Title: Predicting chlorine demand of fresh and fresh-cut produce based on produce wash water properties

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Abstract

This study was conducted to develop models capable of predicting chlorine demand of different fresh and fresh-cut produce wash waters. Ten simulated fruit and vegetable wash waters having different chemical oxygen demands (COD) were prepared. The chlorine demand and wash water quality parameters including pH, oxidation reduction potential (ORP), ultraviolet absorbance at 254 nm (UV254), COD, turbidity, total protein content, total phenolics content and color difference between deionized water and test samples (ΔE) were measured. The correlations between variables were determined. UV254 had the highest correlation coefficient with chlorine demand of various fresh produce wash waters ($R = 0.77$). Further analysis of chlorine demand with UV254 relation showed that two clusters exists, one for produce with high phenolics content and one for low phenolics content. The phenolics-to-protein/ ΔE ratio (PPC) was created to identify in which cluster each produce wash water should be. Empirical equations for predicting chlorine demand were developed as chlorine demand = $295.23 \times \text{UV254} + 6.97$, if $\text{PPC} < 0.6$; or chlorine demand = $119.77 \times \text{UV254} + 2.41$, if $\text{PPC} \geq 0.6$. These two prediction equations were further verified using additional produce wash waters not used for model development. The outcomes of this study demonstrated that the predictive equations developed using water quality parameters can be used to estimate the chlorine demand of different produce wash waters.

Key Words: Fresh produce, washing, chlorine demand, UV254, predictive equations

1. Introduction

Outbreaks associated with the consumption of fresh produce have been widely reported over the past two decades (Olaimat and Holley 2012). The percentage of foodborne outbreaks caused by fresh produce increased from 0.7% in the 1970s to 6% in the 1990s (Sivapalasingam et al. 2004). This number further increased to 14.8% from 1998 to 2007 (CSPI 2009). Compared with meat or poultry products, fresh produce is usually eaten raw or minimally processed. Thus, once fresh produce is contaminated, it is difficult to totally remove or kill those pathogens.

Postharvest washing is one of the most critical processing steps to maintain the safety and quality of fresh and fresh-cut produce (Gil et al. 2015). In most processing lines, fresh fruits and vegetables are immediately dipped into a washing tank to remove dirt, pesticide residues, debris, tissue fluids and microorganisms, as well as to rapidly cool the products (Artes et al. 2009). To further enhance the antimicrobial efficacy and to prevent cross contamination, sanitizers are usually added into the wash water (Gil et al. 2009). Chlorine-based sanitizers are commonly used in the US fresh produce industry (Lu et al. 2014). Generally, chlorine is used in residual concentrations at 50-200 mg L⁻¹ with a 1-2 min dipping time. The pH of chlorine-based sanitizers is usually adjusted to 6-7.5 to maintain the high antimicrobial efficacy and to minimize the generation of toxic chlorine gas (Beuchat et al. 2004).

One concern of using chlorine is its reaction with organic matter. During the industrial washing process, organic compounds accumulate in wash water due to the recycling of wash water (Barrear et al. 2012). The presence of organic compounds can lead to a rapid depletion of hypochlorous acid (HOCl), which is the main active component of a chlorinated wash solution. Although it is reported that the microbial inactivation due to chlorine on fresh produce is limited regardless of concentration (1-2 log reductions) (Beuchat et al. 2004), sufficient residual chlorine

in wash water is a key factor to prevent cross-contamination (Luo et al. 2011). Many studies therefore were conducted to evaluate the impact of organic load on residual chlorine and antimicrobial efficacy of chlorine solutions (Pirovani et al. 2001; Van Haute et al. 2013; Shen et al. 2013; Zhou et al. 2014). Different parameters, such as chemical oxygen demand (COD), turbidity, produce-to-water ratio, oxidation reduction potential (ORP) and ultraviolet absorbance measured at 254 nm (UV254), were used as organic load indicators of fresh produce wash water. However, these studies were designed mostly using only one type of fresh produce, recommendation on relation between wash water properties and organic load/chlorine demand may not be valid for other types of fresh produce wash water. In realistic situations, chemical compositions and reactivity of wash water can vary a lot due to different types of fresh produce, different degree of fresh produce maturity and environmental conditions during planting (Lehto et al. 2014). Therefore, the objectives of our study were to (1) determine the relationship between chlorine demand and produce wash water quality parameters, and (2) explore the feasibility of using wash water quality parameters to estimate chlorine demand for different types of fresh and fresh-cut produce.

2. Materials and Methods

2.1 Preparation of Simulated Produce Wash Waters

Ten different types of fresh produce (iceberg lettuce, romaine lettuce, spinach, celery, mushroom, broccoli, strawberry, grape, cantaloupe, and tomato) were selected based on their botanical classification, chemical composition, and popularity for regular consumption. Produce were purchased from local grocery stores (Griffin, GA, USA) and stored at 4°C until use. Any wilted, discolored leaves or damaged produce were discarded. Iceberg lettuce, romaine lettuce, spinach, celery, mushroom, broccoli, cantaloupe and tomato were cut into small pieces with

clean scissors or knives before use. Approximately 50 g of fresh or fresh-cut produce was placed in Whirl Pak® bags with an equal amount of deionized water (dH₂O). Samples were homogenized in a stomacher (Seward Stomacher®, 80 biomaster, Worthing, UK) for 120 s at 265 rpm, and filtered through filter papers (No. 4, Whatman™, Boca Raton, FL, USA). The COD of each filtered produce extract was determined, and subsequently, solution was diluted to ensure the COD of chlorinated wash water at approximately 50, 400 and 750 mg L⁻¹ to simulate different organic loads in commercial fresh and fresh-cut produce wash waters. Spinach extract was adjusted to a COD of 50, 250 and 450 mg L⁻¹, and mushroom and broccoli to a COD of 50, 150, and 250 mg L⁻¹, to ensure that the chlorine demand at the highest COD would not exceed 100 mg L⁻¹.

2.2 Measurement of Chlorine Demand

Sodium hypochlorite (NaOCl) solution was prepared on the same day of the experiment by diluting 6% regular bleach (RICCA Company, Arlington, TX, USA) in dH₂O to obtain NaOCl solution of 110 mg L⁻¹ free chlorine. The free chlorine concentration was determined using DPD-FEAS titrimetric tests (Hach Company, Loveland, CO, USA). The pH of the solution was adjusted to 6.0 ± 0.2 using 1 N HCl and NaOH. After being stored at 4°C for 2 h, 9 mL of NaOCl solution was mixed with 1 mL of produce wash water in a test tube for 5 min. Deionized water, instead of produce wash water, was used as the control treatment. The chlorine demand of produce wash water was calculated as the difference of residual free chlorine between control and chlorinated produce wash water.

2.3 Analysis of Produce Wash Water Quality

Produce wash water at different COD was diluted 10 times before measuring water quality parameters, so the organic load would be similar to the samples after mixing with chlorine in previous chlorine demand experiments. Wash water quality parameters including pH, ORP, UV254, COD, total phenolics content, total protein content, turbidity, and color difference between samples and dH₂O (ΔE) were determined. Specifically, pH and ORP were measured with a dual channel ACCUMET meter (Model # AR50, Fischer Scientific, Pittsburgh, PA, USA). UV254 was determined using a DU 520 UV-Vis spectrophotometer (Beckman Coulter Inc., Brea, CA, USA) after filtering wash water through a 0.45 μ m pore-size filter (Waterman®, Baco Raton, FL, USA). COD was determined with a DR/90 colorimeter (HACH®, Loveland, CO USA) using a reactor digestion colorimetric method (Hach method 8000). Turbidity was measured using a DR/90 colorimeter (Hach method 8237) and expressed as Formazin Attenuation Unit (FAU).

Total phenolics content was determined using the Folin-Ciocalteu assay (Singleton and Rossi, 1965). Briefly, 1 mL of wash water was added to 9 mL of dH₂O in a test tube plus 1 mL of Folin-Ciocalteu's phenol reagent (Sigma-Aldrich Co., St Louis, MO, USA). After 5 min, 10 mL of 7% Na₂CO₃ solution was added followed by diluting the mixture to 25 mL with dH₂O. After incubating for 90 min at room temperature (20 °C), solutions were read at 750 nm in a 1-cm cuvette with a DU 520 UV-Vis spectrophotometer (Beckman Coulter Inc.). Total phenolics content of wash water was reported as mg L⁻¹ as gallic acid equivalents.

Total protein content was determined using the Bradford assay (Bradford, 1976). Briefly, 1 mL of wash water was mixed with 5 mL of Bradford's reagent (BioWorld, Dublin, OH, USA). After 5 min incubation, solutions were read at 595 nm in a 1-cm cuvette using a DU 520 UV-Vis

spectrophotometer (Beckman Coulter Inc.). Total protein content of produce wash water was reported as mg L⁻¹ as bovine serum albumen (BSA) equivalents.

The color of produce wash water was measured with a MiniScan XET^M colorimeter (Model# 45/0-L, Hunter Associates Laboratory, Reston, VA, USA). Briefly, 25 mL of produce wash water or dH₂O was placed in a 90 mm dia × 15 mm deep plastic petridish. A standard white tile was placed at the bottom of petridish as the background. The distance between colorimeter and surface of solution was adjusted to approximately 2 cm. Color parameters of L*, a* and b* were recorded, where L* represents color lightness, a* represents redness and b* represents yellowness. The ΔE (total color difference) between produce wash water (s) and dH₂O (w) was calculated using the following equation:

$$\Delta E = \sqrt{(L_s^* - L_w^*)^2 + (a_s^* - a_w^*)^2 + (b_s^* - b_w^*)^2} \quad (1)$$

2.4 Chlorine Demand Prediction Equations

A key element in developing prediction equations that can be applied to various fresh produce was to identify the physiochemical parameters of wash water correlated with chlorine demand. Correlations between chlorine demand and water quality parameters were developed based on Pearson correlation coefficient and graphic inspection.

2.5 Model Verification

Two verification experiments were conducted on the usefulness of the chlorine demand prediction equations. The first experiment included six types of fresh produce (iceberg lettuce, romaine lettuce, spinach, mushroom, strawberry and tomato) that were the same as those used for model development, but diluted to different COD levels. The second verification test used three types of fresh produce (apple, romaine hearts, and a commercial mixed leafy vegetable

salad) that were different from the ten types of fresh produce used for model development. Produce wash water was prepared and its quality parameters were measured following the same procedures as described above. Predictive accuracy of each sample and root mean squared prediction error (RMSPE) of each validation test were calculated.

2.6 Statistical Analysis

Two replicate samples from each type of fresh produce were used. Two to three measurements were made on each sample and the model development experiment was duplicated. Correlation and linear regression between chlorine demand and water quality parameters were analyzed using the Corr and REG procedures of SAS 9.4 (SAS Institute, Cary, NC, USA), respectively. The Student's t-test at the probability level of $P < 0.05$ was used for comparisons of means.

3. Results and Discussion

3.1 Correlation between Chlorine Demand and Wash Water Quality Parameters

The water quality parameters of each simulated fresh produce wash water at different COD are shown in Table 1. All produce wash waters were acidic ($\text{pH} < 7$) and had a measurable oxidation potential ($\text{ORP} > 0 \text{ mV}$). For each type of fresh produce, the chlorine demand increased with increasing COD, total protein, total phenolics, UV254 and turbidity (Table 1). This may be the reason that these parameters were successfully used as organic load indicators to predict chlorine loss and microbial reduction in some produce washing studies reported in the literature (Pirovani et al. 2001; Van Haute et al. 2013; Shen et al. 2013; Zhou et al. 2014). However, our results showed that wash water properties varied a lot among different types of fresh produce. For example, although iceberg lettuce and romaine lettuce had similar chlorine

demand at the highest organic load, romaine lettuce wash water contained three times higher protein and phenolics content than iceberg lettuce wash water. In addition, chlorine demand of different fresh produce wash water at COD 750 mg L⁻¹ ranged from 12.00 mg L⁻¹ (grape) to 97.38 mg L⁻¹ (iceberg lettuce). Such large chlorine demand variance indicated that despite the wide use of COD as the organic load indicator for produce wash water (Zhou et al. 2014), COD alone may not correlate well to chlorine demand for all types of fresh produce reported in Table 1.

Correlations between wash water quality parameters and chlorine demand are presented in Table 2. UV254 had the highest correlation coefficient ($R = 0.77$), followed by total phenolics ($R = 0.65$) and total protein content ($R = 0.64$). As reported in the literature, the reactivity of HOCl with organic compounds and different functional groups from high to low is: reduced sulfur moieties > primary and secondary amines > phenols, tertiary amines » double bonds, other aromatics, carbonyls, and amides (Deborde and Gunten, 2008). Due to their high reactivity with chlorine, protein and phenolics were reported to cause significant available chlorine loss when contact with chlorine-based sanitizers (Waters and Hung, 2014). Because UV254 was also correlated with phenolics ($R = 0.94$) and protein content ($R = 0.79$) as indicated in Table 2, it has been widely used as an indicator to predict organic load and chlorine demand during drinking water and waste water disinfection (Fitzgerald et al. 2006). Our results further demonstrated that UV254 was correlated with chlorine demand of various fresh produce wash waters (Table 2). Therefore, UV254 was selected for further analysis.

3.2 Empirical equations for Predicting Chlorine Demand

Fig. 1 shows the scatter plot of chlorine demand versus UV254. Although two variables were positively correlated, produce wash waters could be divided into two clusters. Cluster 1

included iceberg lettuce, mushroom, grape, celery, cantaloupe, broccoli, and tomato with a steep chlorine demand vs. UV254 correlation slope; Cluster 2 included romaine lettuce, spinach, and strawberry with a flat correlation slope. The R^2 between UV254 and chlorine demand for Cluster 1 and 2 was 0.89 and 0.97, respectively. By observing produce wash water quality parameters presented in Table 1, produce wash waters that belonged to Cluster 2 usually had higher phenolics content than that in Cluster 1 at a similar organic load ($P < 0.05$). This is due to the fact that compounds with electron-rich functional groups (such as aromatic rings) absorb more light than others at the 254 nm wavelength. Therefore, at the similar chlorine demand, produce wash water with high phenolics content (Cluster 2) had higher UV254 absorbance than samples with low phenolics content (Cluster 1).

Waters and Hung (2014) reported that the chlorine demand of produce wash water is mainly contributed by phenolics and protein compounds. Since the phenolics content of produce in Cluster 2 was always higher than in Cluster 1 (Table 1), it is reasonable to assume that protein and phenolics compounds were the major components to react with free chlorine in Cluster 1 and 2, respectively. Under this assumption, the ratio of phenolics to protein content for phenolics-dominated produce in Cluster 2 should be higher than protein-dominated produce in Cluster 1, regardless of the organic load. However, the colorimetric assay of total protein content was interfered by the color of samples as supported by the high correlation coefficient between total protein content and ΔE ($R = 0.88$, Table 2). The (total protein/ ΔE) therefore was a better parameter than total protein content, as measured by Bradford assay, to accurately relate chlorine demand with the active protein concentration in produce wash water. Eventually, the phenolics-to-protein/ ΔE or PPC ratio was developed to classify the protein-dominated (Cluster 1) and phenolics-dominated (Cluster 2) produce in Table 1.

$$PPC = \frac{\text{Total Phenolics}}{(\text{Total Protein}/\Delta E)} \quad (2)$$

To determine the best threshold value of PPC, percentage correction (the percentage of samples that were distributed to each cluster) at threshold PPC from 0.1 to 1 is shown in Fig. 2. At threshold PPC = 0.6, all produce wash waters studied had the highest percentage (85%) of samples correctly classified, and percentage correction for both clusters was higher than 75%. PPC values presented in Table 1 further demonstrated that the uncorrected classification only occurred at a low organic load of some produce. Therefore, equations to predict chlorine demand for each cluster were developed as follows:

$$\text{If } PPC < 0.6, \text{ Chlorine demand} = 295.23 \text{ UV}_{254} + 6.97 \text{ with } R^2 = 0.89 \quad (3)$$

$$\text{If } PPC \geq 0.6, \text{ Chlorine demand} = 119.77 \text{ UV}_{254} + 2.41 \text{ with } R^2 = 0.97 \quad (4)$$

3.3 Model Validation

The first validation test was conducted using the same types of produce used for model development, but at different COD levels (Table 3). Specifically, iceberg lettuce, mushroom, and tomato from Cluster 1 and strawberry, spinach, and romaine lettuce from Cluster 2 were used. Each fresh produce wash water was diluted to two COD levels that were different from those in the model development. For example, iceberg lettuce wash water was diluted to COD at 50, 400 and 750 mg L⁻¹ for model development, while the COD levels for validation were 200 and 600 mg L⁻¹.

Measured UV₂₅₄, total protein, total phenolics and ΔE are presented in Table 3. Except the tomato wash water at COD 200 mg L⁻¹, all the tested samples can be correctly distributed to each cluster based on their PPC value. Only 3 out of 12 samples had a predicted residual larger than

10 mg L⁻¹; iceberg lettuce at COD 600 mg L⁻¹ and mushroom and spinach at COD 200 mg L⁻¹.

The absolute value of percentage error of prediction for produce in Cluster 2 was usually smaller than that in Cluster 1, indicating that prediction equations had better predictive performance for Cluster 2. Besides the difference between clusters, there is no clear trend or difference due to the types of fresh produce or organic loads.

The predictive performance of equations was also validated using fresh produce which differed from those used in the model development (Table 4). Based on the calculated PPC value, apple and romaine hearts belonged to Cluster 1, and commercial mixed leafy salad belonged to Cluster 2. Predicted chlorine demand for these three produce wash waters was calculated based on the respective equations for their clusters. It is observed that all the samples had predicted residuals lower than 15 mg L⁻¹. Apple wash water at COD 200 mg L⁻¹ had the highest prediction percentage error (96.49%), however, the predicted residual was only 4.94 mg L⁻¹. Moreover, the RMSPE of two validation experiments was 11.3 and 8.16, respectively, which was close to the MSE for model development (8.23). Validation results indicate that predictive equations based on PPC and UV254 can predict chlorine demand of various fresh produce wash waters with different organic loads.

However, the reader should be cautioned about the limitation of current equations. The cluster selection and chlorine demand prediction equations were developed empirically based on tested samples. Therefore, it is possible that the current method to define the cluster of fresh produce may not be valid or consistent for other types of fresh produce not tested in the current study.

4. Conclusions

259 In conclusion, this study shows that UV254 had the highest correlation coefficient with
260 chlorine demand among tested produce wash water quality parameters. Fresh produce was
261 divided into two clusters based on their PPC values. Empirically developed equations based on
262 UV254 had relatively good prediction accuracy on chlorine demand of various fresh produce
263 wash waters. The results from this study provide a potential way for industry to predict
264 appropriate amount of chlorine-based sanitizers for each type of produce they process. This will
265 ensure sufficient amount of chlorine-based sanitizers are present during fresh and fresh-cut
266 produce postharvest washing process to maintain safety and to avoid adding excess amount of
267 sanitizers that leads to formation of disinfection by-products.

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273 **References**

- 274 Artes, F., Gomez, P., Aguayo, E., Escalona, V., & Artes-Hernandez, F. (2009). Sustainable
275 sanitation techniques for keeping quality and safety of fresh-cut plant commodities. *Postharvest*
276 *Biology and Technology*, 51(3), 287-296.
- 277 Barrera, M. J., Blenkinsop, R., & Warriner, K. (2012). The effect of different processing
278 parameters on the efficacy of commercial post-harvest washing of minimally processed spinach
279 and shredded lettuce. *Food Control*, 25(2), 745-751.
- 280 Beuchat, L. R., Adler, B. B., & Lang, M. M. (2004). Efficacy of chlorine and a peroxyacetic acid
281 sanitizer in killing *Listeria monocytogenes* on iceberg and Romaine lettuce using simulated
282 commercial processing conditions. *Journal of Food Protection*, 67(6), 1238-1242.
- 283 Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram
284 quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, 72,
285 248-254.
- 286 CSPI (Center for Science in the Public Interest) (2009). Outbreak alert. Analyzing foodborne
287 outbreaks 1998 to 2007. <https://cspinet.org/new/pdf/outbreakalertreport09.pdf>. Accessed 23 July
288 2015.
- 289 Deborde, M., & von Gunten, U. (2008). Reactions of chlorine with inorganic and organic
290 compounds during water treatment - Kinetics and mechanisms: A critical review. *Water*
291 *Research*, 42(1-2), 13-51.

292 Fitzgerald, F., Chow, C. W. K., & Holmes M. (2006). Disinfectant demand prediction using
 293 surrogate parameters – a tool to improve disinfection control. *Journal of Water Supply: Research*
 294 *and Technology*, 55(6), 391-400.

295 Gil, M. I., Gomez-Lopez, V. M., Hung, Y.-C., & Allende, A. (2015). Potential of Electrolyzed
 296 Water as an Alternative Disinfectant Agent in the Fresh-Cut Industry. *Food and Bioprocess*
 297 *Technology*, 8(6), 1336-1348.

298 Gil, M. I., Selma, M. V., Lopez-Galvez, F., & Allende, A. (2009). Fresh-cut product sanitation
 299 and wash water disinfection: Problems and solutions. *International Journal of Food*
 300 *Microbiology*, 134(1-2), 37-45.

301 Hach Method 8000. (2014). Oxygen Demand, Chemical-Reactor Digestion Method 8000.

302 Hach Method 8237. (2013). Turbidity, Absorptometric Method 8237.

303 Lehto, M., Sipila, I., Alakukku, L., & Kymalainen, H.-R. (2014). Water consumption and
 304 wastewaters in fresh-cut vegetable production. *Agricultural and Food Science*, 23(4), 246-256.

305 Lu, Y., Joerger, R., & Wu, C. (2014). Similar Reduction of Salmonella enterica Typhimurium on
 306 Grape Tomatoes and Its Cross-Contamination in Wash Water by Washing with Natural
 307 Antimicrobials as Compared with Chlorine Treatment. *Food and Bioprocess Technology*, 7(3),
 308 661-670.

309 Luo, Y., Nou, X., Yang, Y., Alegre, I., Turner, E., Feng, H., Abadias, M., & Conway, W. (2011).
 310 Determination of Free Chlorine Concentrations Needed To Prevent Escherichia coli O157:H7
 311 Cross-Contamination during Fresh-Cut Produce Wash. *Journal of Food Protection*, 74(3), 352-
 312 358.

313 Olaimat, A. N., & Holley, R. A. (2012). Factors influencing the microbial safety of fresh produce:
 314 A review. *Food Microbiology*, 32(1), 1-19.

315 Pirovani, M. E., Guemes, D. R., & Piagnetini, A. M. (2001). Predictive models for available
 316 chlorine depletion and total microbial count reduction during washing of fresh-cut spinach.
 317 *Journal of Food Science*, 66(6), 860-864.

318 Shen, C., Luo, Y., Nou, X., Wang, Q., & Millner, P. (2013). Dynamic Effects of Free Chlorine
 319 Concentration, Organic Load, and Exposure Time on the Inactivation of Salmonella, Escherichia
 320 coli O157:H7, and Non-O157 Shiga Toxin-Producing E. coli. *Journal of Food Protection*, 76(3),
 321 386-393.

322 Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-
 323 Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, 16(3), 144-158.

324 Sivapalasingam, S., Friedman, C. R., Cohen, L., & Tauxe, R. V. (2004). Fresh produce: A
 325 growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997.
 326 *Journal of Food Protection*, 67(10), 2342-2353.

327 Van Haute, S., Samper, I., Holvoet, K., & Uyttendaele, M. (2013). Physicochemical Quality and
 328 Chemical Safety of Chlorine as a Reconditioning Agent and Wash Water Disinfectant for Fresh-
 329 Cut Lettuce Washing. *Applied and Environmental Microbiology*, 79(9), 2850-2861.

330 Waters, B. W., & Hung, Y.-C. (2014). The effect of organic loads on stability of various
 331 chlorine-based sanitisers. *International Journal of Food Science and Technology*, 49(3), 867-875.

- 332 Zhou, B., Luo, Y., Nou, X., & Millner, P. (2014). Development of an Algorithm for Feed-
333 Forward Chlorine Dosing of Lettuce Wash Operations and Correlation of Chlorine Profile with
334 Escherichia coli O157:H7 Inactivation. *Journal of Food Protection*, 77(4), 558-566.

335 Table 1. Quality parameters of simulated fresh produce wash water at different organic loads ^a

Fresh produce	Organic load	COD (mg L ⁻¹)	Chlorine demand (mg L ⁻¹)	pH	ORP (mV)	Protein (mg L ⁻¹)	Phenolics (mg L ⁻¹)	UV254	Turbidity (FAU)	ΔE	PPC
Iceberg lettuce	Low	52.1	6.69	3.68	586.63	2.31	0.78	0.005	5.9	0.23	0.08
	Mediu	403.0	46.19	4.69	531.86	10.90	2.35	0.084	25.4	1.22	0.26
	High	751.2	82.19	5.07	516.85	19.74	3.87	0.215	43.8	2.29	0.45
Mushroom	Low	65.1	13.13	4.87	489.68	4.39	0.85	0.041	9.6	0.34	0.11
	Mediu	147.3	39.31	5.20	471.29	9.71	1.98	0.144	16.1	0.64	0.13
	High	227.3	66.50	5.32	454.25	13.57	3.03	0.227	23.9	1.22	0.27
Grape	Low	51.5	2.94	3.84	533.19	1.28	0.36	-0.003	0.6	0.38	0.39
	Mediu	413.4	7.94	3.84	528.19	1.40	0.93	0.004	1.6	0.22	0.16
	High	725.0	12.63	3.77	527.88	2.08	1.37	0.012	3.1	0.15	0.11
Celery	Low	61.5	3.44	4.20	421.78	0.29	0.41	0.001	4.1	0.20	0.55
	Mediu	394.4	15.56	5.01	364.13	5.23	1.44	0.049	20.0	0.61	0.16
	High	728.8	25.31	5.38	357.95	8.77	2.30	0.096	40.3	1.24	0.32
Cantaloupe	Low	48.0	4.44	4.22	542.61	0.57	0.93	0.002	0.0	0.16	0.71
	Mediu	382.0	29.38	5.61	462.50	5.91	1.69	0.048	1.4	0.34	0.10
	High	727.3	55.19	6.29	428.75	10.23	2.64	0.095	6.4	0.64	0.16
Broccoli	Low	61.3	13.88	4.13	452.35	2.13	1.56	0.027	12.9	0.33	0.30
	Mediu	156.6	40.19	4.57	420.85	11.93	3.30	0.095	34.3	0.67	0.18
	High	255.5	66.81	5.66	363.48	23.72	5.34	0.221	39.5	1.00	0.23
Tomato	Low	70.1	9.88	3.97	452.68	0.16	0.31	0.020	0.4	0.32	0.68
	Mediu	412.3	57.38	4.31	383.51	1.50	1.83	0.169	2.0	0.27	0.53
	High	722.8	94.81	4.46	358.14	2.64	3.18	0.303	3.4	0.25	0.40

Romaine lettuce	Low	56.6	6.25	5.34	518.29	7.12	1.10	0.034	12.3	1.25	0.19
	Mediu	401.4	42.06	6.20	460.20	26.79	5.88	0.330	62.9	9.80	2.18
	High	737.8	74.75	6.29	470.90	41.70	10.53	0.617	113.8	16.23	4.15
Spinach	Low	58.1	13.50	5.06	410.48	13.39	2.59	0.077	31.6	3.77	0.73
	Mediu	212.4	52.81	6.15	342.63	31.81	8.92	0.350	94.0	13.63	3.82
	High	412.4	89.00	6.41	314.74	41.48	17.25	0.698	187.6	24.42	10.15
Strawberry	Low	61.6	4.56	4.06	452.88	2.08	1.20	0.030	3.0	0.35	0.20
	Mediu	416.6	28.75	3.89	384.58	4.06	6.88	0.260	7.0	0.83	1.36
	High	764.8	55.31	3.87	363.35	5.39	12.59	0.488	11.3	1.00	2.29

336 ^a Data are presented as a mean of two samples.

337 ^b PPC = Total phenolics/(Total protein/ Δ E).

338 Table 2. Pearson correlation of chlorine demand and water quality parameters

	Chlorine demand (mg L ⁻¹)	pH	ORP (mV)	Protein (mg L ⁻¹)	Phenolics (mg L ⁻¹)	UV254	COD (mg L ⁻¹)	Turbidity (FAU)	ΔE
Chlorine demand	1.00	0.52	-0.43	0.64	0.65	0.77	0.59	0.55	0.48
pH		1.00	-0.34	0.78	0.46	0.52	0.23	0.68	0.64
ORP			1.00	-0.31	-0.49	-0.47	-0.25	-0.35	-0.31
Protein				1.00	0.76	0.79	0.26	0.92	0.88
Phenolics					1.00	0.94	0.40	0.81	0.81
UV254						1.00	0.48	0.79	0.80
COD							1.00	0.21	0.17
Turbidity								1.00	0.96
ΔE									1.00

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340 Table 3. Prediction of chlorine demand of produce wash water used for model development but at different COD

Fresh produce (Cluster #)	Targeted COD (mg L ⁻¹)	UV254	Protein (mg L ⁻¹)	Phenolics (mg L ⁻¹)	ΔE	PPC	Observed chlorine demand (mg L ⁻¹)	Predicted chlorine demand (mg L ⁻¹)	Predicted residual ^a (mg L ⁻¹)	Prediction percentage error ^b
Iceberg lettuce (1)	200	0.047	5.32	1.20	0.57	0.13	26.13	20.70	-5.43	-20.77
	600	0.142	13.68	2.57	1.20	0.23	71.88	48.97	-22.91	-31.87
Mushroom (1)	100	0.080	5.92	1.44	0.51	0.12	22.75	30.66	7.91	34.78
	200	0.192	11.84	2.37	0.80	0.16	42.75	63.65	20.90	48.90
Tomato (1)	200	0.101	0.36	1.29	0.20	0.71	28.00	36.86	8.86	31.65
	600	0.290	3.18	2.91	0.31	0.28	83.38	92.51	9.14	10.96
Spinach (2)	200	0.251	33.11	7.62	12.60	2.90	46.50	32.50	-14.00	-30.10
	600	0.743	52.39	22.18	30.14	12.76	92.88	91.40	-1.47	-1.59
Romaine lettuce (2)	200	0.137	18.82	2.86	4.69	0.71	17.13	18.76	1.63	9.54
	600	0.424	29.64	7.52	13.12	3.33	48.25	53.13	4.88	10.12
Strawberry (2)	200	0.097	0.17	2.91	0.39	6.73	16.25	14.03	-2.22	-13.67
	600	0.312	2.93	8.35	0.55	1.57	49.13	39.81	-9.32	-18.96

341 ^a Predicted residual = Predicted chlorine demand – Observed chlorine demand.

342 ^b Prediction percentage error = (Prediction error)/(Observed chlorine demand) × 100.

343 Table 4. Prediction of chlorine demand of produce wash water use different produce from model development

Fresh produce (Cluster #)	Targeted COD (mg L ⁻¹)	UV254	Protein (mg L ⁻¹)	Phenolics (mg L ⁻¹)	ΔE	PPC	Observed chlorine demand (mg L ⁻¹)	Predicted chlorine demand (mg L ⁻¹)	Predicted residual (mg L ⁻¹)	Prediction percentage error
Apple (1)	200	0.011	1.14	1.29	0.17	0.20	5.13	10.07	4.94	96.49
	600	0.039	3.18	3.01	0.59	0.56	13.50	18.34	4.84	35.82
Romaine hearts (1)	200	0.089	7.58	1.64	0.50	0.11	24.13	33.10	8.97	37.19
	600	0.256	19.24	3.65	1.47	0.28	75.75	82.40	6.65	8.78
Mixed leafy (2)	100	0.170	22.32	4.14	5.72	1.06	25.13	22.71	-2.41	-9.61
	300	0.495	39.58	11.25	15.51	4.40	76.50	61.64	-14.86	-19.43

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Figure 1. Scatter plot of chlorine demand of produce wash water at different UV254.

Figure 2. Percentage of correct classification of produce wash water to two clusters based on different threshold PPC value.