar R. Kothapalli, and Parthasarathy Srinivasan

Abstract: Controlling the free chlorine (FC) availability in wash water during sanitization of fresh produce enhances our ability to reduce microbial levels and prevent cross-contamination. However, maintaining an ideal concentration of FC that could prevent the risk of contamination within the wash system is still a technical challenge in the industry, indicating the need to better understand wash water chemistry dynamics. Using bench-scale experiments and modeling approaches, we developed a comprehensive mathematical model to predict the FC concentration during fresh-cut produce wash processes for different lettuce types (romaine, iceberg, green leaf, and red leaf), carrots, and green cabbage as well as *Escherichia coli* O157:H7 cross-contamination during fresh-cut iceberg lettuce washing. Fresh-cut produce exudates, as measured by chemical oxygen demand (COD) levels, appear to be the primary source of consumption of FC in wash water, with an apparent reaction rate ranging from 4.74×10^{-4} to 7.42×10^{-4} L/mg·min for all produce types tested, at stable pH levels (6.5 to 7.0) in the wash water. COD levels increased over time as more produce was washed and the lettuce type impacted the rate of increase in organic load. The model parameters from our experimental data were compared to those obtained from a pilot-plant scale study for lettuce, and similar reaction rate constant (5.38×10^{-4} L/mg·min) was noted, supporting our hypothesis that rise in COD is the main cause of consumption of FC levels in the wash water. We also identified that the bacterial transfer mechanism described by our model is robust relative to experimental scale and pathogen levels in the wash water. Finally, we proposed functions that quantify an upper bound on pathogen levels in the water and on cross-contaminated lettuce, indicating the maximum potential of water-mediated cross-contamination. Our model results could help indicate the limits of FC control to prevent cross-contamination during lettuce washing.

Keywords: COD, cross-contamination, free chlorine, mathematical modeling, produce wash

Introduction

Foodborne illnesses affect millions of people in the United States every year according to the Center for Disease Control (Scallan et al., 2011). The association of these outbreaks to food products varies from raw meat and fresh produce contamination, to the consumption of undercooked and poorly packaged foods. In terms of fresh-cut produce, outbreaks of *Escherichia coli* and *Salmonella* have been linked to the consumption of leafy greens as well as fruit (Jung, Gao, Jang, Guo, & Matthews, 2017; Zhou, Luo, Nou, Lyu, & Wang, 2015). While it has been suggested that the primary source of these outbreaks most likely occurred during preharvest, the postharvest wash process has the potential to play a significant role in secondary contamination (Murray, Wu, Shi, Jun Xue, & Warriner, 2017). The critical issue here is that when produce is washed, there is a possibility of bacterial cross-contamination from a bacteria contaminated batch to other uncontaminated ones that were subsequently washed in the same water. Therefore, while the postharvest wash step is limited in completely decontaminating produce (even with potentially high levels of sanitizer in the wash water), the current focus of washing operations is to prevent this secondary contamination or cross-contamination between produce lots (Gombas et al., 2017; Murray et al., 2017).

Chlorine is a commonly used water disinfectant in the produce industry as it is an inexpensive and effective disinfectant (Luo et al., 2011). However, maintaining a stable FC concentration during washing is challenging (Ruiz-Cruz, Acedo-Félix, Díaz-Cinco, Islas-Osuna, & González-Aguilar, 2007). Chlorine can be consumed by organic matter released from the fresh-cut produce and the bactericidal activity will be reduced (Van Haute, Sampers, Holvoet, & Uyttendaele, 2013). If the organic load, measured as chemical oxygen demand (COD), is too high, the sanitizing effect of chlorine is reduced (Gómez-López, Lannoo, Gil, & Allende, 2014). Likewise, chlorine levels (measured as FC) must be continuously regulated to remain at sufficient concentrations to prevent cross-contamination (Gil, Selma, López-Gálvez, & Allende, 2009).

There are commercial systems that could maintain almost constant free chlorine (FC) levels at an industrial scale due to the relatively short lag-time between FC measurements and chlorine addition. However, to optimize these systems from predictive framework as well as to provide independent validation of the control of FC variability relative to specified set points, the underlying mechanisms that dictate FC concentration (relative to organic load and pH dynamics) during fresh-cut produce wash operations must be more specifically quantified. Part of the problem is that the relationships between chlorine levels and such water quality parameters have only been described through experimental and correlative approaches (Barrera, Blenkinsop, & Warriner, 2012; Beuchat, Adler, & Lang, 2004; Chen & Hung, 2016, 2017; Gómez-López et al., 2014; Luo et al., 2011; Pirovani, Guemes, & Piagnetini, 2001; Shen, Luo, Nou, Wang, & Millner, 2013; Van Haute et al., 2015). While these results have clear value, they cannot be used to make precise predictions of FC concentration and antimicrobial capacity in dynamically changing circumstances.

Connected with maintaining sufficient sanitizer levels in the wash water, there have been many *observational* studies directly examining pathogen cross-contamination at the lab scale (Fu, Li, Awad, Zhou, & Liu, 2018; Holvoet et al., 2014; López-Gálvez, Gil, Truchado, Selma, & Allende, 2010; Luo et al., 2011) and at the pilot scale (Luo et al., 2012). Also, a number of recent studies indirectly address pathogen contamination at different scales (Jensen, Friedrich, Harris, Danyluk, & Schaffner, 2015; López-Gálvez et al., 2010; Luo et al., 2012; Murray, Aldossari, Wu, & Warriner, 2018). However, given the potential for wash water to promote pathogen cross-contamination, there remains a critical need to determine mechanisms involved with pathogen transfer during produce washing (Gombas et al., 2017). Furthermore, considering the diversity of experimental procedure and scale in these prior studies, as pointed out by Gil et al. (2009), a standardized approach that can synthesize current results, providing both a unifying perspective as well as a direction toward increased predictive capacity is necessary to advance fresh-produce safety.

To address some of these limitations, this study aims to (a) assess the dynamic changes in water quality during the washing process of lettuce, carrots, and green cabbage by measuring parameters such as FC, total chlorine, pH, and COD; (b) develop a mathematical model to predict chlorine decay in the wash water as well as *E. coli* O157:H7 cross-contamination during a simulated batch lettuce wash procedure; and (c) utilize the developed model to investigate the reaction rate constant for the reaction between FC and organic matter (COD) and the *E. coli* transfer rate from water to lettuce.

Previously, using the data provided by Luo et al. (2012), we have developed a mathematical model to predict FC concentration and pathogen levels in pilot plant washing systems (Munther, Luo, Wu, Magpantay, & Srinivasan, 2015). In this study, our aim is to test and develop the model mechanisms to justify that the relevant chlorine consumption and pathogen transfer mechanisms at the pilot scale are similar to the bench-scale during batch process washing for iceberg lettuce. Moreover, we also demonstrate that a similar mechanism, with a similar rate constant, is also valid for romaine/green leaf/red leaf lettuce, carrots, and green cabbage. We illustrate how mathematical modeling tools can provide a reference point to address how experimental scale affects FC dynamics and mechanisms of cross-contamination during fresh-cut produce washing. In terms of FC decay, we first determine the apparent reaction rate of FC with organics during washing by conducting bench-top experiments and develop a corresponding predictive mathematical model. In addition to quantifying the FC decay dynamics relative to four types of lettuce (romaine, iceberg, green leaf, and red leaf), carrots (imperator type), and green cabbage, we simultaneously validate our model and address scaling questions by comparing our results with experimental data at the pilot/commercial scale (Luo et al., 2012). Next, we use our model in conjunction with *E. coli* O157:H7 data from lab and pilot scale experiments (López-Gálvez et al., 2010; Luo et al., 2011, 2012) to specify processing conditions in which water-mediated cross-contamination dominates the pathogen transfer dynamic during washing. Finally, we use our model to illustrate the effectiveness of FC control to prevent cross-contamination and highlight specific experimental needs to improve such control.

Materials and Methods

Produce preparation

Four types of lettuce (romaine, iceberg, green leaf, and red leaf), carrots (imperator type), and green cabbage were selected for analysis. Prebagged produce was purchased from a local supermarket and stored at 4 °C before the experiments and used within 2 days of purchasing. Exterior leaves of the lettuce and cabbage were trimmed out and discarded, and the rest were cut to 1″ × 1″ size using a chopper. Carrots were chopped into two different shapes—stick cut (0.25″ × 0.25″ × 1″) and disk cut (thickness 0.25″).

Fresh-cut produce washing system

Produce washing experiments were carried out using a bench-top wash system consisting of a container holding 3 L of tap water, using experimental conditions similar to those reported in the literature. Before starting each experiment, 1.4 mL of concentrated (4.5%) sodium hypochlorite (BCS Chemicals, Redwood City, CA, USA) was added to the wash water to achieve approximately 20 mg/L FC concentration in the wash water (20 °C). The pH was adjusted to 6.5 for all experiments using 1 M citric acid. The cut produce (approximately 600 g) was washed through six consecutive batches, with each batch weighing 100 g and a dwelling time of 30 s. After washing each batch, the cut produce was removed from the washing solution using a sieve and drained above the container for 30 s. Samples (wash water aliquots) were taken for analysis after washing each batch (100 g) of produce. The total experimental time for washing lettuce was 45 min, and that for carrots and cabbage was 30 min.

Evaluation of water quality

FC was measured immediately after washing each batch, based on a N, N-diethyl-p-phenylenediamine (DPD) method, using a Chlorine Photometer (CP-15, HF Scientific Inc., Ft. Myers, FL, USA). The pH was measured right after each batch was washed using a digital pH meter (Orion^{TM} 2-Star, ThermoFisher Scientific, Grand Island, NY, USA). The COD was determined using a reactor digestion method (Luo et al., 2011).

Mathematical model for FC dynamics in the wash water

A variety of factors could affect FC concentration, but organic load is possibly the primary source of consumption of FC (Munther et al., 2015; Van Haute et al., 2013). As produce was introduced into the wash water, organic material from the cut produce enters the water and COD increases significantly in water. For all produce in our study, we found that as more produce was washed, the COD in wash water increases linearly. So, the relation between COD and time could be written as:

$$\frac{dO}{dt} = k_0 \tag{1}$$

where t denotes time (min), O (mg/L) is the COD in the wash water, and k_0 (mg/L·min) is the slope of increasing COD over time. If the washing process is continuous and there is no stop between the batches, then the integrated form of Eq. 1 is

$$O = k_0 t + O_0 \tag{2}$$

where O_0 is the initial COD concentration. However, this study was carried out within a batch process and there were pauses between batches for data collection. To account for this, a step function for the rate of change of COD is used. This function is a constant when the produce is not immersed in the wash water (time for sampling and measuring pH, FC, and COD, between batches), and is linearly increasing when the produce is being washed. Therefore, there are two steps for modeling each batch of the washing system: wash 100 g of chopped produce for 30 s and drain for 30 s and collect aliquots to measure parameters. In this case, if we modify Eq. 1 to reflect this two-step batch process, the integrated form of the model becomes

$$O = \begin{cases} k_0 t + O_0 - (n-1)\, k_0 \tau_2, & (n-1)(\tau_1 + \tau_2) \le t \le n\tau_1 + (n-1)\,\tau_2 \\ n k_0 \tau_1 + O_0, & n\tau_1 + (n-1)\,\tau_2 \le t \le n(\tau_1 + \tau_2) \end{cases} \quad (3)$$

Here n is the batch number, τ_1 is the amount of time for which the produce is washed (0.5 min), and τ_2 is the time taken between the sample collection and measurements (7 min for lettuce, and 4.5 min for carrots and cabbage).

Using COD as an indirect measure for the organic load (R), we consider the following apparent reaction (Deborde & von Gunten, 2008; Munther et al., 2015):

$$\mathrm{HOCl} + \mathrm{R} \rightarrow \mathrm{Products}$$

which accounts for FC reduction in the wash water. It is assumed that this reaction is both first- and second-order (Deborde & von Gunten, 2008; Munther et al., 2015), so the rate of change of FC is given by:

$$\frac{dC}{dt} = -\lambda_c C - \beta_c OC \quad (4)$$

where C (mg/L) indicates the FC concentration in the wash water, λ_c is the natural decay rate of chlorine in tap water, and β_c is the reaction rate of the above reaction. Typically, β_c is a function of pH and temperature, but since the temperature was held constant during the experiments and the change in pH was small, we assume that β_c remains constant. On the other hand, there are different types of organic and inorganic matter in the wash system that react with chlorine (for example, produce extract, bacteria, soil, ammonia, humic acid). Because the reaction of chlorine with each of these materials has a different rate constant, β_c is considered to be the average of all these constants.

Using the change in COD levels described by Eq. 3 and integrating, we obtain

$$C = \begin{cases} C_0 e^{-\lambda_c t - \beta_c [(k_0 t/2 + O_0 - (n-1) k_0 \tau_2) t + \varphi_n (n-1) k_0 \tau_2 / 2]} & (n-1)(\tau_1 + \tau_2) \le t \le n\tau_1 + (n-1)\,\tau_2 \\ C_0 e^{-\lambda_c t - \beta_c [(n k_0 \tau_1 + O_0) t - \varphi_n n k_0 \tau_1 / 2]} & n\tau_1 + (n-1)\,\tau_2 \le t \le n(\tau_1 + \tau_2) \end{cases} \quad (5)$$

where $\varphi_n = n\tau_1 + (n-1)\tau_2$. Eq. 3 and 5 can predict the dynamic changes of COD and FC levels for batch washing systems based on time.

Statistical analysis and parameter fitting

All experiments were done in triplicate and the average ± standard deviation of the three independent runs was reported. The resulting data were used to find k_0 and β_c parameters. The *fminsearch* function in MATLAB R2016b (MathWorks Inc., Natick, MA, USA) was used for curve fitting.

Data and modeling for *E. coli* O157:H7 cross-contamination

Following our earlier model (Munther et al., 2015), we built a modified mathematical model that accounts for the batch-wash timing described in the experimental procedure in section "Fresh-cut produce washing system" with the focus of quantifying *E. coli* O157:H7 concentration in the wash water as well as cross-contamination dynamics at the lab scale (see Appendix A for details concerning model assumptions and description). To inform the new model's parameters and justify its mathematical forms at this scale, we utilize *E. coli* O157:H7 data from lettuce wash studies by Luo et al. (2011, 2012), and *E. coli* (CECT 471, 516, and 533) data from a lettuce wash study by López-Gálvez et al. (2010).

Complete model for water chemistry and *E. coli* O157:H7 cross-contamination

Combining the FC dynamics as well as the pathogen cross-contamination dynamics (see Appendix A), our complete model (CM) for the batch-wash system is defined by the following system of equations:

$$\frac{dO}{dt} = k_0 \quad (6a)$$

$$\frac{dC}{dt} = -\lambda_c C - \beta_c OC \quad (6b)$$

$$\frac{dX_W}{dt} = \hat{\beta}_{WS} - \hat{\beta}_{LW} \frac{L}{V} X_W - \alpha X_W C \quad (6c)$$

$$\frac{dX_{L_n}}{dt} = b^n_{LW} X_W - \alpha X_{Ln} C \chi_n \quad (6d)$$

$$\hat{\beta}_{WS} = \begin{cases} \beta_{WS}, & (n-1)(\tau_1 + \tau_2) \le t \le n\tau_1 + (n-1)\,\tau_2 \\ 0, & n\tau_1 + (n-1)\,\tau_2 \le t \le n(\tau_1 + \tau_2) \end{cases}$$

$$\hat{\beta}_{LW} = \begin{cases} \beta_{LW}, & (n-1)(\tau_1 + \tau_2) \le t \le n\tau_1 + (n-1)\,\tau_2 \\ 0, & n\tau_1 + (n-1)\,\tau_2 \le t \le n(\tau_1 + \tau_2) \end{cases}$$

$$b^n_{LW} = \begin{cases} \beta_{LW}, & (n-1)(\tau_1 + \tau_2) \le t \le n\tau_1 + (n-1)\,\tau_2 \\ 0, & \text{else} \end{cases}$$

for $n \in \{1, 2, \ldots, N\}$, on the state space where O, C, X_W, X_{L_n} are all nonnegative. By inspection, one can see that the model is positively invariant on this space, and therefore, model solutions are biologically realistic.

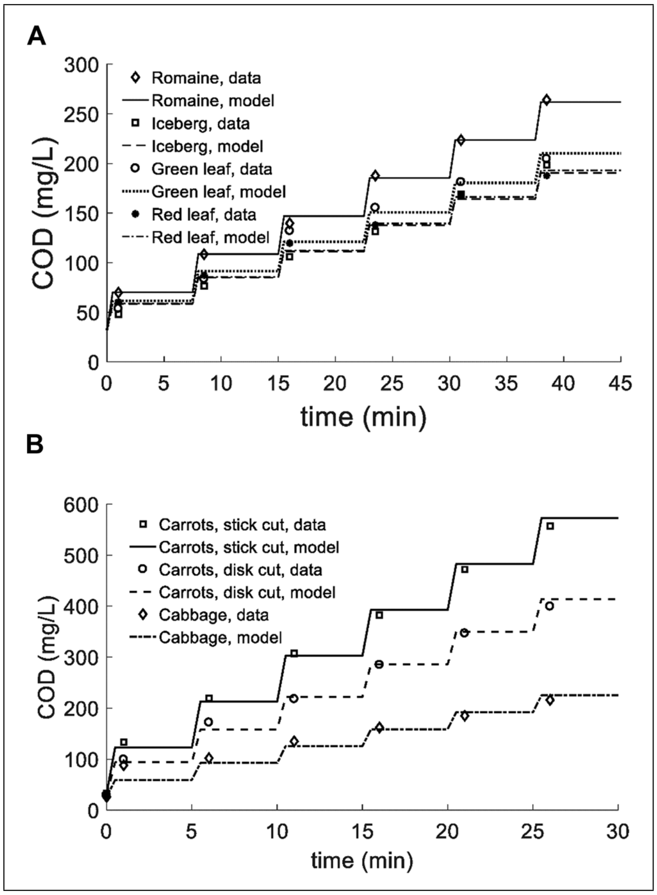

Figure 1–COD profile over time from washing (A) different types of lettuce and (B) carrots and cabbage. The lines are from the model fit described in Eq. 3, and symbols represent experimental data.

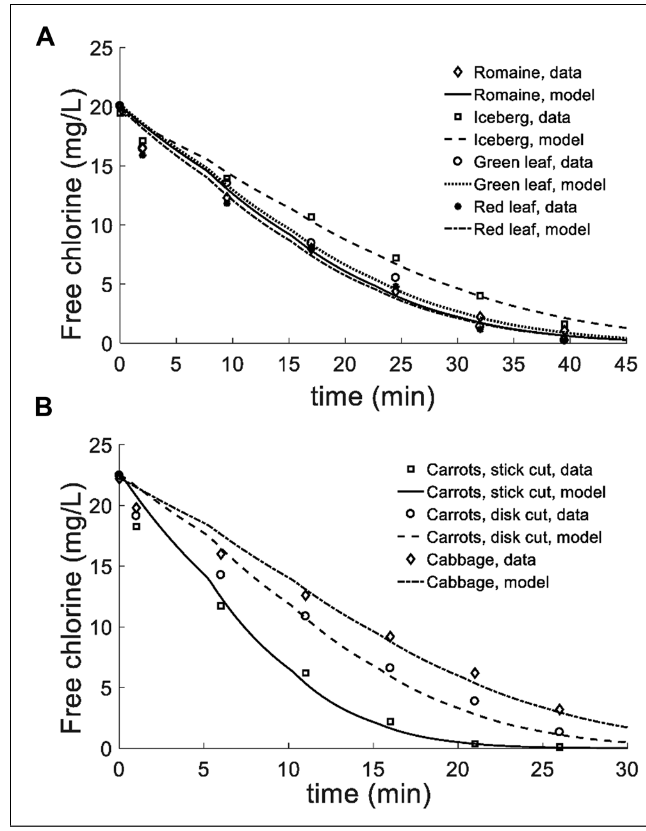

Figure 2–Free chlorine dynamics over time when (A) different types of lettuce were washed (pH level of 6.5), and (B) different cuts of carrots and cabbage were washed (pH level of 6.5). The lines were from the model described in Eq. 5, and symbols represent experimental data.

Results and Discussion

Water quality and free chlorine concentration dynamics during washing

Results from the batch-wash experiments indicate that as the cut produce was sequentially washed in the container, COD levels increased in the wash solution (Figure 1). Under the operating conditions present in this experiment, the increase in COD linearly corresponded to the amount of produce washed, and hence the amount of exudates and other organic material from the washed vegetables released into the wash system. Although there were variations among different batches of produce, the overall trend remained similar for each produce type (Figure 1). COD dynamic changes remained similar for all produce types.

As chopped produce was introduced into the washing system, the increase in COD was accompanied by a decline in residual FC concentration (Figure 2A: lettuce; Figure 2B: sliced and stick-cut carrots and cabbage). The initial concentration of 20 to 22 mg/L of FC was nearly depleted by the end of each wash trial, when approximately 600 g of chopped produce was washed in 3 L of tap water. Interestingly, no significant effect of wash water temperature (2 °C compared with 20 °C) on FC levels was observed when disc cut carrots were washed under similar conditions (Figure S1).

Washing cut produce in chlorinated water also affects the water pH. In general, the pH of vegetable extract ranges from 6.1 to 6.3 (Nou et al., 2011). Depending on the initial pH of the wash solution, washing lettuce gradually changes the solution pH toward that of the produce pulp pH (Van Haute, Tryland, Escudero, Vanneste, & Sampers, 2017). The pH level of the wash water is also a function of the amount of produce extract and sodium hypochlorite added (Tomás-Callejas et al., 2012). At the beginning of each experiment in this study, the pH of chlorinated water for washing was adjusted to 6.5 using citric acid. As the amount of produce introduced into the water increased, the organic material in the washing solution increased as well. The changes in pH and FC were impacted by both the wash water conditioning (adding chlorine and citric acid) and the washing processes (produce exudate and debris). The pH was relatively stable during washing, with a slight decrease in pH for all products tested. The pH was below the recommended upper limit of 7.0 for maximizing the concentration of hypochlorous acid, the form of chlorine with the highest efficacy against microorganisms (Deborde & von Gunten, 2008; Gombas et al., 2017). We expect no significant changes in our model parameters due to this pH change.

Fitting the FC model to the data

We noted earlier (Figure 1) that the COD in the wash water increased linearly with the introduction of chopped produce to the wash system. The curve fitting for COD levels (Figure 1) was performed using experimental data presented earlier as well as our model described in Eq. 3. In all experiments, the initial value of COD was approximately 32 mg/L. As noted from the curve-fitting results in Table 1, the model fitted the data well for each lettuce type. The R^2 values for COD curve fitting (Table 1)

Table 1–Parameter values for different types of lettuce, carrots, and green cabbage, where the parameters O_0 and Cl_0 were measured in the experiments. The parameter k_0 was fitted to minimize the distance between Eq. 3 and measured values of COD levels for each produce type. This k_0 value was subsequently used to obtain the parameter βc when fitted to minimize the distance with Eq. 5 and measured FC levels for each produce type. RMSE indicates Root Mean Square Error.

Produce type	O_0 (mg/L)	Cl_0 (mg/L)	k_0 (mg/L·min)	β_c (L/mg·min)	R^2 (COD)	R^2 (FC)	RMSE (COD)	RMSE (FC)
Romaine	31.8	20.0	76.62	5.62×10^{-4}	0.99	0.98	161.1	0.85
Iceberg	31.5	19.5	52.86	4.72×10^{-4}	0.98	0.99	116.6	0.61
Green leaf	31.5	20.1	59.36	6.17×10^{-4}	0.99	0.98	129.8	0.97
Red leaf	32.0	19.8	53.66	7.43×10^{-4}	0.99	0.98	120.1	1.02
Carrots, stick cut	33.0	22.4	179.77	7.38×10^{-4}	1.00	0.98	343.18	1.05
Carrots, disk cut	30.0	22.5	127.92	5.03×10^{-4}	0.99	0.97	247.2	1.33
Green cabbage	28.7	22.2	54.3	3.81×10^{-4}	0.98	0.96	111.1	0.95

suggest that the model we developed represented well the COD changes in wash solution as more produce entered the wash water.

Utilizing the calculated COD rate increase (k_0 values in Table 1) together with Eq. 5 and FC data, the parameter β_c was determined for each respective produce type. The best fit curves corresponding to each produce type were shown in Figure 2, and Table 1 lists β_c values for different produce types. Also, as noted in this table, the experimental data were well represented by the model for decaying FC levels, with only minor deviation between them, as indicated by the R^2 and Root Mean Square Error (RMSE) values from the respective curve fittings for all the produce types. This suggests that Eq. 3 and 5 adequately describe the decay dynamics of FC in the wash water.

Among the numerous reactions that could happen in this system, it was initially assumed that multiple reactions between FC and organic matter might exist, and β_c is the average of all those reaction rate constants. Since the organic matter entering the wash solution for different types of produce was not the same, slight differences between β_c values could be expected. Our assumption that the main reaction in the wash system is of second order between COD and FC gives an excellent fit for the data and yields very similar reaction rates not only for various produce types but also across different scales (as illustrated in section "FC model validation and predictability"). We take advantage of this by considering the same β_C value for all lettuce types. We used an average β_c value of 6×10^{-4} L/mg·min from all produce types to predict the dynamics of FC in the wash water for each type of produce. The results show only a very minor deviation between predicted results and experimental data shown in Figure 2, and the R^2 values were 0.95 or higher for each produce type. Moreover, deviation between predicted values by model and experimental data at each data point for all produce types was less than 5% in each case, showing that there is a very good match between the predicted FC and experimental data.

FC model validation and predictability

All the experiments in this study were performed at a bench scale (3 L water tank). To validate our model, we determined the parameters from the data by Luo et al. (2012) and compared to those obtained in our work. Some of the operating conditions of that study were similar to our experimental conditions including the initial pH that was 6.5 in both studies, the use of sodium hypochlorite as the sanitizer agent, and cut iceberg lettuce as the produce washed. On the other hand, some conditions differed: the scale of the washing system (3200 L compared with 3 L), the type of process (continuous compared with batch), the amount of produce washed per liter of water per minute, and the initial COD level (307 mg/L compared with 32 mg/L). However, because the differences between the two studies are accounted for as variables of our model, these changes do not affect the proposed mechanisms for consumption of FC in the wash water. Therefore, we used the data from Luo et al. (2012) to validate our model forms for these mechanisms.

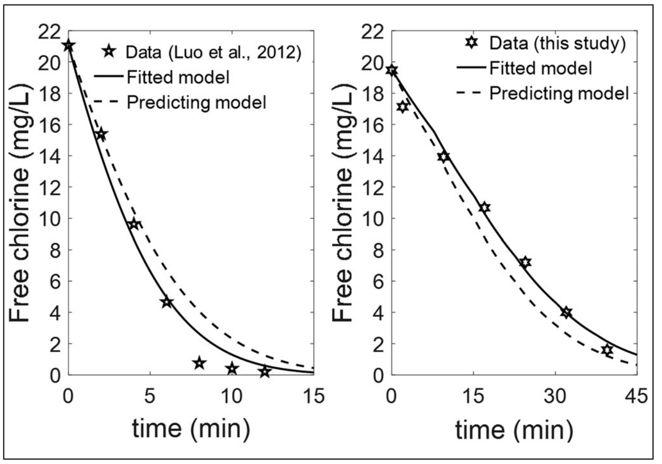

Figure 3–Free chlorine dynamics during washing of iceberg lettuce using data from (A) (Luo et al., 2012) and (B) the current study. The lines in (A) represent the fitting using a βc value of 5.38×10^{-4} (solid line) from Munther et al. (2015), and a value of 4.74×10^{-4} L/mg·min (dashed line) as a predicting model from this study, and vice versa for (B). The symbols represent experimental data.

Equations 3 and 5 are appropriate forms of our model to describe the dynamic changes of COD and FC concentration of the washing system and can be used even for continuous systems by just taking τ_1 to be the duration of the experiment, $\tau_2 = 0$, and $n = 1$. Using these versions of Eq. 3 and 5, and experimental data from Luo et al. (2012), we earlier reported the values of k_0 and β_c (Munther et al., 2015).

The values of β_c found in the current study can be used to predict the FC concentration in experiments by Luo et al. (2012), and vice versa. The R^2 values calculated for these predictions were shown in the last column of Table 2 and these curve fittings were shown in Figure 3. Figure 3A compares the fitting of data from Luo et al. (2012), using the value of β_c from Munther et al. (2015) (solid line) to this study (dashed line) as a predicting model. Figure 3B compares the fitting from our current study using the β_c found in our study (solid line), and its value from Munther et al. (2015) (dashed line) as a predicting model. We report $R^2 \geq 0.98$ in both the studies, indicating an excellent match between the fitted model and the experimental data. Moreover, the deviation between the predicting model and the curve-fitting model was less than 5% for each data point, with a combined R^2 value of

Table 2–Comparison of curve-fitting results in this study with that from Luo et al. (2012) for iceberg lettuce processing. Using data from Luo et al. (2012), we earlier calculated and reported parameters k_0 and β_c (Munther et al., 2015).

	O_0 (mg/L)	Cl_0 (mg/L)	k_0 (mg/L·min)	β_c (L/mg·min)	R^2 (Fitting)	R^2 (Prediction)
Current study	32	20	52.86	4.74×10^{-4}	0.99	0.95
(Munther et al., 2015)	307	21	32.3	5.38×10^{-4}	0.98	0.95

0.95 for all the data points in both the cases. This suggests that the predictive model does very well in matching the experimental data from both studies.

The numbers from Table 2 show a significantly lower value for k_0 from Luo et al. (2012) compared to that in our work for iceberg lettuce. The main factor that affects the COD levels (and thereby the k_0 value) is the rate at which lettuce entered the washing water relative to the wash tank volume, which was lower in that study compared to ours. Other factors that may be relevant include the farm source of lettuce, its age, variations in cut sizes of the pieces, and season of the year (Barrera et al., 2012; Chen & Hung, 2017). As may be expected, during different runs, we observed minor variations in k_0 values for the same lettuce type even under the same experimental conditions. Differences in the initial value of COD (O_0) and its rate of increase (k_0) are to be expected, as they depend on the produce used and the experimental setup. However, it is not clear if variations in β_c values are to be expected *a priori* due to potential scaling effects (Ding et al., 2017). As can be seen in Table 2, the value of β_c using data from Luo et al. (2012) is close to the β_c in our study.

Our model also allows us to predict the FC levels by only knowing the initial value of COD in the wash water and its steady rate of increase. Our model could be extended to larger operation scales such as commercial wash systems, where the produce may not be introduced to the wash water at a nearly constant rate and requires constant monitoring of COD to accurately predict FC. Since real-time monitoring of COD is not feasible, fresh-cut produce processing companies could try to predict the COD present in the wash water by using product type and throughput and water replenishment data. In this context, our model could be used to help validate set points and test efficiency of FC dosing strategies *via* intermittent collection of COD data during specific washing durations.

Water-mediated cross-contamination dynamics

An important question regarding cross-contamination concerns which mode or modes of pathogen transfer significantly contributes to the dynamics during washing. Among the possibilities for cross-contamination, the top three suspects for mediation are: (i) by water, which involves pathogen transfer from water to the surface of uncontaminated produce; (ii) by particles in the water, that is, pathogens attach to the surface of small produce debris in water and they, in turn, attach to the surface of uncontaminated produce; and (iii) by produce-to-produce contamination, that is, contact between contaminated produce and uncontaminated produce in the same wash batch (Gombas et al., 2017).

Note that we earlier (Munther et al., 2015) assumed that water-mediated cross-contamination played a dominant role in pathogen transfer to uninoculated iceberg lettuce. Using our batch model for pathogen dynamics, Eq. 6c and 6d in section "Complete model for water chemistry and E. coli O157:H7 cross-contamination" as a gauge, and data from prior studies in this regard (López-Gálvez et al., 2010; Luo et al., 2011, 2012;), we can justify this assumption by comparing relative values of the *E. coli* in water-to-lettuce transfer rate, β_{LW}. We earlier determined that $\beta_{LW} = 0.38$ mL/g·min (Munther et al., 2015). Note that in the study by López-Gálvez et al. (2010); hitherto referred as *study A*), produce-to-produce contamination during the prewash step was impossible as inoculated lettuce was dipped into the wash tank for 1 min, removed, and then noninoculated lettuce was dipped for 1 min into the same water (López-Gálvez et al., 2010). Using data from Figure 1 and 4 of *study A*, we estimate that $\beta_{LW} = 0.19$ mL/g·min. In contrast, Luo et al. (2011); hitherto referred as *study B*) washed both inoculated and noninoculated lettuce simultaneously and using data from Table 2 and 3 of *study B*, we estimate on average that $\beta_{LW} = 9.04$ mL/g·min.

The above analysis concerning β_{LW} indicates a few points. First, considering the experimental procedure in *study A*, β_{LW} indeed represents the rate of *E. coli* transfer *via* contaminated water. Since the values of β_{LW} from *study A* and the pilot scale study by Luo et al. (2012) are extremely close (note that this comparison is across three orders of magnitude in water volume), we conclude that water-mediated cross-contamination *dominates* the bacterial transfer dynamic during the pilot scale experiment in the latter study. This is not to say that produce-to-produce contamination did not occur during the pilot scale study, but that its contribution toward the observed cross-contamination levels on the lettuce was minimal as compared with water-mediated transfer. This notion is reinforced by the fact that β_{LW} corresponding to *study B*, in which product-to-product contact was permitted during washing, is at least an order of magnitude greater. It is also interesting to note that in *study A*, the bacterial levels in the wash water are significantly higher, the water agitation/flow rate is much lower, and the produce mass/water volume ratio is significantly lower, compared to a study by Luo et al. (2012); however, the β_{LW} values are remarkably similar. This suggests that the bacterial transfer mechanism described by the model is robust relative to experimental scale and pathogen levels in the wash water. Therefore, we propose that lab scale wash experiments for fresh-cut lettuce, in which product-to-product contamination is controlled against or at least limited (by lowering the produce to water ratio), may provide adequate representation of the cross-contamination dynamic via water that might occur in typical commercial wash processes.

Insights toward cross-contamination control

Another important question in the industry is: what level of FC is sufficient to prevent cross-contamination during fresh-cut produce washing? Using model (CM), we can gain insight to this question by assessing the efficacy of FC in preventing water-mediated cross-contamination. Numerically mimicking an experimental procedure similar to that described in section "Fresh-cut produce washing system," we set the initial FC level to 20 mg/L in the tank (3 L) and successively introduced 100 g batches of produce, 1% of which is inoculated with level σ (MPN/g) of *E. coli* O157:H7. The wash time is set for 30 s and the total experimental time is 45 min.

Table 3–List of parameters and their values used in the complete model (CM) simulations to generate Equations (8a) (8b) in section "Insights toward cross-contamination control."

Parameter	Description	Values and units	Reference
k_0	COD increase rate	52.86 mg/L·min	This study
λ_c	Natural FC decay rate	2×10^{-3} /min	(Hua, West, Barker, & Forster, 1999)
β_c	FC apparent reaction rate	5.38×10^{-4} L/mg·min	(Munther et al., 2015)
β_{LW}	*E. coli* binding rate: water to lettuce	0.38 mL/g·min	(Munther et al., 2015)
β_{WS}	Effective *E. coli* input rate to wash water	[1,105] MPN/mL·min	This study
L	Amount of lettuce in wash tank	0.1 kg	This study
V	Volume of wash water	3 L	This study
α	Kill rate of *E. coli* via FC	0.75 L/mg·min	(Munther et al., 2015)

Among other factors, the efficacy of a certain FC level to prevent cross-contamination depends on the amount of pathogen available for transfer in the wash system. To explore the interplay between FC dynamics and pathogen transfer (*via* water) when varying levels of pathogen are introduced to the wash system, we vary the parameter β_{WS} (the rate at which pathogens are introduced to the wash water) stepwise, and use model (CM) to compute the FC level, C^* (mg/L), and the corresponding time, T^* (min), when cross-contamination on uninoculated lettuce is first detectable during the six-batch wash cycle (see Table 3 for parameter values used in model [CM] simulations). We set the level of detection to be 0.12 MPN/g (Source: FDA website).

Notice that β_{WS} can be described as a function of σ, the average pathogen level on prewashed lettuce, considering that pathogens shed from inoculated prewashed produce into the water during washing (see Appendix B for more details). Using the functional relationship between β_{WS} and σ, the model (CM) predictions (Figure 4) illustrate the ability of FC to control water-mediated cross-contamination relative to various levels of prewash inoculation. For instance, for $\sigma \leq 4.7$ Log_{10} (MPN/g), pathogens will not be detectable on uninoculated lettuce until $T^* \approx 38$ min with a corresponding FC level of $C^* \approx 1.9$ mg/L in the wash water. For lettuce inoculated at $\sigma > 5.9\text{Log}_{10}$ (MPN/g), even 20 mg/L of FC may not be sufficient to prevent cross-contamination via water.

We can also use model (CM) to provide a simple relationship between the maximum level of pathogens in the water X_W^{Max} (MPN/mL) and the maximum level of cross-contamination on produce leaving the wash X_L^{Max} (MPN/g). Calculating these terms from model (CM) outputs as functions of the input inoculation level σ, we find that:

$$X_W^{\text{Max}} = 4.4 \times 10^{-5} e^{2.3\sigma} \tag{8a}$$

$$X_L^{\text{Max}} = 3.8 \times 10^{-6} e^{2.3\sigma} \tag{8b}$$

Not only do these functions provide a convenient upper bound on pathogen levels in the water and on cross-contaminated lettuce, but we can use them to infer the maximum cross-contamination potential (via water). Equation 8a and 8b can provide a simple reference point for the upper limit of water-mediated cross-contamination. In the context of experimental practice, these equations indicate that if X_L has at least the same or greater order of magnitude than X_W, water-mediated cross-contamination may not be solely responsible for the pathogen transfer dynamic during washing. For instance, in the study by Luo et al. (2011)), $X_W = 5.6$ (MPN/mL) and $X_L = 12.4$ (MPN/g). Since $X_L = 2.2\ X_W$, it provides evidence to support the hypothesis that direct contact between inoculated and uninoculated produce played a significant role in pathogen transfer to uninoculated produce during washing.

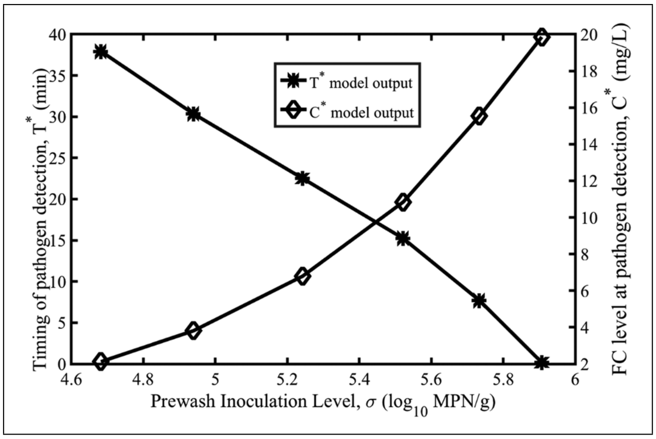

Figure 4–Model results illustrating the experimental timing T^* (min) and FC level C^*(mg/L) when water-mediated cross-contamination of *E. coli* O157:H7 is first detected as a function of prewash inoculation level σ ($\log_{10}$ CFU/g).

Model limitations

First, we discuss some of the limitations of our model for COD and FC levels in the wash water (Equations 6a and 6b). We observe that while our model for FC for iceberg lettuce washing is valid at both the laboratory scale and the pilot-plant scale, this may not hold true for other produce types. Moreover, FC levels are pH-dependent, and can vary widely if the pH of the system varies, which our model does not currently account for. We note that this is difficult to do using our approach unless the causes for the variability in pH are known. It is also possible that the rate of FC decay is sensitive to its initial level in the water. While our model may still be valid if this initial FC level is larger than 40 mg/L, more detailed studies varying these initial levels need to be performed to understand how this may affect the decay rate of FC. There are other sources of variability that may occur due to differences in the regions in which the fresh produce is grown and the season of the year, which our model does not currently account for, and which may be important in certain conditions.

The main dynamics contributing to water-mediated cross-contamination involve the introduction of pathogen into the wash water (β_{WS}), the contact/binding rate from pathogens in the water to the produce (β_{LW}), and the kill rate of pathogens in wash water and on produce via FC (α). Note that from equation in Appendix B, β_{WS} depends on the pathogen shed rate b (1/min) that may be produce-/cut-type-dependent. Furthermore, β_{LW} may be produce-/cut-type-dependent. This indicates that additional studies should be performed to classify these parameters relative to various produce/cut combinations.

Another important notion centers on the question of quantifying the killing rate of *E. coli* via FC in the wash water and on the produce relative to various parameters such as experimental scale, produce/ volume ratio, and magnitude of pathogen levels on produce and in wash water. Let $f_W(\alpha, \vec{X})$ represent the killing rate of pathogen in the water, where α denotes the efficacy of FC, and $\vec{X}$ is a vector quantifying the FC level in the water (C), the pathogen level in the water (X_W), and potentially other parameters. Let $f_P(\alpha, \vec{Y})$ represent the killing rate of pathogens via FC on produce, where $\vec{Y}$ is a vector quantifying the FC level in the water, the pathogen level on the produce, and so on. The question above relates to determining the functional form of both f_W and f_P. It should be noted that the efficacy of sanitizers in produce wash water depends primarily on their concentration as they have very short time span (order of seconds) to influence cross-contamination (Gombas et al., 2017). In the context of fresh-cut produce washing, this indicates that the forms of f_W and f_P should explicitly depend on C. In our earlier study (Munther et al., 2015), these killing rates were modeled by using a well-mixing assumption implying that $f_w(\alpha, \vec{X}) = \alpha X_W C$ and $f_P(\alpha, \vec{Y}) = \alpha X_L C$, where α (L/(mg min)) represents a *constant* efficacy rate of FC. However, more studies need to be conducted to ascertain the potential-scale dependence of this parameter.

Conclusions

In this study, the dynamic changes of water quality during the washing process of four different types of lettuce (romaine, iceberg, green leaf, and red leaf), carrots and green cabbage were first studied. Results showed that COD levels increased over time as more produce was washed and, in particular, the lettuce type impacted the rate of increase in organic load. As the produce was introduced to the washing solution, FC concentration decreased mainly due to consumption of chlorine by organic matter. FC concentration is of high importance in washing of fresh-cut produce as it inactivates bacteria, and such depletion of FC might lead to cross-contamination in the washing water. Using data from a bench-top experimental setup, a mathematical model was developed for FC dynamics in the washing solution to describe the mechanism by which FC changes in the washing of fresh-cut produce. The reaction rate constant between chlorine and COD was calculated based on the experimental data. Results showed that the apparent reaction rate constant (β_c) ranges from 4.74×10^{-4} to 7.42×10^{-4} L/mg·min for lettuce, carrots, and cabbage, when the pH of the wash water is maintained at stable levels of 6.5. We compared the model parameters from our experimental data to those obtained from a pilot-plant scale study for lettuce (Luo et al., 2012; Munther et al., 2015), and observed a very similar β_c value of 5.38×10^{-4} L/mg·min. This strongly supports our hypothesis that rise in COD is the main cause of consumption of FC levels in the wash water. Given the variety of produce types we have used in our study, we believe that our model and the value of β_c will be comparable to the range we have obtained in this study as long as the pH of the water is stable at ambient temperatures, and that the chlorine levels can be estimated well using our model by only knowing the rate of change of the COD levels under these conditions.

In addition to water quality dynamics, we used our CM to not only confirm the utility of lab-scale experiments in quantifying water-mediated cross-contamination, but also illustrate how our model can provide guidelines for future experiments to establish quantifiable guidelines for cross-contamination control. Further studies under a wider range of operational conditions need to be conducted to corroborate these results, so that they may be used to understand the dynamics of FC and cross-contamination during the wash process, and to potentially improve existing industrial practices.

Acknowledgments

P.S., C.K., and D.M. acknowledge funding support from the Natl. Inst. of Food and Agriculture Grant No. 2017-67018-26225, from the Center for Produce Safety Award No. 2018CPS06, and the Office of Research at Cleveland State Univ. We gratefully acknowledge Dr. Yaguang Luo (USDA) for useful discussion, and anonymous reviewers for making useful suggestions to improve the quality of our manuscript.

Author Contributions

All authors participated in the design of experiments. M.D., A.A., and C.K. performed the experiments. M.D., D.M., and P.S. developed and implemented the model for parameter estimation. All authors participated in data analysis, manuscript preparation, and editing.

References

Barrera, M. J., Blenkinsop, R., & Warriner, K. (2012). The effect of different processing parameters on the efficacy of commercial post-harvest washing of minimally processed spinach and shredded lettuce. *Food Control*, *25*(2), 745–751.

Beuchat, L. R., Adler, B. B., & Lang, M. M. (2004). Efficacy of chlorine and a peroxyacetic acid sanitizer in killing Listeria monocytogenes on iceberg and romaine lettuce using simulated commercial processing conditions. *Journal of Food Protection*, *67*(6), 1238–1242.

Chen, X., & Hung, Y. C. (2016). Predicting chlorine demand of fresh and fresh-cut produce based on produce wash water properties. *Postharvest Biology and Technology*, *120*, 10–15.

Chen, X., & Hung, Y. C. (2017). Effects of organic load, sanitizer pH and initial chlorine concentration of chlorine-based sanitizers on chlorine demand of fresh produce wash waters. *Food Control*, 77, 96–101. https://doi.org/10.1016/j.foodcont.2017.01.026

Deborde, M., & von Gunten, U. (2008). Reactions of chlorine with inorganic and organic compounds during water treatment—Kinetics and mechanisms: A critical review. *Water Research*, *42*(1), 13–51. https://doi.org/10.1016/j.watres.2007.07.025

Ding, D., Benson, D. A., Fernandez-Garcia, D., Henri, C. V., Hyndman, D. W., Phanikumar, M. S., & Bolster, D. (2017). Elimination of the reaction rate "scale effect": Application of the Lagrangian reactive particle-tracking method to simulate mixing-limited, field-scale biodegradation at the Schoolcraft (MI, USA) site. *Water Resources Research*, *53*(10), 411–432.

Fu, T. J., Li, Y., Awad, D., Zhou, T. Y., & Liu, L. (2018). Factors affecting the performance and monitoring of a chlorine wash in preventing Escherichia coli O157: H7 cross-contamination during postharvest washing of cut lettuce. *Food Control*, *94*, 212–221.

Gil, M. I., Selma, M. V., López-Gálvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: Problems and solutions. *International Journal of Food Microbiology*, *134*(1), 37–45. https://doi.org/10.1016/j.ijfoodmicro.2009.05.021

Gombas, D., Luo, Y., Brennan, J., Shergill, G., Petran, R., Walsh, R., . . . Deng, K. (2017). Guidelines to validate control of cross-contamination during washing of fresh-cut leafy vegetables. *Journal of Food Protection*, *80*(2), 312–330. https://doi.org/10.4315/0362-028X.JFP-16-258

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. https://doi.org/10.1016/j.foodcont.2014.01.034

Holvoet, K., De Keuckelaere, A., Sampers, I., Van Haute, S., Stals, A., & Uyttendaele, M. (2014). Quantitative study of cross-contamination with Escherichia coli, E. coli O157, MS2 phage and murine norovirus in a simulated fresh-cut lettuce wash process. *Food Control*, *37*, 218–227.

Hua, F., West, J. R., Barker, R. A., & Forster, C. F. (1999). Modelling of chlorine decay in municipal water supplies. *Water Research*, *33*(12), 2735–2746.

Jensen, D. A., Friedrich, L. M., Harris, L. J., Danyluk, M. D., & Schaffner, D. W. (2015). Cross Contamination of Escherichia coli O157:H7 Between lettuce and wash water during home-scale washing. *Food Microbiology*, *46*, 428–433.

Jung, Y., Gao, J., Jang, H., Guo, M., & Matthews, K. R. (2017). Sanitizer efficacy in preventing cross-contamination during retail preparation of whole and fresh-cut cantaloupe. *Food Control*, *75*, 228–235. https://doi.org/10.1016/j.foodcont.2016.12.009

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 pre-washing cannot be controlled after subsequent washing with chlorine dioxide or sodium hypochlorite. *Food Microbiology*, *27*(2), 199–204.

Luo, Y., Nou, X., Millner, P., Zhou, B., Shen, C., Yang, Y., . . . 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. *International Journal of Food Microbiology*, *158*(2), 133–139. https://doi.org/10.1016/j.ijfoodmicro.2012.07.008

Luo, Y., Nou, X., Yang, Y., Alegre, I., Turner, E., Feng, H., . . . Conway, W. (2011). Determination of free chlorine concentrations needed to prevent Escherichia coli O157:H7 cross-contamination during fresh-cut produce wash. *Journal of Food Protection*, *74*(3), 352–358. https://doi.org/10.4315/0362-028X.JFP-10-429
Munther, D., Luo, Y., Wu, J., Magpantay, F. M. G., & Srinivasan, P. (2015). A mathematical model for pathogen cross-contamination dynamics during produce wash. *Food Microbiology*, *51*, 101–107. https://doi.org/10.1016/j.fm.2015.05.010
Murray, K., Aldossari, H., Wu, F., & Warriner, K. (2018). Dynamic changes in free-chlorine levels within a commercial post-harvest wash and prevention of cross-contamination between shredded lettuce batches. *Food Control*, *85*, 127–134. https://doi.org/10.1016/j.foodcont.2017.09.029
Murray, K., Wu, F., Shi, J., Jun Xue, S., & Warriner, K. (2017). Challenges in the microbiological food safety of fresh produce: Limitations of post-harvest washing and the need for alternative interventions. *Food Quality and Safety*, *1*(4), 289–301.
Nou, X., Luo, Y., Hollar, L., Yang, Y., Feng, H., Millner, P., & Shelton, D. (2011). Chlorine stabilizer T-128 enhances efficacy of chlorine against cross-contamination by *E. coli* O157:H7 and Salmonella in fresh-cut lettuce processing. *Journal of Food Science*, *76*(3), M218–224. https://doi.org/10.1111/j.1750-3841.2011.02046.x
Pirovani, M. E., Guemes, D. R., & Piagnetini, A. M. (2001). Predictive models for available chlorine depletion and total microbial count reduction during washing of fresh-cut spinach. *Journal of Food Science*, *66*(6), 860–864.
Ruiz-Cruz, S., Acedo-Félix, E., Díaz-Cinco, M., Islas-Osuna, M. A., & González-Aguilar, G. A. (2007). Efficacy of sanitizers in reducing Escherichia coli O157:H7, Salmonella spp. and Listeria monocytogenes populations on fresh-cut carrots. *Food Control*, *18*(11), 1383–1390. https://doi.org/10.1016/j.foodcont.2006.09.008
Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M., Roy, S. L., . . . Griffin, P. M. (2011). Foodborne illness acquired in the United States—Major pathogens. *Emerging Infectious Disease*, *17*(1), 7–15.
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. *Journal of Food Protection*, *76*(3), 386–393.
Tomás-Callejas, A., López-Gálvez, F., Sbodio, A., Artés, F., Artés-Hernández, F., & 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*(2), 325–332. https://doi.org/10.1016/j.foodcont.2011.07.022
Van Haute, S., López-Gálvez, F., Gómez-López, V. M., Eriksson, M., Devlieghere, F., Allende, A., & Sampers, I. (2015). Methodology for modeling the disinfection efficiency of fresh-cut leafy vegetables wash water applied on peracetic acid combined with lactic acid. *International Journal of Food Microbiology*, *208*, 102–113.
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. *Appllied Environmental Microbiology*, *79*(9), 2850–2861.
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 Science and Technology*, *75*, 301–304. https://doi.org/10.1016/j.lwt.2016.09.002
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 Microbiology*, *50*, 88–96. https://doi.org/10.1016/j.fm.2015.03.004

Appendix A: Model for *E. coli* O157:H7 Cross-Contamination

Following our earlier paper (Munther et al., 2015), we consider the following mechanisms connected to the pathogen level in the water X_W (MPN/mL) during batch experiments: (i) pathogens on inoculated produce shed into the water during washing (which we assume occurs at a constant rate given that produce is inoculated with the same level of pathogens on average), (ii) pathogens in the wash water can transfer/attach to produce during washing, and (iii) pathogens can be inactivated by FC in the wash water. Incorporating these three mechanisms, we build the following equation:

$$\frac{dX_W}{dt} = \hat{\beta}_{WS} - \hat{\beta}_{LW}\frac{L}{V}X_W - \alpha X_W C$$

In the above equation,

$$\hat{\beta}_{WS} = \begin{cases} \beta_{WS}, & (n-1)(\tau_1+\tau_2) \le t \le n\tau_1 + (n-1)\tau_2 \\ 0, & n\tau_1 + (n-1)\tau_2 \le t \le n(\tau_1+\tau_2) \end{cases}$$

$$\hat{\beta}_{LW} = \begin{cases} \beta_{LW}, & (n-1)(\tau_1+\tau_2) \le t \le n\tau_1 + (n-1)\tau_2 \\ 0, & n\tau_1 + (n-1)\tau_2 \le t \le n(\tau_1+\tau_2) \end{cases}$$

where β_{WS} (MPN/mL·min) is the entry rate of the pathogen into the water, β_{LW} (mL/g·min) is the rate of pathogen transfer from the water to the produce, L/V (g/mL) is the produce to water ratio in during washing, α (L/mg·min) is the kill rate of pathogens via FC, and C (mg/L) is the FC level in the wash water. Note that we assume that complete mixing occurs during washing and thus successful transfer of pathogens from water to produce occurs at a rate proportional to the amount of pathogen in the water X_W (MPN/mL) and the amount of produce in the water L(g). To incorporate the batch-washing dynamic, $\hat{\beta}_{WS}$ and $\hat{\beta}_{LW}$ are defined to be "on" during washing and "off" in between washes.

In terms of quantifying cross-contaminated pathogen levels on produce batch n, we have the following equation for $n \in \{1, 2, \ldots, N\}$:

$$\frac{dX_{L_n}}{dt} = b^n_{LW} X_W - \alpha X_{L_n} C \chi_n$$

$$b^n_{LW} = \begin{cases} \beta_{LW} & (n-1)(\tau_1+\tau_2) \le t \le n\tau_1 + (n-1)\tau_2 \\ 0 & \text{else} \end{cases}$$

where X_{L_n} (MPN/g) represents the average pathogen level *via* cross-contamination on produce batch n during washing, the first term (on the right-hand side of the equation) quantifies pathogen transfer onto lettuce (again defined to account for the batch process) and the second term tracks the killing of pathogens via FC on the produce surface during washing.

Appendix B: Functional relationship between β_{WS} and σ

Assuming that the extent of time *E. coli*, inoculated onto prewash lettuce, remains on the lettuce during washing is exponentially distributed, we define β_{WS} as the following function of σ:

$$\beta_{WS} = \frac{\sigma(1 - \exp(-\tau_1 * b))\theta N}{V}$$

where $\tau_1 = 0.5$ (min) is the average dwell time in the wash tank for each produce batch, $b = 0.43$ (1/min) is the average shed rate of *E. coli* from iceberg lettuce (this was determined from data from figure 1 of López-Gálvez et al., 2010), $\theta = 0.01$ is the fraction of inoculated produce, $N = 0.2$ (kg/min) is the amount of produce washed in the tank per minute, and $V = 3$ L is the volume of the tank.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Free chlorine dynamics over time when 150 g of disc carrots (0.1″ thickness) were washed in 3 L of tap water with initial free chlorine level of 25 mg/L (pH~6.5) for 30 s, at 2 and 20 °C, and the decay of chlorine in the water monitored for the next 30 min. Experimental data were presented as average ± standard deviation.