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Abstract: Disinfection of fresh produce process wash water (PWW) needs to be optimized to guarantee the microbiological safety while minimizing the presence of disinfection by-products (DBPs) that can be uptaken by the washed produce. In this study, the occurrence of DBPs including trihalomethanes (THMs) and chlorate in the PWW of shredded lettuce, baby spinach and shredded carrots disinfected with sodium hypochlorite (NaClO), and electrochemical disinfection (ED) was assessed. The potential of activated carbon (AC) filtration treatment for the reduction of DBPs was also evaluated. Results showed that both disinfection treatments caused the accumulation of DBPs in PWW, reaching concentrations above the legal limits for potable water. However, there were significant differences in the concentration of DBPs due to the type of washed product and disinfection treatment. There was a lower accumulation of THMs in spinach PWW compared with lettuce and carrot PWWs. Electrochemical disinfection generated higher concentrations of DBPs in the PWW, but a significant reduction was observed when using AC. Such reduction led to a lower accumulation of chlorate in the washed produce, but it lowered the levels of free chlorine. The presence of THMs in PWW was not relevant when using these chlorine-based disinfection treatments as there was a limited transfer of THMs to the washed produce. On the contrary, a significant migration of chlorate from the PWW to the washed product occurred, even when the AC was used as a mitigation treatment.

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**G. Campbell-Platt**
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**September 20th, 2018.**

**Dear G. Campbell-Platt,**

Please find enclosed the manuscript entitled "**Disinfection by-products generated by sodium hypochlorite and electrochemical disinfection in different process wash water and fresh-cut products and their reduction by activated carbon**", submitted for publication in the journal Food Control. The aim of the present study was the evaluation of the accumulation of disinfection by-products (DBPs), particularly THMs and chlorate, in process wash water and washed fresh produce using sodium hypochlorite and electrochemical disinfection. The reduction of DBPs by active carbon filtration was also evaluated. We hope you find the manuscript interesting and suitable to be accepted in this journal.

Thank you for your consideration.

Yours sincerely,

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# Disinfection by-products generated by sodium hypochlorite and electrochemical disinfection in different process wash water and fresh-cut products and their reduction by activated carbon

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## Abstract

Disinfection of fresh produce process wash water (PWW) needs to be optimized to guarantee the microbiological safety while minimizing the presence of disinfection by-products (DBPs) that can be uptaken by the washed produce. In this study, the occurrence of DBPs including trihalomethanes (THMs) and chlorate in the PWW of shredded lettuce, baby spinach and shredded carrots disinfected with sodium hypochlorite (NaClO), and electrochemical disinfection (ED) was assessed. The potential of activated carbon (AC) filtration treatment for the reduction of DBPs was also evaluated. Results showed that both disinfection treatments caused the accumulation of DBPs in PWW, reaching concentrations above the legal limits for potable water. However, there were significant differences in the concentration of DBPs due to the type of washed product and disinfection treatment. There was a lower accumulation of THMs in spinach PWW compared with lettuce and carrot PWWs. Electrochemical disinfection generated higher concentrations of DBPs in the PWW, but a significant reduction was observed when using AC. Such reduction led to a lower accumulation of chlorate in the washed produce, but it lowered the levels of free chlorine. The presence of THMs in PWW was not relevant when using these chlorine-based disinfection treatments as there was a limited transfer of THMs to the washed produce. On the contrary, a significant migration of chlorate from the PWW to the washed product occurred, even when the AC was used as a mitigation treatment.

Keywords: *chemical risk, microbial safety, process water, minimally processed, cross-contamination, fresh produce*.

# 1. Introduction

The fresh-cut produce industry requires significant quantities of water for the washing operation of fresh salads, and thus water is recycled to meet the high water demand (Casani, Rouhany, & Knøchel, 2005). When water is reused, the microbiological safety of the process wash water (PWW) needs to be guaranteed by using suitable disinfection technologies (Gil, Selma, López-Gálvez, & Allende, 2009). However, the use of chemical disinfectants can generate disinfection by-products (DBPs) that can accumulate in the PWW and be uptaken by the produce, affecting its chemical safety (Gil, Marín, Andujar, & Allende et al., 2016).

Chlorine as sodium hypochlorite (NaClO) is the disinfectant most frequently used to guarantee the microbiological safety of PWW (Teng et al., 2018). Electrochemical disinfection (ED) is another powerful technique for the inactivation of microorganisms in PWW (Gómez-López, Gil, & Allende, 2017; López-Gálvez et al., 2012). The disinfectant capacity of ED is based on the generation of hypochlorous acid and hydrogen peroxide as well as other reactive oxygen species and the direct oxidation at the surface of the anode (Anglada, Urtiaga, & Ortiz, 2009). The main disadvantage of using chlorine-based disinfectants is the formation and accumulation of both organic DBPs such as trihalomethanes (THMs) and haloacetic acids (HAA) and inorganic DBPs as chlorate (Gil et al., 2016; Gómez-López, Lannoo, Gil, & Allende, 2014; Gómez-López et al., 2017; Shen, Norris, Williams, Hagan, & Li, 2016). During washing of fresh-cut produce, exudates are released into the PWW, increasing the concentration of organic matter and therefore the disinfectant demand. In most of the cases, the presence of organic matter in the chlorinated wash water is linked to the formation of organic DBPs like THMs (Gil et al., 2009). However, not all organic matter reacts in the same way with the disinfectants (Chen & Hung, 2016; Toivonen & Lu, 2013). Fan & Sokorai

(2015) observed that the formation of THMs depended on the reactivity of the organic matter released from the product exudates.

Different studies have shown that the DBPs present in the PWW can be uptaken by the produce during washing, although rinsing the product can help to alleviate this problem (Fan & Sokorai, 2015; Gómez-López et al., 2017; López-Gálvez et al., 2010; Nitsopoulos, Glaumer, & Friedle, 2014). The presence of DBPs linked to chlorinated water in commercial samples has evidenced a potential risk for consumers (Coroneo et al., 2017; Kaufmann-Horlacher et al., 2014). A possible mitigation treatment to reduce the presence of DBPs in PWW is by filtration through activated carbon (AC). This technology is useful in the removal of THMs and their precursors (organic matter) (USEPA, 1981, 1999). Activated carbon has also shown promising results for the removal of chlorate in drinking water treated with chlorine dioxide (Gonce & Voudrias, 1994; Sorlini, Biasibetti, Gialdini, & Collivignarelli, 2016; USEPA, 2016).

In the present study, the accumulation of THMs and chlorate in the process wash water and washed product by sodium hypochlorite and electrochemical disinfection was assessed. The reduction of DBPs by AC filtration was also evaluated. PWWs generated from the three main fresh-cut products, shredded lettuce, baby spinach, and shredded carrots, were compared.

## 2. Materials and methods

### *2.1. Process wash water and pilot plant trials*

Three different types of PWWs were generated from shredded iceberg lettuce (*Lactuca sativa* L.), baby spinach (*Spinacia oleracea* L.) and shredded carrots (*Daucus carota* L.). Fresh vegetables were obtained from a local grocery store and kept refrigerated until their use the same day of purchase. In the case of lettuce heads, outer and damaged leaves were discarded, and the remaining leaves were cut in ≈6 mm strips. Baby spinach leaves were used without further processing. Carrots were peeled and shredded manually. To generate the PWWs, aliquots of each product were placed in a stomacher bag with filter and tap water was added in a ratio product weight:water volume of 1:2. Then, the mix was homogenized in a stomacher for 2 min. After that, the homogenate was poured through the filter to obtain the PWW.

The disinfection trials were performed in a cold room in a temperature range of 4-8 °C using a dynamic system previously described (Gómez-López, Marín, Medina-Martínez, Gil, & Allende, 2013; Gómez-López et al., 2014). This dynamic system simulates a fresh produce wash system in which disinfectant and the organic matter (including the microbial load) changed over time. The system comprises a polypropylene tank for concentrated PWW, a second tank with the disinfectant solution (NaClO), two peristaltic pumps for dosing the PWW and the disinfectant, a stainless steel deposit (30 L) simulating the washing tank, a cooling system consisting in a stainless steel heat exchanger connected to a refrigerated water bath (model LTDG6, Grant, Cambridge, UK), a centrifuge pump for water recirculation, a control board with an overflow valve, rotameter, and a flow control valve. Process temperature was controlled by placing the heat exchanger of the cooling system into the treatment tank. In all the trials, the water recirculation rate was adjusted to 750 L/h.

### 2.2. *Sodium hypochlorite and electrochemical disinfection*

Process wash water was treated with sodium hypochlorite (NaClO) and electrochemical disinfection (ED). In both cases, a volume of 6 L of PWW was placed into the washing tank before starting the experiment. Each test lasted 8 h, and water samples were taken after 3, 6 and 8 h. To study the uptake of DBPs by fresh-cut produce, baby spinach and shredded lettuce (200 g each replicate) were hand-washed for 1 min in the PWW that was disinfected previously for 6 h as an example of a worst-case scenario for the accumulation of DBPs. After washing, produce was rinsed with tap water for 30 s and dewatered for 1 min using a manual salad spinner.

### *2.2.1. Sodium hypochlorite*

Chlorine as NaClO was applied as explained in Gómez-López et al. (2014). Briefly, a chlorine solution of NaClO was prepared by diluting food grade bleach (Industrias Gamer, Murcia, Spain) in distilled water to reach a free chlorine concentration of ≈300 mg/L. Chorine was dosed as needed using a peristaltic pump to keep the free chlorine concentration between 3 - 5 mg/L. The inflow rate of the NaClO solution ranged between 1.2 and 1.8 L/h. The pH was adjusted to ≈6.5 using citric acid.

### *2.2.2. Electrochemical disinfection*

This treatment was applied using the equipment provided by WaterDiam France S.A.S. (Franken, France) coupled with the dynamic system described before. This equipment included a power supply, control board, and an electrolytic cell (Diacell 401) equipped with 4 cells placed in parallel containing boron-doped diamond (BDD) in the cathode and anode. The inter-electrode gap between the anodic and cathodic compartment was 1 mm. PWW was pumped from the treatment tank through the electrolytic cell returning to the tank and starting the cycle again. In these electrochemical disinfection tests, NaCl was initially added to the tank to reach a concentration of 0.25 g/L in the water to obtain the desired residual free chlorine concentration (3 - 5 mg/L).

### 2.3. Activated carbon (AC)

The effectiveness of an AC filter treatment for the removal of DBPs in PWW treated with chlorine and electrochemical disinfection was assessed. A granular AC filter was coupled in the pilot plant system for this purpose. Disinfection treatments were performed as explained before for NaClO and with some modifications for the electrochemical disinfection trials (**Table 1**). In ED, PWW was passed through the electrolytic cell, and a small part was diverted into the AC filter before returning to the treatment tank. In these trials, higher NaCl concentration (0.5-1.5 g/L) was added due to the higher chlorine demand of the PWWs. In these tests, an electrolytic cell Diacell 201 with an overall effective anode surface area of 67 $cm^2$ was used instead of the Diacell 401 mentioned before following the manufacturer recommendations.

### 2.4. Physicochemical analyses of process wash water

Free and total chlorine were measured using a photometer (Spectroquant NOVA 60, Merck, Darmstadt, Germany) with the appropriate kits. Temperature, pH, and ORP were performed using a multimeter (pH and redox 26; Crison, Barcelona, Spain). Chemical oxygen demand (COD) was determined by the standard photometric method (APHA, 1998) using a photometer (Spectroquant NOVA 60, Merck) with the appropriate kits. Turbidity was measured using a turbidimeter (Turbiquant 3000 IR, Merck) and conductivity was determined using a conductivity meter (CM35 Crison, Barcelona, Spain). Alkalinity was measured by potentiometric titration until pH 4.3 with HCl and a pH meter (Crison, Barcelona, Spain).

### 2.5. Microbiological analyses of process wash water

At each sampling time, water samples (n=3) were taken in sterile bottles containing enough solid sodium thiosulphate pentahydrate (0.5–1.5 g) (Scharlau, Barcelona, Spain) for the quenching of disinfectant residuals (López-Gálvez, Tudela, Allende, & Gil, 2018). Serial dilutions were prepared as needed using buffered peptone water (BPW, 2 g/L) (Oxoid, Basingstoke, UK). Counts of cultivable aerobic mesophilic bacteria and total coliforms were assessed by plating in the appropriate culture media. For the assessment of aerobic mesophilic counts, samples were plated on plate count agar (PCA, Scharlab, Barcelona, Spain) incubated at 30 °C for 36-48 h. For total coliforms, Chromocult coliform agar (Merck) plates were incubated at 37 °C for 24 h.

### *2.6. Disinfection-by products in process wash water and washed product*

At each sampling time, samples of PWW (n=3) and fresh-cut product (n=4) were taken for THM and chlorate analyses as previously described (López-Gálvez et al., 2018). Water samples (120 mL) were taken in amber glass bottles filled up to the top to minimize headspace and kept tightly closed under refrigeration until analysis. Trihalomethanes (THMs) were analyzed by GC–MS (Gómez-López et al., 2013) using a calibration mix (EPA 501/601, Supelco, Bellefonte, PA, USA). The analysis of THMs was carried out by GC-MS (Gómez-López et al., 2013) and the concentration of individual THMs including trichloromethane (TCM), bromodichloromethane (BDCM), dibromochloromethane (DBCM), and tribromomethane (TBM) was expressed as total THMs. For chlorate, samples of 30 mL were taken in 50 mL polypropylene tubes and analyzed by UPLC-MS (Gil et al., 2016). Results were expressed in µg/L for THMs and in mg/L for chlorates.

In the case of the fresh-cut product, samples of 30 g each were chopped (Moulinex A320, Moulinex, Ecully, France). For THM analysis, chopped produce (1 g) was transferred to SPME vials. Samples were analyzed by GC–MS as explained before and results were expressed in µg/kg of fresh weight (FW) (Gómez-López et al., 2013). For chlorate analysis, chopped produce (10 g) was extracted with a solution of formic acid (1%) in methanol, sonicated vortexed and centrifuged. The supernatant was filtered through 0.22 µm filters and analyzed by UPLC-MS (Gil et al., 2016). Results were expressed in mg/kg FW.

### 2.7. *Statistical analysis*

For statistical analyses, IBM SPSS statistics 24 was used. The Shapiro–Wilk test was performed to assess the normality of the data (P >0.05). Levene's test was used to test the homogeneity of variance. When normality was assumed, T-tests or ANOVA were used to compare treatments. Tukey's HSD or Dunnett's were used as post hoc tests depending on the homogeneity of the variances. When data was not following a normal distribution, non-parametric tests (Kruskal-Wallis, Mann-Whitney) were applied.

## 3. Results

### 3.1. *Chlorine demand of process wash water*

The physicochemical characteristics of the PWWs induced significant differences on the chlorine demand (**Table 2)**. The pH, ORP, turbidity, COD and mesophilic bacteria counts were similar in lettuce and spinach PWWs and different from carrot PWW (**Table 2**). On the other hand, conductivity and alkalinity were similar for lettuce and carrot PWWs and different from spinach PWW. For total coliforms, carrot PWW showed the highest counts (**Table 2)**. The chlorine demand of PWWs from shredded lettuce, baby spinach, and shredded carrots was estimated. Lettuce-PWW showed higher chlorine demand than spinach and carrot PWWs (**Figure 1**). The addition of more than 70 mg of free chlorine (from NaClO) per liter of PWW was needed to reach a residual free chlorine concentration of 5 mg/L in lettuce PWW. On the contrary, the addition of ≈20 mg/L of free chlorine (from NaClO) in spinach and carrot PWWs was enough to reach the free chlorine level of 5 mg/L established (**Figure 1**).

### 3.2. *Generation of disinfection-by products by sodium hypochlorite and electrochemical disinfection in different process wash waters*

To understand the generation of DBPs, the physicochemical characteristics of the PWWs (shredded lettuce, baby spinach, and shredded carrot) over 8 h of disinfection were examined (**Table 3**). The average pH was similar for all the PWWs disinfected with NaClO and spinach PWW treated with ED (6.7-6.8) while the pH was lower ($p<0.05$) in lettuce and carrot PWWs treated with ED (5.2 and 6.1, respectively). It was remarkable that the average ORP was significantly higher ($p<0.05$) in those PWWs disinfected with NaClO compared with ED (**Table 3)**. The highest average COD was detected in NaClO carrot PWW (1743 mg $O_2$/L), and the lowest in spinach PWW treated with ED (759 mg $O_2$/L). After the whole 8 h experiment, free chlorine levels were always maintained higher when NaClO was used compared with ED (**Table 3**). The average concentration of total chlorine in PWWs from the highest to the lowest was: NaClO-lettuce>NaClO-spinach>NaClO-carrot=ED-lettuce>ED-spinach>ED-carrot.

When THM formation was evaluated, it was observed that the average concentration of THMs differed significantly depending on the disinfection treatment and PWWs (**Figure 2)**. As a general trend, the accumulation of THMs was always higher ($p<0.05$) in ED than in NaClO disinfected PWWs (**Figure 2**). The concentration of THMs in the different PWWs can be ordered as carrot>lettuce>baby spinach in both

NaClO and ED treatments. The highest concentration of THMs was accumulated in carrot PWW treated with ED (1300 µg/L) while the lowest level was accumulated spinach PWW disinfected with NaClO (≈100 µg/L). The concentration of individual THMs such as trichloromethane (TCM), bromodichloromethane (BDCM), dibromochloromethane (DBCM) and tribromomethane (TBM) was also examined (data not shown). TCM represented more than 90% of all the THMs detected. The concentration of TBM was higher in all PWWs treated with NaClO than those treated with ED. However, only in carrot PWW, a higher concentration of BDCM was observed when treated with NaClO than ED. Also, there were no differences in the accumulation of DBCM in any of the PWWs due to the different disinfection technologies.

### *3.3. Removal of disinfection-by products generated by sodium hypochlorite in lettuce wash water and reduction in the washed product by using activated carbon (AC)*

For the assessment of the potential use of AC for the mitigation of DBP accumulation, the physicochemical characteristics of lettuce PWW over 6 h disinfected with NaClO were examined (**Table 4**). The average pH was in the range 6.2-6.6. Average COD value was significantly higher in NaClO without AC ($p<0.05$). Free chlorine was similar in NaClO filtered or not through AC.

Accumulation of THMs and chlorate in lettuce PWW disinfected with NaClO filtered or not through AC is shown in **Figure 3A**. In the case of NaClO treated lettuce PWW, the use of AC decreased 57.3% and 81.5% the concentrations of THMs and chlorate, respectively. When the individual THMs were examined it was observed that the concentration of TCM was significantly higher ($p<0.05$) in PWW treated with

NaClO without AC filtration. Regarding the concentrations of BDCM, DBCM, and TBM, there were no differences between NaClO with or without AC (**Figure 3A**). When the content of chlorate was examined in lettuce PWW, AC filtration decreased this content significantly ($p<0.05$) compared with that without AC.

The transfer of DBPs from the PWW to the washed product was studied as well as the mitigation effect of AC. The concentration of THMs and chlorate in lettuce washed in PWW disinfected with NaClO filtered or not through AC is shown in **Figure 3B**. The concentration of TCM was significantly reduced ($p<0.05$) when lettuce was washed in PWW treated with NaClO filtered with AC. However, no differences in the concentration of BDCM, DBCM, and TBM were observed in lettuce washed in PWW filtered or not through AC, probably because of the low content of these individual THMs. Regarding chlorate concentration, it was significantly higher ($p<0.05$) in lettuce washed in PWW disinfected with NaClO without filtered through AC.

### *3.4. Removal of disinfection-by products generated by electrochemical disinfection in spinach wash water and the washed product by activated carbon (AC)*

To assess the potential of AC to mitigate the accumulation of DBPs in spinach PWW treated with ED over 6 h, the physicochemical characteristics were first examined (**Table 4**). The average pH and COD were significantly higher in the trials with AC ($p<0.05$) while the redox potential (ORP) was significantly higher ($p<0.05$) in the experiments performed without AC. On the other hand, no significant differences in free chlorine and total chlorine ($p>0.05$) between treatments were detected (**Table 4**).

When the removal of THMs and chlorate generated in spinach PWW treated with ED by AC was studied it was observed that the concentration of both DBPs was significantly lower ($p<0.05$) when the PWW was filtered through AC (**Figure 4A**). The concentration of total THMs and chlorate was 95.6% and 90.4% lower, respectively, in filtered spinach PWW.

The content of DBPs in baby spinach washed in PWW treated with ED for 6 h and filtered or not through AC is shown in **Figure 4B**. The concentration of THMs in the washed product was very low (<10 µg/kg). No differences were observed in the concentration of TCM and BDCM when the PWW was filtered or not through AC. In contrast, the concentration of DBCM and TBM was lower in spinach samples washed in water treated with AC. Baby spinach washed in PWW treated with ED and filtered or not through AC had a similar chlorate content.

## 4. Discussion

In the present study, lettuce PWW showed the highest chlorine demand despite having similar COD than that of spinach PWW and even lower than that of carrot PWW. The chlorine demand of PWW is mainly caused by the presence of organic matter (Chen & Hung, 2017; Weng et al., 2016). Thus, different constituents of the organic matter affect in a different way the chlorine demand and therefore the generation of DBPs. Several plant constituents such as phenolic compounds have higher chlorine demand than carbohydrates (Chen & Hung, 2016; Toivonen & Lu, 2013). Considering the composition of the three types of PWWs studied, it is clear that lettuce PWW exerted the highest chlorine demand due to the high content on caffeoylquinic acids, such as chlorogenic acid, that can be easily oxidized by chlorine, showing a higher chlorine demand (Llorach, Martínez-Sánchez, Tomás-Barberán, Gil, & Ferreres, 2008). In the case of spinach PWW, even though the content of some constituents such as flavonoids is high, they cannot be oxidized as easily as the phenolic acids (Gil, Ferreres & Tomás-Barberán, 1999). Also, spinach shows a higher content of ascorbic acid than lettuce which can prevent the oxidation of constituents (Bunea et al., 2008; Llorach et al., 2008; Rothwell et al., 2013; USDA, 2017).

During the experiments performed to test the formation of THMs in different PWWs, TCM (chloroform) was the predominant THMs as shown in previous studies with other chlorinated disinfectants (Gómez-López et al., 2013; Gómez-López et al., 2017; Shen et al., 2016). In our case, we observed that even though the free chlorine was higher in the NaClO tests, the concentration of THMs was significantly higher in PWWs treated with ED. In a previous study, Gómez-López et al. (2013) reported no significant differences between the concentration of THMs in PWW treated with NaClO and ED, when using the same treatment conditions used in the current study. The observed differences could be due to variances in the composition of the used PWWs or the final free chlorine concentrations reached in each study (Table 2).

When the different PWWs were compared, we observed that the higher concentration of THMs in carrot>lettuce>spinach could be partially explained by the differences in the COD as the free chlorine levels were similar. However, organic matter concentration is not always correlated with the formation of THMs. Fan & Sokorai (2015) observed a higher formation of TCM in lettuce PWW compared with onion PWW, although the latter showed higher COD and turbidity. Shen et al. (2016) detected in lettuce PWW disinfected with chlorine a correlation between the concentration of THMs in PWW with the free chlorine but not with COD.

When PWW was filtered through AC, COD decreased because of the removal of organic matter (USEPA, 1981, 1999). In the case of spinach and lettuce PWWs filtered through AC, the concentration of THMs decreased to levels accepted for water intended for human consumption in the EU (<100 µg/L; EU, 1998). The potential of AC for the removal of THMs in drinking water has been observed for a long time (USEPA, 1981). However, to the best of our knowledge, the AC filtration has not been applied for PWW. In contrast, the chlorate concentrations in all disinfected PWW were above the level that the World Health Organization guideline recommended for drinking water (0.7 mg/L; WHO, 2011). However, when AC was combined with the disinfection treatment, a significant reduction in the concentration of chlorate in the PWW was achieved. Accordingly, some studies have shown that AC has some efficacy for the removal of chlorate present in drinking water (Gonce & Voudrias, 1994; Sorlini et al., 2016).

In the present study, low concentration of THMs (<10 µg/kg) was detected in the washed product even though there was a high concentration of THMs (>200 µg/L) in the PWWs. The concentration of THMs detected in the washed product, in µg/kg, was always lower than the maximum concentration acceptable in drinking water for human consumption (100 µg/L; EU, 1998). It should be taken into account that there is no a maximum residue level (MRL) for THMs in fresh produce. In previous lab scale studies in our lab, low concentrations of THMs (<10 µg/L) were observed in produce washed in PWW with a high concentration of THMs >200 µg/L (Gómez-López et al., 2017; Gómez-López et al., 2013; López-Gálvez et al., 2010). In contrast, when using longer produce-water contact times, Huang & Batterman (2010) observed a higher transfer of THMs from water to produce. However, the relationship between this lab scale results and the exposure of consumers to THMs present in commercial minimally processed fresh produce is not well characterized. Coroneo et al. (2017), for example, detected concentrations of THMs of ≈77 µg/kg in commercial samples of ready to eat vegetables washed in chlorinated water. In our study, the removal of THMs in the PWWS by AC was evidenced. However, in the case of chlorate, the mitigation of its accumulation by

AC was not enough, and therefore the concentration of chlorate in washed produce was higher than the levels proposed by the European Commission (0.01 mg/kg) (AHBD, 2017). Gil et al. (2016) reported lower concentrations of chlorate in washed shredded lettuce at commercial scale probably because of the high water replenishment, avoiding the accumulation of COD and reducing the chlorine demand.

## 5. Conclusions

This study provides additional evidence that in fresh produce wash water, not only the amount but also the type of organic matter affects the chlorine demand. Links were identified between the concentration of DBPs and the type of vegetable wash water and/or the type of disinfection technology applied. Treated lettuce PWW had more THMs than that of carrot and spinach PWWs. In general, ED was associated with a higher accumulation of DBPs than NaClO. Chlorinated disinfection technologies lead to the occurrence of DBPs in PWW above the legal or recommended limits of these compounds in water for human consumption. The transfer of THMs to the washed produce was limited, while the transfer of chlorate was significant. The use of activated carbon reduced significantly the concentration of DBPs in water, with consequences in the concentration of chlorate in the washed produce.

## Competing interests statement

The authors have no conflicts of interest to declare.

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**Table 1.** Conditions performed in lettuce and spinach process wash water (PWWs) treated with sodium hypochlorite (NaClO) and electrochemical disinfection (ED) for the activated carbon (AC) test.

| PWW | Disinfection technology | AC | Filter flow (L/h) | pH regulation | NaCl (%) |
|---|---|---|---|---|---|
| Lettuce | NaClO | No | 0 | Phosphoric acid | 0 |
| Lettuce | NaClO | Yes | 30 | Phosphoric acid | 0 |
| Spinach | ED | No | 0 | Citric acid | 0.05 |
| Spinach | ED | Yes | 50 | Citric acid | 0.05 |

**Table 2.** Physicochemical and microbiological characteristics of process wash water (PWW) for the assessment of chlorine demand.

| **PWW** | **pH** | **ORP (mV)** | **Conductivity (µS/cm)** | **Turbidity (NTU)** | **COD (mg/L)** | **Alcalinity (mEq/L)** | **Mesophilic bacteria (log cfu/mL)** | **Total coliforms (log cfu/mL)** |
|---|---|---|---|---|---|---|---|---|
| **Lettuce** | 7.4±0.0 | 253±11 | 1422±11 | 56±2 | 2685±35 | 183±00 | 6.7±0.1 | 4.3±0.3 |
| **Spinach** | 7.8±0.0 | 230±00 | 2403±11 | 97±15 | 2328±13 | 970±28 | 6.9±0.0 | 5.5±0.3 |
| **Carrot** | 6.3±0.0 | 142±17 | 1165±26 | 298±5 | 3765±49 | 163±9 | 7.7±0.2 | 6.4±0.1 |

Data are the mean (n=2) ±standard deviation. No statistical analysis was performed due to the limited number of data available.

**Table 3.** Physicochemical characteristics of process wash water (PWWs) treated with sodium hypochlorite (NaClO) or electrochemical disinfection (ED) for the assessment of trihalomethanes formation.

| Disinfection technology | PWW | pH | ORP (mV) | COD (mg $O_2$/L) | Free chlorine (mg/L) | Total chlorine (mg/L) |
|---|---|---|---|---|---|---|
| **NaClO** | Lettuce | 6.8±0.1a | 685±252ab | 1140±364b | 5.3±5.7ab | 48.8±43.2a |
| **NaClO** | Spinach | 6.8±0.1a | 519±132bc | 1177±155b | 7.1±3.3a | 29.3±12.2a |
| **NaClO** | Carrot | 6.7±0.2a | 792±236a | 1743±188a | 7.2±3.6a | 15.9±6.9b |
| **ED** | Lettuce | 5.2±0.4c | 371±116d | 1315±448b | 1.9±1.2b | 16.4±11.7b |
| **ED** | Spinach | 6.7±0.1a | 177±91e | 759±413c | 3.0±3.2b | 11.9±6.4b |
| **ED** | Carrot | 6.1±0.2b | 229±130e | 1236±543b | 1.3±0.6b | 5.2±2.9c |

Data are the mean (n= 9)±standard deviation of the values measured over 8 h of disinfection period. Different letters within the same column indicate significant differences ($p<0.05$). ORP: Oxidation-reduction potential. COD: Chemical oxygen demand.

**Table 4.** Physicochemical characteristics of lettuce process wash water treated with sodium hypochlorite (NaClO), and spinach process wash water treated with electrochemical disinfection (ED) with and without activated carbon (AC) filtration.

| Disinfectant | AC | pH | ORP (mV) | COD (mg/L) | Free chlorine (mg/L) | Total chlorine (mg/L) |
|---|---|---|---|---|---|---|
| **NaClO** | **No** | 6.6±0.3a | 902±100a | 592±227a | 3.3±1.2a | 18.4±9.4a |
| **NaClO** | **Yes** | 6.4±0.4b | 843±153a | 327±92b | 3.6±1.4a | 15.5±6.7a |
| **ED** | **No** | 6.8±0.2B | 331±141A | 141±60B | 2.8±2.6A | 7.8±3.3A |
| **ED** | **Yes** | 9.3±0.2A | 172±1B | 265±91A | 1.5±0.7A | 3.5±1.5A |

Data are the mean (n=7)±standard deviation of the values measured over 6 h in two independent tests. Different letters in the same column for the same process wash water indicate significant differences ($p<0.05$).

## Figure captions

**Figure 1.** Chlorine demand represented as residual free chlorine versus chlorine added as NaClO (mg/L) to the different process wash water (See Table 2 for PWW characterization).

**Figure 2.** Concentration of trihalomethanes (µg/L) accumulated in the different process wash water treated with NaClO or electrochemical disinfection (ED). Different letters indicate significant differences ($p<0.05$). (See Table 3 for PWW characterization).

**Figure 3**. Concentration of trihalomethanes (µg/L) and chlorate (mg/L) in lettuce process wash water (A) treated with NaClO combined or not with activated carbon (AC) and in the washed product (B). Bars are the mean ($n=7$)±standard deviation of samples taken over 6 h treatment. Different letters indicate significant difference ($p<0.05$). (See Table 4 for PWW characterization).

**Figure 4**. Concentration of trihalomethanes (µg/L) and chlorate (mg/L) in spinach process wash water (A) treated with electrochemical disinfection (ED) combined or not with activated carbon (AC) and in the washed product (B). Bars are the mean ($n=6$)±standard deviation of the samples taken over 6 hours treatment. Different letters indicate significant difference ($p<0.05$). (See Table 4 for PWW characterization).

540 **Figure 1.**

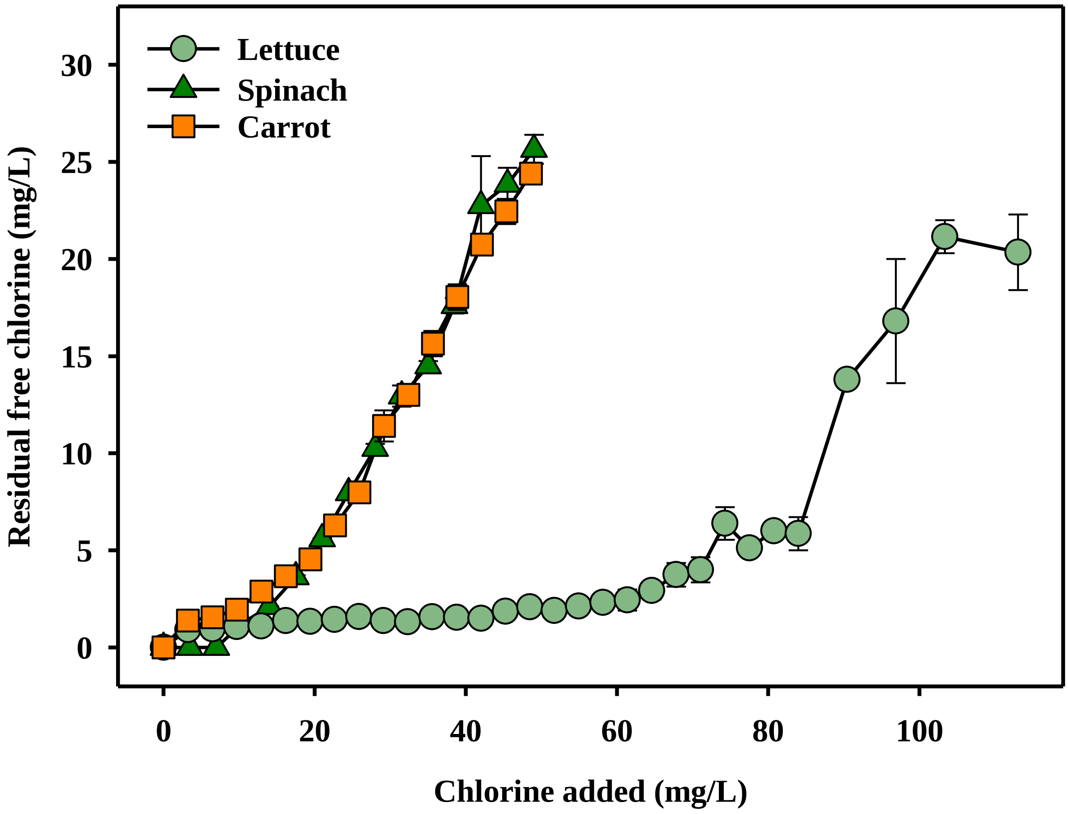

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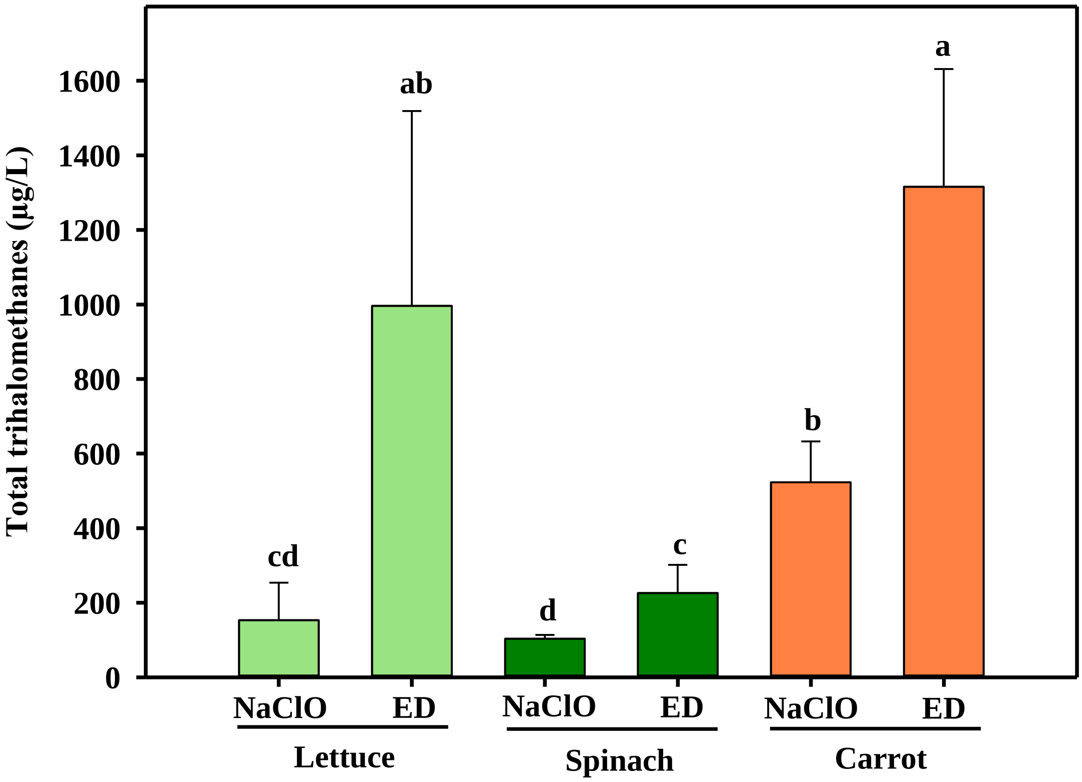
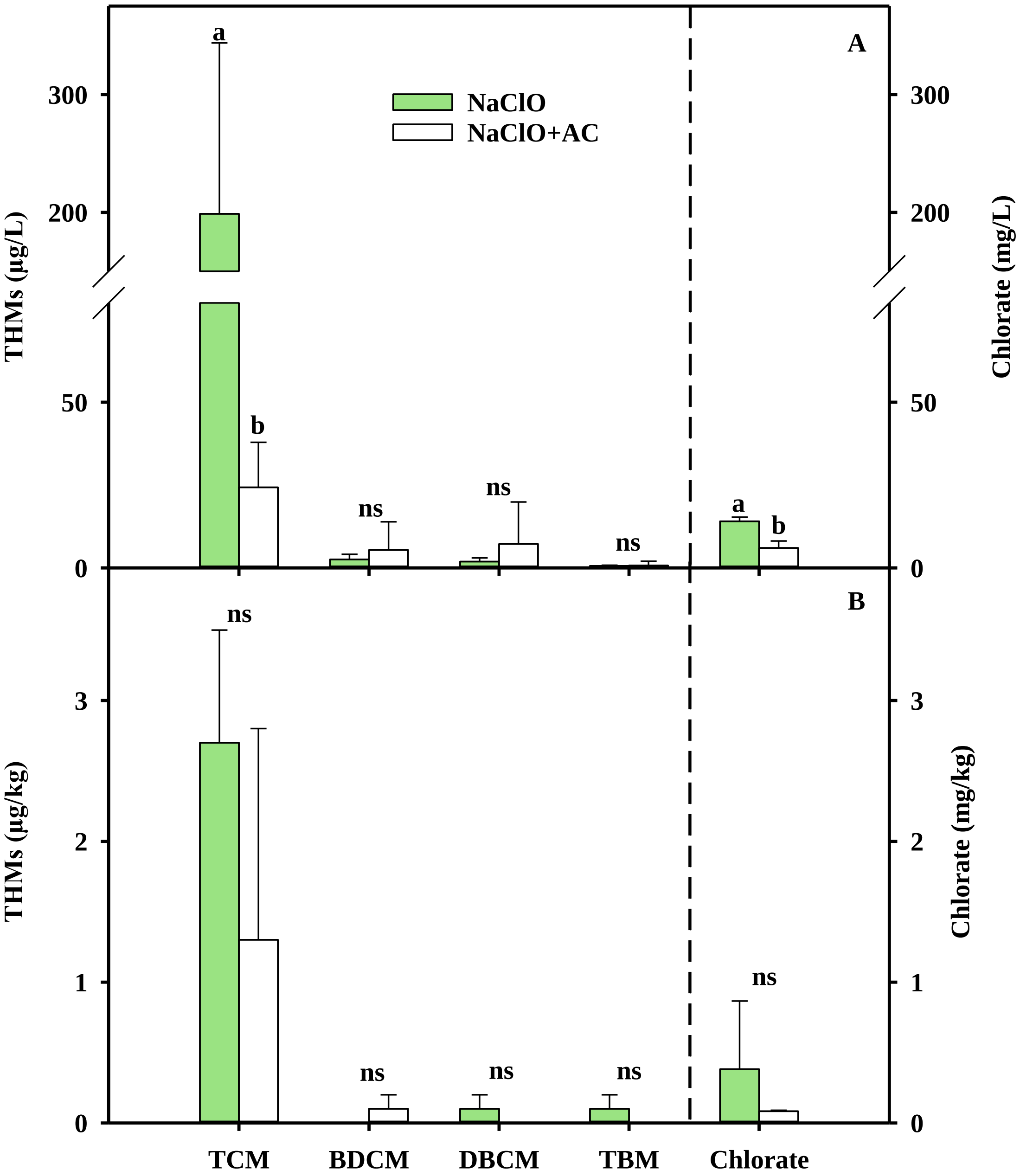
**Figure 2**

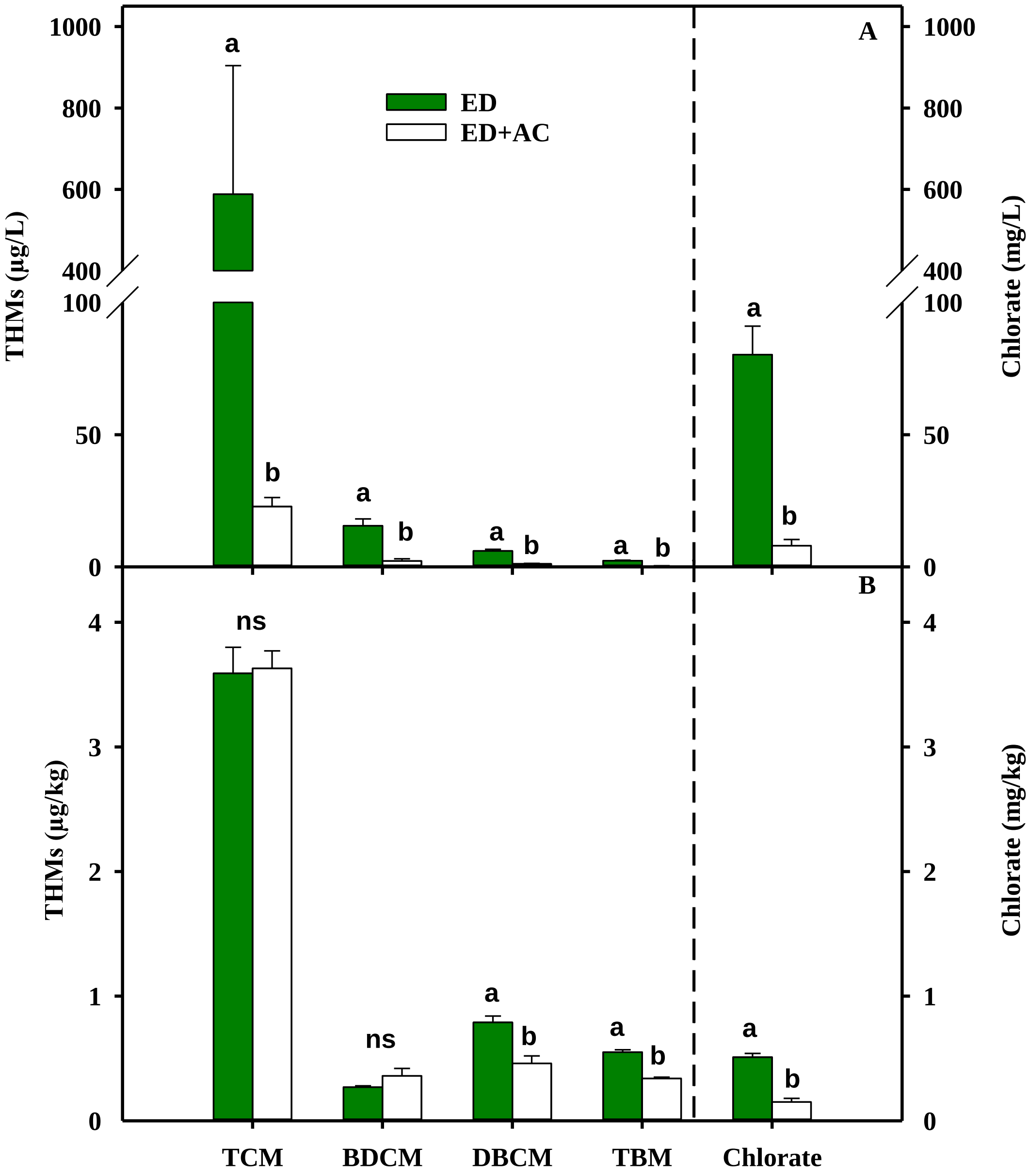

Total trihalomethanes (µg/L)
0
200
400
600
800
1000
1200
1400
1600
cd
ab
d
c
b
a
NaClO
ED
NaClO
ED
NaClO
ED
Lettuce
Spinach
Carrot

544 **Figure 3**



545

**Figure 4**


A
B
ED
ED+AC
THMs (µg/L)
Chlorate (mg/L)
THMs (µg/kg)
Chlorate (mg/kg)
1000
800
600
400
100
50
0
4
3
2
1
a
b
ns
TCM
BDCM
DBCM
TBM
Chlorate

## Highlights

- Electrochemical disinfection leads to higher levels of disinfection by-products
- Occurrence of trihalomethanes is affected by the type of fresh produce wash water
- Activated carbon filtration reduces disinfection by-products in produce wash water
- Limited transfer of trihalomethanes from produce wash water to the washed product
- Significant transfer of chlorate from produce wash water to the washed product