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Can UV absorbance rapidly estimate the chlorine demand in wash water during fresh-cut produce washing processes?

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ABSTRACT

Free chlorine is used in industrial fresh-cut produce washing to avoid cross-contamination from pathogenic and spoilage microorganisms, although chlorine dosing typically depends on feedback control. Control of free chlorine levels in fresh-cut produce wash water could be improved if chlorine demand (CLD) could be determined real-time, during processing. Previous research has shown that the CLD of non-chlorinated fresh produce wash water (CLD_{max}) correlates with UV absorbance (UVA) at 254 nm (UVA254). The goal of this study was to estimate CLD for produce wash conditions that are in-progress, i.e., when the chlorine concentration in water partially meets the CLD, as is the case during industrial, continuous produce washing. This was done for cabbage, carrot, green leaf lettuce and onion. UVA changed with both CLD_{max} and remaining CLD. Two wavelengths were necessary to predict the CLD:UVA_{min}, which changed minimally due to chlorination and had maximum correlation with CLD_{max} and UVA_{max}. The CLD_{max} and UVA_{max} changed maximally with chlorination and had maximum correlation with the fraction of the remaining CLD. Results showed that UVA_{min} and UVA_{max} were between 240–290 nm, and the exact wavelength depended on the vegetable. However, free chlorine itself influences UVA, and at a residual above 25 mg/L the chlorine interfered with the estimation of CLD. A case study on green leaf lettuce showed that CLD can be predicted by a model of the form $f(UVA_{min}) \times g(UVA_{max}/UVA_{min})$. Using external validation data, optimal predictability of the model was obtained when both f and g were expressed as quadratic equations (SD/RMSE = 3.55; R^2 = 0.93). The described UVA method for predicting CLD shows promise for online application. Further studies should incorporate the possible variability in crop composition as well as other possible interferences with the UVA signal.

1. Introduction

Washing of fresh produce after cutting is a standard process that typically reduces microbial load 1–2 logs on the produce, but this level of reduction is insufficient to guarantee food safety (López-Gálvez et al., 2010). Reliable, cost-effective microbial inactivation technologies that preserve the desired quality of the fresh commodities are not yet available. However, addition of sanitizer to wash water, as a processing aid, may prevent microbial cross-contamination during washing (Castro-Ibáñez et al., 2016; Gil et al., 2009; Van Haute et al., 2015; Gombas et al., 2017).

Chlorine, the most commonly used and cost-effective water disinfectant, rapidly inactivates vegetative bacteria in water (Van Haute et al., 2013). However, reaction of organic compounds, that are inherently present in recirculating wash water at fresh-cut produce facilities, with chlorine reduces the efficacy of chlorine sanitizers substantially. Free chlorine ($CL_{residual}$) consumed or changed to less active forms (e.g., organic chloramines) through reaction with these organic compounds is defined as the chlorine demand (CLD). During a processing run, the majority of the water is recirculated, which leads to an ever-increasing organic content (soil, debris, and exudates from cut tissues) in the water over time, despite the practice of replacing a relatively small volume of recirculated water with clean water.

Fresh-cut processors currently use frequent chlorine dosing in an attempt to maintain the desired $CL_{residual}$ in the wash water, since the continuous influx of organics adds CLD. Processors often utilize a

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feedback control system that automatically adds chlorine to the water when the measured free chlorine level drops below the set-point. However, previous work (Luo et al., 2018) has demonstrated the difficulty of maintaining a consistent free chlorine level using this type of feedback system, especially during dynamic, commercial fresh-cut produce processing where high amounts of produce exudate enter the wash water. Large variability in $CL_{residual}$ during washing is sub-optimal and subject to problems that may result from both chlorine over- and under-dosing. Chlorine over-dosing (in combination with a pH below 4.5) could compromise worker safety by off-gas production and potentially lead to an excessive production of potentially carcinogenic disinfection by-products in the wash water. Conversely, chlorine under-dosing could lead to increased food safety risks due to the increased potential of pathogen cross-contamination via the wash water (Nou et al., 2010; Luo et al., 2011; Van Haute et al., 2013).

Free chlorine levels in fresh-cut produce wash water could be better controlled if CLD could be determined real-time, during processing. Previous work investigated the potential for predicting CLD using various parameters, including oxidation reduction potential, protein content, phenolic content, pH, UVA at 254 nm (UVA_{254}), chemical oxygen demand (COD), and color change. Among all previously mentioned parameters, UVA_{254} showed promise for predicting CLD, as it was highly correlated with CLD (Chen & Hung, 2016; Chen & Hung, 2017). However, that research was conducted using static conditions (i.e., estimation of the CLD was solely based on a mixture of non-chlorinated organic compounds in water) with simulated wash water.

Currently, the majority of commercial fresh-cut washing processes involve continuous addition of produce and chlorine in the washing tank and recirculation of the chlorinated wash water, which also contains non-chlorinated organic molecules liberated from cut produce tissues. The organic molecules that have already reacted with chlorine would not contribute to the CLD of the wash water. Conversely, non-chlorinated organic molecules, i.e., those that have not yet reacted with chlorine, potentially would still contribute to the CLD of the wash water. Therefore, a method for estimating CLD will only work in practice when there is a distinction between chlorinated organics that have no further contribution to CLD and non-chlorinated organics that still contribute to the CLD. Research reports now show that based on non-chlorinated organics, estimation of CLD can be made accurately (Chen & Hung, 2016, 2017), but the influence of chlorinated organics on CLD has not been investigated yet.

The goal of this research was to evaluate the use of UV absorbance to estimate CLD by using wash water as it occurs during fresh-cut produce washing, i.e., partially chlorinated wash water. The objectives were to i) determine the relationship between CLD and UVA for fresh-cut produce wash waters obtained from a range of vegetables with diverse chemical oxygen demand, and chlorine demand, as represented by green leaf lettuce, cabbage, carrots and onions, and ii) develop and validate a model to predict CLD using UVA for lettuce wash water from a dynamic washing process.

2. Materials and methods

2.1. Production of fresh-cut produce wash water

Cabbage (*Brassica oleracea* L. var. *capitata*), carrots (*Daucus carota* L. subsp. *sativus*), onions (*Allium cepa* L.), and green leaf lettuce (*Lactuca sativa* L.; Lafta et al., 2017) were purchased from a local wholesale market in Jessup, MD, USA, and stored at 4 °C for 24 h before processing. Prior to dicing, the heads of cabbage were manually de-cored and cut in half, and root hairs of carrots were manually removed. Onions were de-cored in the packing house and were diced without additional preparation. Lettuce was prepared by trimming the leaf edges and removing the stems (Luo, 2007).

The trimming, cutting, and washing procedures followed typical practices of commercial processors. The organic load in terms of COD and turbidity were within the range reported during commercial operations (Luo et al., 2018). Cabbage, carrots, and onions were diced into 0.32 cm cubes using a commercial vegetable cutter (Urschel Sprint2 Dicer, Chesterton, IN) by manually adding 10 kg of each vegetable into the cutter within 20 ± 2 s. The diced vegetables were transferred via a conveyor belt (Coastal Manufacturing, Watsonville, CA) to a washing basin containing 20 L of tap water. The water inside the washing basin was continuously, manually agitated as cut produce was added. The produce and water was continuously stirred for an additional minute after the last piece of produce dropped into the washing basin from the conveyor belt (total washing time of 2 min). Afterwards, the vegetable pieces were removed from the water using a sieve (1 × 1 mm mesh opening) and the wash water was collected.

Lettuce was shredded into 0.32 cm width pieces at a rate of 1 kg min^{-1} using a commercial vegetable cutter (Nichimo Seven Chefs ECD-302, Tokyo, Japan). Five 1 kg-batches (total 5 kg) of lettuce were shredded and washed consecutively in 10 L of tap water with continuous agitation. After each batch was washed for 2 min, lettuce was removed from the water tank using a sieve (1 × 1 mm) and the wash water was collected. A wash water sample was collected after washing each batch of lettuce, to obtain water samples with a range of organic load. Water samples had product-to-water ratios of 1:10, 2:10, 3:10, 4:10, and 5:10.

2.2. Measurement of chlorine demand

CLD is the amount of chlorine (mg L^{-1}) that can be consumed through reaction with substances (e.g., organic materials) present in the water. During fresh-cut produce washing, the CLD of the wash water varies with changing organic load and chlorination level. For each wash water sample, the CLD was described using two parameters: the maximum potential CLD before any chlorination has occurred (CLD_{max}) and the remaining CLD after some chlorination (chlorine dose > 0 mg L^{-1} and < CLD_{max}) has occurred (CLD):

$$CLD_{\max} = CL_{excessdose} - CL_{ressidual} \quad (1)$$

where $CL_{excess\ dose}$ is the excess chlorine dose, i.e., the excess amount of chlorine added to the water (mg L^{-1}); and $CL_{residual}$ is the chlorine residual, i.e., amount of free chlorine (mg L^{-1}) after 30 min contact time. During industrial fresh-cut produce washing, the produce is washed for only a short period (30 s to 2 min), but the wash water is continuously recycled, with some amount of clean, fresh water added to compensate for volume loss during processing. As such, the wash water is chlorinated during the production time, with the organics (exudates from last batch of produce added) being chlorinated for minutes up to hours (exudates from the first batch of produce added). Due to accumulation of organics, a high CLD can build up in the wash water over an 8-h shift period. Thus, in an experimental setting, a high added chlorine dose during a relatively long contact time is needed to measure the CLD so that it would represent prolonged exposure of the produce and ample reaction time between chlorine and organic exudates. Experimentally, the maximum potential CLD, CLD_{max}, for each water sample was determined by combining 10 mL of fresh-cut produce wash water and 10 mL of a concentrated chlorine stock solution (Clorox 8.25% sodium hypochlorite, Clorox Professional Products Company, CA), which was added to achieve 1000 mg L^{-1} free chlorine; the pH was adjusted to 6.5 using 0.05 mol L^{-1} phosphate buffer. The produce wash water sample and chlorine solution were thoroughly mixed at room temperature (22 °C) for 30 min.

The $CL_{residual}$ was measured using the N,N-diethyl-p-phenylenediamine (DPD) method (Eaton and Franson, 2005) with a Chlorine Photometer (HF Scientific Inc., FT. Myers, FL). Measuring $CL_{residual}$ in fresh-cut wash water needs to be done very rapidly due to the rapid chlorine decay. The DPD photometric method is very fast and therefore the chosen method in these types of waters. Some interference occurs from breakthrough of chloramines, but this is limited if the $CL_{residual}$ is high, as was the case in this study. CLD_{max} was calculated using Eq. (1).

The CLD was defined as:

$$CLD = CLD_{max} - (CL_{doses} - CL_{residual}) \tag{2}$$

where CL_{dose} is the chlorine dose, i.e., the amount of chlorine added to the water (mg L^{-1}). Experimentally, the remaining CLD for each water sample was determined using the same method as described above for CLD_{max}, (i.e., by combining 10 mL of fresh-cut produce wash water and 10 mL of a concentrated chlorine solution) except a less concentrated CL_{dose} (> 0 mg L^{-1} chlorine and < CLD_{max}) was added for 30 min, the concentration depended on the targeted remaining CLD after chlorination.

2.3. Measurement of physicochemical parameters

Three physicochemical parameters were quantified for the wash water samples: chemical oxygen demand (COD), turbidity, and UVA. All measurements were conducted at room temperature (22 °C). COD and turbidity of the wash water samples were measured before chlorination on undiluted samples. COD was measured according to the small-scale sealed-tube method (HR COD digestion vials, Hach, CO). Turbidity was measured with a turbidimeter (Orion AQ4500, Thermo Scientific, MA).

The UVA of the wash water samples was measured after dilution using equal volumes (10 mL each) of the wash water sample and buffered chlorine solution, as described above in Section 2.2. This dilution helped ensure accurate UVA readings for samples with high absorbance (> 2). UVA was measured from 200 to 400 nm using a scanning UV–vis spectrophotometer with 1 nm steps (DU 730, Beckman Coulter, NJ); blank measurement was done with phosphate buffer 0.05 mol L^{-1}; samples were measured after filtration through a 0.45 μm polyethersulfone filter (VWR, PA) in quartz cuvettes (ZCUV001X, Vernier, OR).

2.4. Correlation between UVA and chlorine demand of fresh-cut produce wash water at a constant CLD_{max}

Cabbage, carrot, lettuce, and onion wash water with product to water ratio of 10 kg in 20 L was produced (Section 2.1). The CLD_{max} of each of these water samples was determined (Section 2.2). Based on this, the wash water samples were exposed to a chlorine dose of 0 (non- chlorinated), 0.2 × CLD_{max}, 0.4 × CLD_{max}, 0.6 × CLD_{max}, 0.7 × CLD_{max}, and 0.8 × CLD_{max} for 30 min (Section 2.2). Initially, equally spaced chlorine dosages (interval of 0.2 × CLD_{max}) were chosen. However, because initial trials with a ratio of 0.8 × CLD_{max}, showed that at unwanted $CL_{residual}$ (with possible influence on UVA readings) remained in the wash water after 30 min, a chlorine dose equal to 0.8 × CLD_{max} or higher was not used, but was replaced with 0.7 x CLD_{max}. This could be explained by the much higher (excess) chlorine dose that was applied to estimate CLD_{max}, compared to the chlorine dose in subsequent trials. A very high chlorine dose has been shown to yield a correspondingly higher chlorine consumption during a fixed contact time (Chen & Hung, 2017).

The UV spectra were recorded (200–400 nm). The UVA at each wavelength was correlated (Pearson R^2) with the CLD in order to identify wavelengths at which minimum and maximum correlation occurred. To assess at which wavelength the minimum and maximum response occurred due to chlorination, the change in UVA (ΔUVA), was calculated at each wavelength:

$$\Delta UVA = |UVA_{CLD100\%} - UVA_{CLD30\%}| \tag{3}$$

UVA_{CLD} 100% = UVA of wash water when remaining CLD is 100% (i.e., non-chlorinated); UVA_{CLD} 30% = UVA of wash water when remaining CLD is 30% UVA at 30% remaining CLD (i.e., after a dose of 0.7 × CLD_{max}) was used because of the presence of undesired $CL_{residual}$ at chlorine dose of 0.8 × CLD_{max} or higher as described above.

These analyses were done with SPSS 22 (IBM, NY). Each trial was executed in triplicate.

For the four vegetables, the data from the above trial, along with additional data from chlorine doses higher than CLD_{max}, were also used to study the influence of free chlorine on UVA. This was performed by measuring the free chlorine concentration and UVA for each chlorine dose after 30 min of contact time. Each trial was conducted once as this was not the main focus of this research.

2.5. Correlation between UVA and chlorine demand of fresh-cut produce wash water at a variable CLD_{max}

For model development, lettuce wash water samples with product-to-water ratios of 2:10, 3:10, 4:10, and 5:10 were produced (Section 2.1) and CLD_{max} of each was determined (Section 2.2). Based on this, the wash water samples with varying CLD_{max} were exposed to a chlorine dose of 0 (non-chlorinated), 0.1 × CLD_{max}, 0.3 × CLD_{max}, 0.5 × CLD_{max}, or 0.7 × CLD_{max} for 30 min (Section 2.2). Statistical analysis was conducted as described in Section 2.4. Each trial was executed in triplicate.

2.6. Prediction of chlorine demand in lettuce wash water: a case study

A calibration with independent validation approach was used to develop a robust prediction model for CLD, i.e., a model for predicting CLD in multiple batches (validation data) that were not used to construct the model, and not solely for the single batch used to make the model (calibration data). This was done to evaluate the accuracy of the model's performance with variations among the batches studied. A separate model was constructed for each of the vegetables. For lettuce, the UVA data (at different CLD_{max} values, Section 2.5) were used as calibration data (Table 1, calibration data). These data were used to construct a prediction model, i.e., to predict the CLD based on UVA data. To validate the model with independent data, another set of wash water samples was produced from a distinctly separate batch of lettuce, as described in Section 2.1 (Table 1, validation data). Calculations and predictive modeling were done with SPSS and Excel 2016. Linear, quadratic, and cubic functions were applied to estimate CLD. Pearson squared correlation coefficients (R^2) were used to assess the models' goodness-of-fit by comparing the predicted and measured CLD values. The ratio of prediction-to-deviation (RPD) was also used to assess how well each model fit the data; RPD is the ratio of the standard deviation of the measured CLD values to the root mean square error of the predicted CLD values. An increasing RPD indicated an increase in the prediction accuracy compared to the use of the mean CLD value to predict all CLD values. A model with RPD > 3 was considered to have a good prediction potential; RPD ranging from 1.5 to 3 was considered to have a moderate prediction potential, and an RPD < 1.5 indicated an insufficient prediction potential (Karoui et al., 2007).

Table 1
Turbidity, COD and CLD_{max} of the lettuce wash water used for calibration and validation of the CLD prediction model (n = 3).

product-to-H2O ratio (kg 10 L^{-1})	Turbidity (NTU)	COD (mg L^{-1})	CLD_{max} (mg L^{-1})
SET 1: calibration (1/24/2017)			
2	200 ± 15	998 ± 9	183 ± 24
3	359 ± 57	1588 ± 13	313 ± 19
4	518 ± 25	2100 ± 13	360 ± 5
5	645 ± 11	2718 ± 38	473 ± 12
SET 2: validation (2/2/2017)			
2	133 ± 33	848 ± 31	162 ± 13
3	318 ± 20	1275 ± 31	303 ± 15
4	441 ± 38	1911 ± 13	370 ± 9
SET 3 validation (12/21/2016)			
5	217 ± 9	1982 ± 56	486 ± 15

3. Results

3.1. Chlorine demand as a function of UVA for fresh-cut produce wash water at a constant CLD_{max}

For a given vegetable, the UVA increased when CLD_{max} increased, as shown for carrot wash water (Fig. 1a), and this scaling of UVA with CLD occurred over the full range 200 to 400 nm. When comparing crops (Fig. 1b), it was observed that the relationship between UVA and CLD_{max} is not universal for wash water from all vegetables. When using the UVA at 254 nm as an example, the ratios of CLD_{max} to UVA at 254 nm for the vegetables become: 1014 mg/L for cabbage, 397 mg/L for carrot, 348 mg/L for lettuce and 1476 mg/L for onion. Clearly, the ratio of CLD_{max} and UVA of the vegetable wash water was dependent on the vegetable. For onion and cabbage, UVA for a given CLD_{max} was lower than for lettuce and carrot wash water. Chlorination also changed the UVA as shown for carrot (Fig. 1c) in proportion to the added chlorine dose (to achieve the targeted remaining CLD). Only part of the UVA disappeared when the CLD was consumed, i.e., there was some remaining amount of UVA even when the CLD had been met (Fig. 1c).

The influence of chlorination on the UVA was studied for the four vegetable wash waters (Fig. 2). For each vegetable, wavelengths with minimum changes in UVA (minimum ΔUVA) and minimum correlation with CLD were identified (UVA_{min}). Wavelengths with maximum ΔUVA and maximum correlation with remaining CLD were also identified for each vegetable (UVA_{max}). The results showed that at certain UV wavelengths, CLD and UVA were correlated strongly at a fixed CLD_{max} (a water sample with a fixed organic load).

3.2. Chlorine demand as a function of UVA for fresh-cut produce wash water at variable CLD_{max}

For lettuce, the same experiment was repeated for four different CLD_{max} values with corresponding physicochemical parameters of the wash water of the lettuce samples (Table 1, calibration set). As the amount of washed lettuce increased, the particulate and dissolved organic matter that was transferred to the wash water also increased, which resulted in an increase in the CLD_{max}. For all CLD_{max} levels tested for the lettuce wash water, the minimum correlation between CLD and UVA was found at about 252 nm (Fig. 3a). There was some variation in the wavelength of UVA_{max} for the different CLD_{max} values (Fig. 3a). By taking the mean R^2 at different CLD_{max} for wavelengths ranging from 200 to 400 nm, maximum correlation peaks were encountered for UVA_{max} at 238, 279 nm and 339 nm. Graphs of CLD as a function of UVA_{max} at 279 nm for the different CLD_{max} values, show that at a fixed CLD increased UVA corresponds to increased CLD_{max} (Fig. 3b). A similar trend occurred for UVA_{max} at 238 and 339 nm. The use of the absorbance at a single UV wavelength did not correlate well with CLD. This was expected because results showed that UVA_{max} was influenced by at least two variables, CLD and CLD_{max}, as illustrated in Fig. 1a and c respectively. Therefore, another approach was needed.

3.3. The use of UVA_{max} and UVA_{min} to predict chlorine demand

The UVA_{max} and UVA_{min} wavelengths were determined for wash water from the different vegetables (Fig. 2). Because UVA_{min} changes minimally with chlorination, it could potentially serve as an indicator of the organic load (or CLD_{max}) of fresh-cut vegetable wash water. Lettuce was used to study the correlation of CLD_{max} with UVA_{min}. When correlating the CLD_{max} with the UVA for all calibration samples, a peak was encountered at 252 nm (Fig. 4a). A similar high correlation was encountered at 252 nm (Fig. 4a) when the CLD_{max} was assessed using the validation data set (Table 1). Thus, UVA_{min} (Fig. 3a) could be used to predict the CLD_{max}. The high peak at 215 nm (Fig. 4a) was not considered because the absorbance of the wash water was too high to be usable in that range (Fig. 1b).

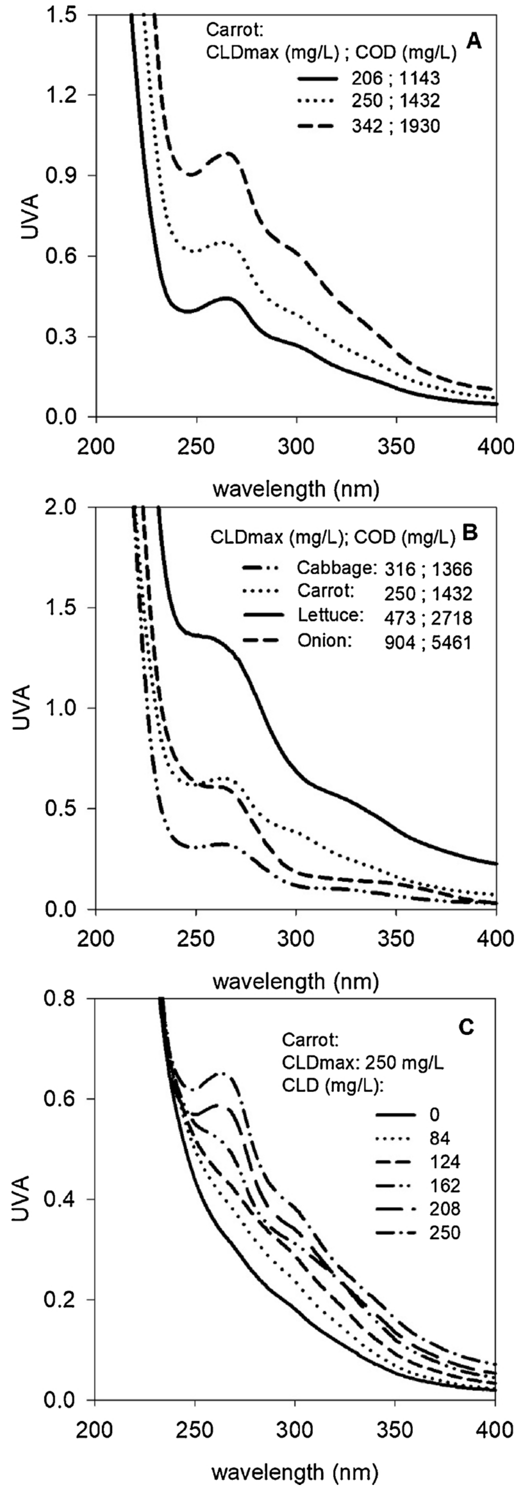

Fig. 1. UVA spectra of fresh-cut produce wash water. A) non-chlorinated carrot wash waters with varying CLD_{max} and COD (first number in Figure legend is CLD_{max}, the second COD); B) comparison of UVA spectra of non-chlorinated wash water from cabbage, carrot, lettuce and onion; C) comparison of UVA spectra from chlorinated carrot wash water (CLD_{max} = 250 ± 12 mg L^{-1}) with varying remaining CLD.

Because the UVA_{max} was influenced by the CLD_{max}, UVA_{max} as a sole parameter is insufficient to determine CLD. The ratio UVA_{max} /UVA_{min} will help in estimating the remaining fraction of the CLD (i.e., CLD_{ratio}), as such removing the influence of UVA_{max}. The ratio UVA_{max}/UVA_{min} will be independent of the CLD_{max} of the wash water, because UVA_{min} scales with CLD_{max}. For each vegetable, UVA_{max} /UVA_{min} was

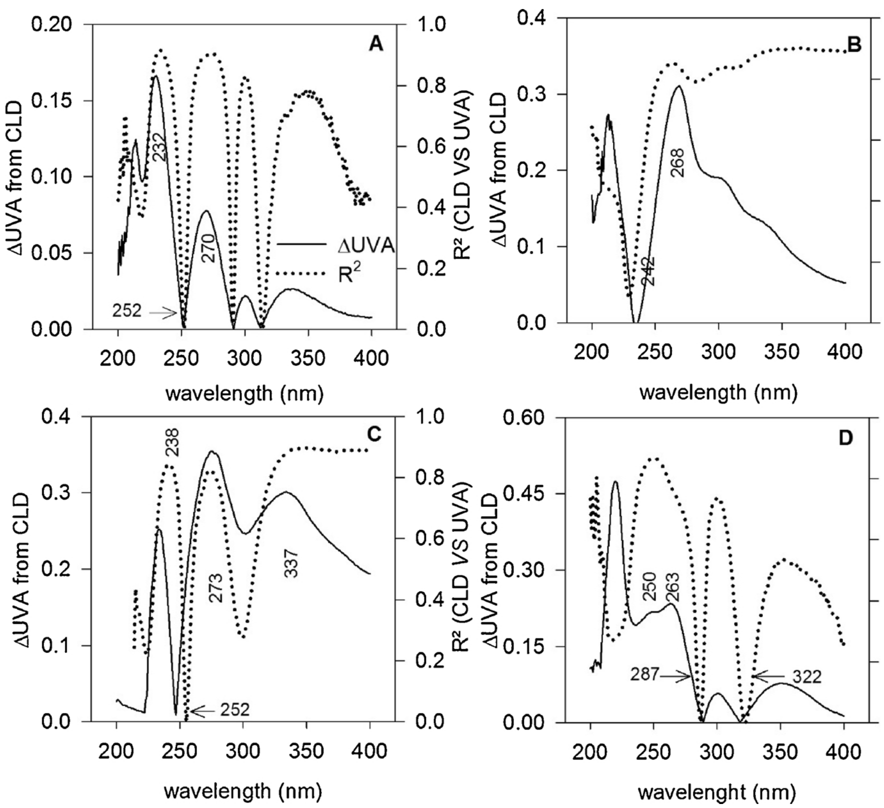

Fig. 2. The influence of chlorination on the UVA of wash water. A) cabbage (CLD_{max} = 316 ± 6 mg L^{-1}, COD = 1366 ± 16 mg L^{-1}, Turbidity = 78 ± 6 NTU); B) carrot (CLD_{max} = 250 ± 12 mg L^{-1}, COD = 1432 ± 12 mg L^{-1}, Turbidity = 55 ± 4 NTU); C) lettuce (CLD_{max} = 473 ± 12 mg L^{-1}, COD = 2718 ± 38 mg L^{-1}, Turbidity = 645 ± 11 NTU); D) onion (CLD_{max} = 904 ± 28 mg L^{-1}, COD = 5461 ± 360 mg L^{-1}, Turbidity = 45 ± 3 NTU). Values expressed as ΔUVA (200–400 nm), as well as correlation (R^2) between CLD and UVA.

correlated with the CLD_{ratio} (Table 2) at a constant CLD_{max}.

For cabbage, carrot and lettuce, a similar correlation profile was observed with the optimal prediction (highest RPD) of CLD_{ratio} using the UVA_{max} at 270, 268, and 273 nm, respectively, combined with the UVA_{min} at 252, 242, and 252 nm, respectively. For onion, the situation was different, with optimal prediction of CLD_{ratio} using the UVA_{max} at 250 nm and UVA_{min} at 287 nm. For cabbage (UVA_{max} at 232 nm) and lettuce (UVA_{max} at 238 nm), the negative coefficient indicates that the absorbance increased due to chlorination, in contrast with the other applied UVAv wavelengths (Table 2). These results showed that for each vegetable, there was a strong correlation between UVA_{max} /UVA_{min} and CLD_{ratio} at a constant CLD_{max}. For lettuce, this was further studied at different CLD_{max} levels. By using the CLD_{ratio}, which is a fraction, all the lettuce calibration data (Table 1, Fig. 3a) was studied together, irrespective of the CLD_{max}. The maximum correlation was found at 238, 279, and 339 nm (Fig. 4b). When plotting CLD_{ratio} as a function of UVA_{max} /UVA_{min} (i.e., UVA_{279}/UVA_{252}), a strong correlation was observed that was independent of CLD_{max} (Fig. 4c). For both UVA_{max} = UVA_{238} or UVA_{339} a similar strong correlation occurred, with a negative correlation in the case of UVA_{238}.

These results indicated that by splitting the CLD into a CLD_{max} and a CLD_{ratio}, a prediction of CLD could be performed using UVA_{min} and UVA_{max} :

$$CLD_{ratio} - \frac{CLD}{CLD_{max}} \tag{4}$$

$$CLD = CLD_{max} \times CLD_{ratio} \tag{5}$$

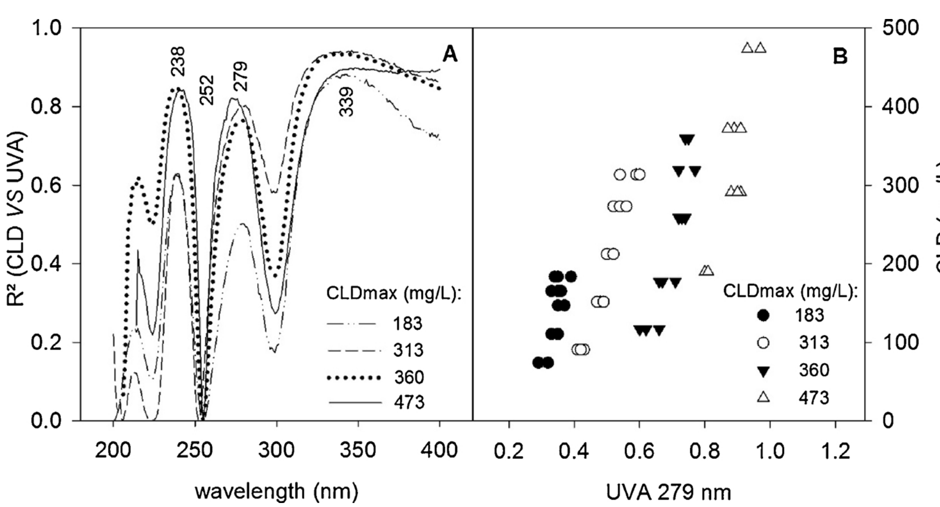

Fig. 3. The effect of CLD_{max} on the correlation between CLD and UVA in lettuce wash water. A) the correlation between CLD and UVA (200–400 nm) at different CLD_{max}; B) CLD as a function of UVA at 279 nm.

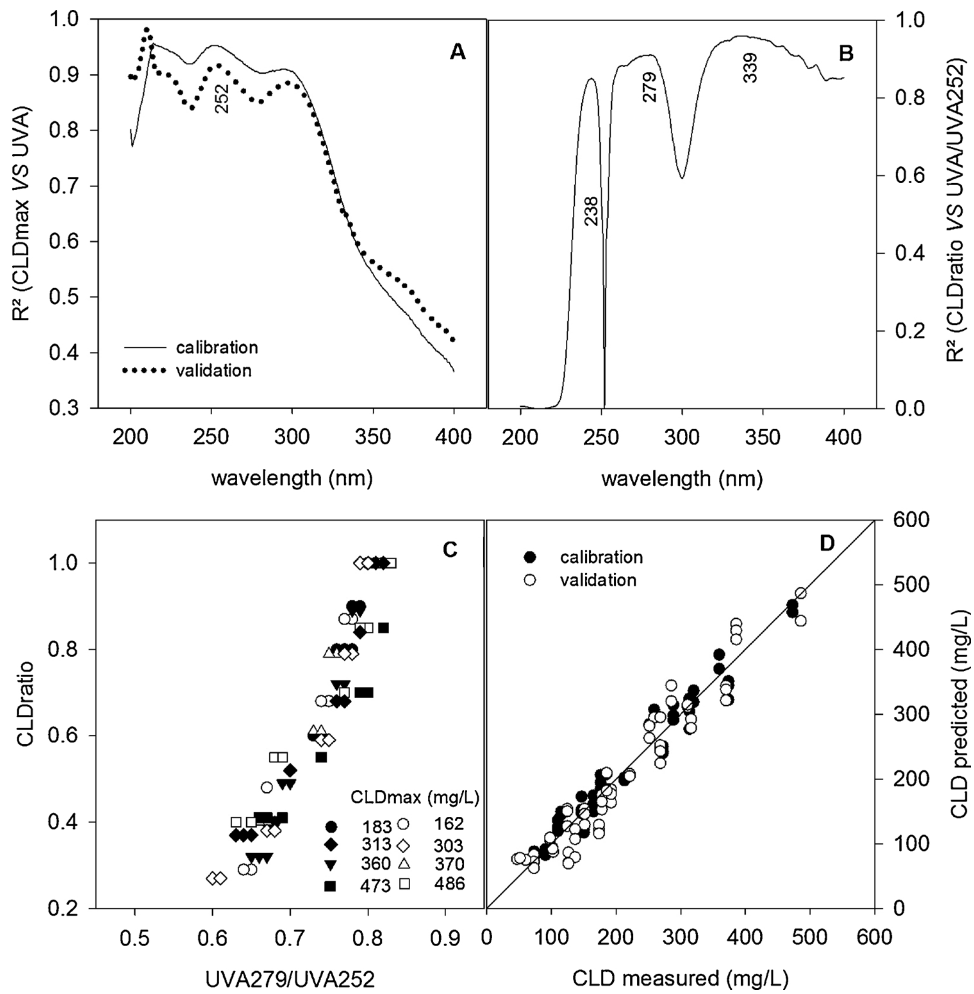

Fig. 4. Predictive modeling of CLD in lettuce wash water. A) correlation of CLD_{max} and UVA for all calibration and validation samples; B) correlation of CLD_{ratio} with the ratio UVA/UVA_{252} for all calibration samples; C) correlation of CLD_{ratio} with UVA_{279}/UVA_{252} (UVA_{max}/UVA_{min}) for the different CLD_{max}; D) the CLD values predicted with the best predictive model (2nd order, UVA_{max} = 279 nm, UVA_{min} = 252 nm) as a function of the measured CLD values, the line denotes the perfect fit line.

Table 2
Correlation of UVA_{max} /UVA_{min} with CLD_{ratio} for wash water from fresh-cut cabbage, carrot, lettuce, and onion.

	UVA_{max} (nm)	UVA_{min} (nm)	CLD_{ratio}[a]	R^2[b]	RPD[b]
cabbage	232	252	−0.7.UV232/UV252 + 2.0	0.85	2.68
	270	252	2.5.UV270/UV252 − 1.5	0.91	3.33
carrot	268	241	1.8.UV268/UV242 − 0.8	0.97	6.26
lettuce	238	252	−3.1.UV238/UV252 + 4.1	0.84	2.64
	273	252	2.3.UV270/UV252 − 1.3	0.98	7.94
	337	252	3.7.UV337/UV252 − 0.29	0.94	4.44
onion	250	287	1.5.UV250/UV287 − 2.1	0.94	4.15
	263	287	1.5.UV263/UV287 − 1.9	0.93	3.93
	250	322	0.6.UV250/UV322 − 1.5	0.75	2.06
	263	322	0.6.UV263/UV322 − 1.37	0.74	2.12

[a] $CLD_{ratio} = \frac{CLD}{CLD_{max}}$.

[b] R^2: Pearson's correlation coefficient; RPD: the ratio of prediction-to-deviation, i.e., the standard deviation of the measured CLD values to the root mean square error of the predicted CLD values.

$$CLD = f(UVA_{min}) \times g(UVA_{max}/UVA_{min}) \quad (6)$$

The use of UVA_{max} and UVA_{min} to predict the CLD was assessed for lettuce. The model was constructed with the use of the calibration data set and the prediction quality of the model was assessed with the validation data sets (Table 1). The absorption values at 279 nm and 339 nm were tested as options for the UVA_{max} of the model, as described in Eq. (5). The correlation peak at 238 nm was not considered because of the relatively low predictability of CLD_{ratio} (Table 2). The models are shown in Table 3. Regarding the efficacy of the calibration data, the prediction accuracy (RPD) increased with higher order terms ($x^3 > x^2 > x$) as expected, because the model was developed and based on the same data that were used for prediction. However, when applying the independent validation data set, it became clear that with a UVA_{max} of 279 nm, the validation data could be predicted more adequately than with a UVA_{max} of 339 nm (Table 3). Moreover, when using second order terms, the prediction of CLD in the validation set improved (RPD = 3.55). The model with second order terms predicted the measured CLD without systematic bias and the spread around the perfect fit line did not show a pattern (Fig. 4d). When third order terms were used, the RPD of validation dropped to 1.92, and showed a large difference with RPD of the calibration (5.21), which indicated overfitting, i.e., the model used information from the calibration set that was not universal for all validation data sets.

Table 3
Prediction quality of the models for CLD (product of CLD_{max} and CLD_{ratio}) in fresh-cut lettuce wash water based on different UVA_{max} values and the number of terms in the polynomial equations, with calibration data and validation data.

UVA_{max}	UVA_{min}	CLD_{max}				CLD_{ratio}				Calibration		Validation	
		X^3	X^2	X^a	intercept	X^3	X^2	X^a	intercept	R^{2b}	RP[b]	R^2	RPD
279	252			366	32			4.2	−2.4	0.95	4.34	0.89	2.69
279	252		−108	544	−33		5.2	−3.3	0.4	0.95	4.56	0.93	3.55
279	252	1310	−3342	3040	−628	−67	151	−110	26	0.96	5.21	0.85	1.92
340	252			366	16			3.8	−0.3	0.95	4.18	0.84	2.33
340	252		−108	544	−33		−1.5	4.5	−0.4	0.95	4.22	0.87	2.66
340	252	1310	−3342	3040	−628	−92	69	−13	1.0	0.96	4.87	0.79	1.53

[a] For CLD_{max}: X = UVA_{min}; for CLD_{ratio}: X = UVA_{max} /UVA_{min}.
[b] R^2: Pearson's correlation coefficient; RPD: the ratio of prediction-to-deviation.

3.4. To what extent does free chlorine interfere with UVA

$CL_{residual}$ absorbs in the UV wavelength range (Fig. 5). Due to the essential presence of $CL_{residual}$ during washing, its effect on the UVA needs to be insignificant for a successful prediction model based on UVA.

For the four studied vegetable wash waters, the effect of $CL_{residual}$ on UVA_{max} was significant when the $CL_{residual}$ reached a high enough concentration (Fig. 6). For cabbage, the influence on UVA_{max} was negligible up to 25 mg L^{-1} $CL_{residual}$, but was detrimental at 45 mg L^{-1} or more. For carrot, the influence was negligible up to at least 28 mg L^{-1} $CL_{residual}$, but very significant at 90 mg L^{-1}. For lettuce, there was no detrimental influence up to at least 35 mg L^{-1} of $CL_{residual}$, but very significant at 270 mg L^{-1}. For onion, it can only be said that 53 mg L^{-1} $CL_{residual}$ had a detrimental influence on the UVA_{max}. For cabbage, carrot and lettuce, the UVA_{max} was not influenced by a $CL_{residual}$ up to at least 25 mg L^{-1}. For onion, insufficient information was available to estimate an acceptable $CL_{residual}$.

4. Discussion

If UVA is to be used as an indicator of CLD during fresh-cut produce washing, the model should reflect the remaining CLD in partially chlorinated water, i.e. when there are both non-chlorinated and chlorinated materials (molecules altered by electrophilic substitution or oxidation by chlorine) (Deborde & von Gunten, 2008). This type of model is necessary because current fresh-cut produce washing practices involve dynamic, continuous processes, in which very large amounts of plant tissue and their organic exudates simultaneously enter the wash water tanks. The UVA of the fresh-cut wash water samples correlated with CLD_{max}. This relationship has been observed in previous studies, specifically for UVA_{254} (Chen & Hung, 2016, 2017). The relationship between UVA and CLD_{max} observed in this study was vegetable dependent. This was also indirectly observed in a previous study, as the antimicrobial efficacy of chlorine was negatively correlated with the UV_{254} when considering wash water from one leafy vegetable, but was not correlated when comparing wash water from several leafy vegetables (Van Haute et al., 2013). UVA also changed with chlorination; chlorination decreased the UVA at certain wavelengths, but the UVA did not drop to 0 when all the CLD had been met. UVA was impacted by both the CLD_{max} and remaining CLD. Water with a certain CLD had a higher UVA when the CLD_{max} was higher. Therefore, at least two wavelengths were required to assess CLD in chlorinated water. For the tested vegetable wash waters, UVA_{max} and UVA_{min} were determined and the ratio correlated with CLD_{ratio}, i.e., independent of the CLD_{max}. UVA_{max} was in the range 250–280 nm for each type of vegetable wash water. At higher and lower wavelengths, the prediction of CLD_{ratio} was less accurate. In the case-study with lettuce, the prediction of validation data using UVA_{279} was more accurate than when UVA_{339} was used. The better prediction quality of UVA_{279} illustrates the importance of validation and incorporating the potential influence of variability in crop composition. The chemical composition of a crop can vary with the degree of maturity, and environmental conditions during crop production (Lehto et al., 2014), such as variation in phenolic compounds in lettuce varieties among different harvest events (Nicolle et al., 2004). The constructed model for lettuce wash water in this study is likely not optimal for all processed lettuce crops of the green leaf lettuce variety. Ultimately, a robust model could be built by applying the methodology described in this study on a larger dataset, as such incorporating the possible variations in the processed crops due to chemical constitution and soil.

The reaction rate of $CL_{residual}$ with organics occurs insss the following order from high to low: reduced sulfur moieties > primary/secondary amines > phenols, tertiary amines > double bonds, other aromatics > carbonyls and amides (Deborde & von Gunten, 2008). In the studied food systems, amino acids (especially cysteine) and phenolic compounds would be significant contributors to CLD (Toivonen & Lu, 2013). Protein and phenolic compounds have been suggested as the groups of molecules with the highest impact on CLD in food related systems and the relationship to CLD_{max} has been indicated (Waters & Hung, 2014; Chen & Hung, 2016, 2017). The UVA in the range of wavelengths between about 240 and 290 nm constituted a peak in the UV spectra of the wash water of the four vegetables (Fig. 1a) and provided the spectral information used in this study. Relevant organic molecules do have a maximum absorbance peak in their UV spectrum in that range, including flavonoids (group of polyphenolic plant compounds) in the range 250–290 nm (Tsimogiannis et al., 2007), and the aromatic amino acids phenylalanine (255–260 nm), tryptophan, and tyrosine (270–300 nm) (Mach & Middaugh, 1994; Knapik et al., 2015).

The results in this study showed that $CL_{residual}$ up to 25 mg L^{-1} was

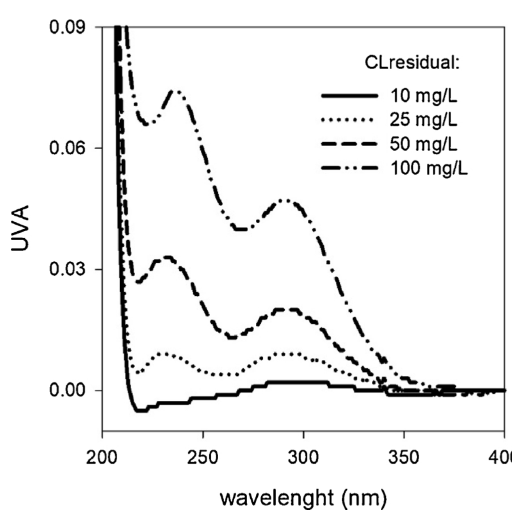

Fig. 5. UVA spectra of $CL_{residual}$ in phosphate buffer (pH 6.5).

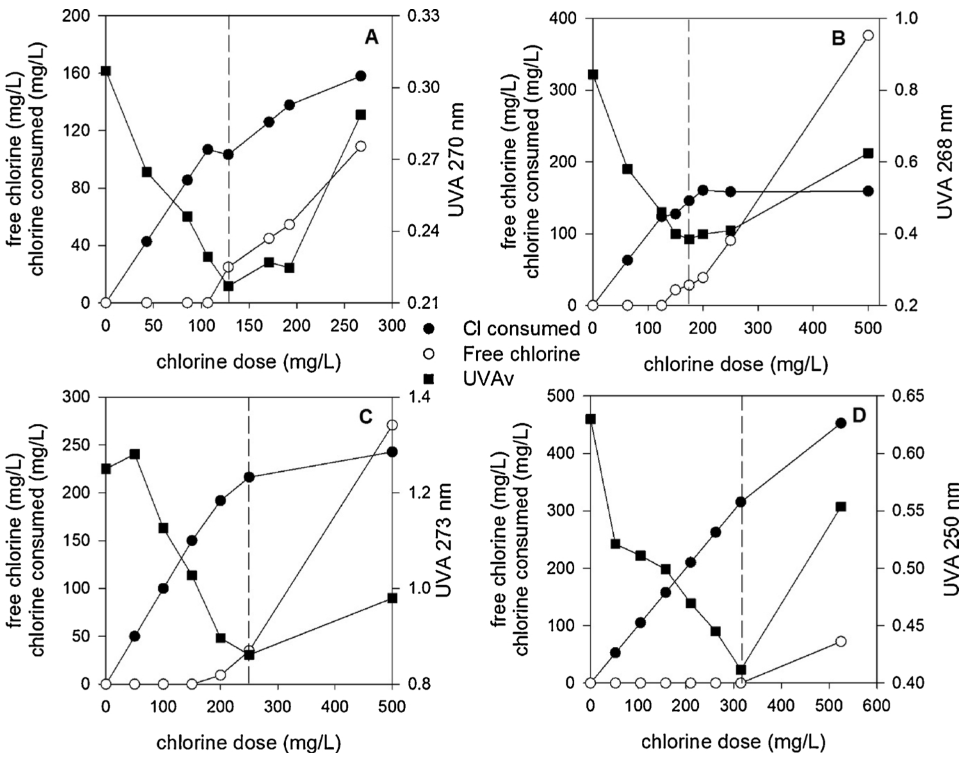

Fig. 6. The influence of chlorine dose on $CL_{residual}$, chlorine consumption, and UVA_{max} for wash water with constant CLD_{max}. A) cabbage (CLD_{max} = 158 ± 3 mg L^{-1}); B) carrot (CLD_{max} = 160 ± 8 mg L^{-1}); C) lettuce (CLD_{max} = 243 ± 7 mg L^{-1}); and D) onion (CLD_{max} = 452 ± 14 mg L^{-1}). The dashed lines indicate chlorine dose at which UVAv approximately stops decreasing.

acceptable in cabbage, carrot and lettuce wash water, whereas for onion wash water no acceptable $CL_{residual}$ could be determined based on the data generated. Target $CL_{residual}$ of 5 to 10 mg L^{-1} are common in fresh-cut produce washing operations, but sometimes higher target $CL_{residual}$ are applied, e.g. some companies in the USA use a target of 40 mg L^{-1} for washing cut cabbage, and even higher $CL_{residual}$ during tomato washing (Gereffi et al., 2015). The $CL_{residual}$ level at which the UVA method starts to suffer from interference in a specific fresh-cut produce wash water will help determine the feasibility of this method. The pH in fresh-cut produce washing with $CL_{residual}$ as a sanitizer is generally controlled at a pH in the range of 5.0 to 6.5 to optimize hypochlorite efficiency. Adding a phosphate buffer in order to force the pH to a desired value before measuring UVA, as was done in this study, seems a good strategy to exclude pH effects. An additional issue is the dosing of acidulants. In order to control the pH, an acidulant needs to be dosed to cope with the influence of the alkaline pH of sodium hypochlorite. If phosphoric acid is used, the influence of the acid on UVA would be low. In addition, T-128 (mainly composed of phosphoric acid and propylene glycol) (Lemons and Taylor Fresh Food Inc., 2009; Shen et al., 2012), which is the acidulant in SmartWash® systems that are used more and more frequently during industrial fresh-cut produce washing, has also very low influence on UV absorbance in the region 200 to 400 nm (Fig. S1). If other (organic) buffering agents are applied, this could potentially cause significant interference with UVA measurements. For example, citric acid has a CLD and reaction with chlorine produces trichloromethane which is a disadvantage (Fan & Sokorai, 2015), although citric acid does not absorb UVA in the range of interest between 240 and 300 nm. Lactic acid on the other hand, which reacts considerably slower with $CL_{residual}$, absorbs in the range 210 to 300 nm, would generally cause interference (Poerwono et al., 2001; Toivonen & Lu, 2013). In general, the influence of $CL_{residual}$ and added pH regulators on UVA_{max} and UVA_{min} should be insignificant in order to make the UVA method feasible.

5. Conclusion

Research on predicting CLD during fresh-cut produce washing based on UVA (exclusively UV_{254}) has provided evidence that CLD_{max} correlates with UVA_{254}. In this study, CLD was estimated using UVA when part of the CLD has already been met, as is the case in industrial, continuous fresh-cut produce washing operations. UVA was influenced by chlorination, but the absorbance did not decrease to zero when the CLD was met. UVA changed with both CLD_{max} and remaining CLD. At least two wavelengths were necessary to predict the CLD: UVA_{min}, which changed minimally due to chlorination and was strongly correlated with CLD_{max} and UVA_{max}, which changed maximally with chlorination. These parameters were determined for all four vegetables and there was a strong correlation between UVA_{max}/UVA_{min} and CLD_{ratio}. The $CL_{residual}$ absorbed in the relevant UVA range (240–300 nm) so there is a limit to how high the $CL_{residual}$ can be for the predictive method to be accurate; this issue requires further attention. The case study with wash water of lettuce showed that the methodology works, but some variability in the correlation of CLD with UVA among different crops of the same vegetable variety exists. A robust model could be developed in the future, that considers this variability via representative calibration and validation data sets (considering climate, stage of maturity, presence of soil etc.). Future research should also consider whether relevant acidulants interfere with the UVA method. Ultimately, it needs to be determined whether CLD estimation based on UVA actually contributes as a supporting measurement to the ORP/DPD/amperometric method that is used to measure and control the $CL_{residual}$ during current fresh-cut produce washing.

Conflict of interest

Mention of trademark or propriety products in this manuscript does not constitute a guarantee or warranty of the property by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.postharvbio.2018.02.002.

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