Research Article

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Disinfection by-products in baby lettuce irrigated with electrolysed water

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Abstract

BACKGROUND: Irrigation water disinfection reduces the microbial load but it might lead to the formation and accumulation of disinfection by-products (DBPs) in the crop. If DBPs are present in the irrigation water, they can accumulate in the crop, particularly after the regrowth, and be affected by the postharvest handling such as washing and storage. To evaluate the potential accumulation of DBPs, baby lettuce was grown using irrigation water treated with electrolysed water (EW) in a commercial greenhouse over three consecutive harvests and regrowths. The impact of postharvest practices such as washing and storage on DBP content was also assessed.

RESULTS: Use of EW caused the accumulation of chlorates in irrigation water (0.02–0.14 mg L^{-1}), and in the fresh produce (0.05–0.10 mg kg^{-1}). On the other hand, the disinfection treatment had minor impact regarding the presence of trihalomethanes (THMs) in water (0.3–8.7 μg L^{-1} max), and in baby lettuce (0.3–2.9 μg kg^{-1} max).

CONCLUSIONS: Disinfection of irrigation water with EW caused the accumulation of chlorates in the crop reaching levels higher than the current maximum residual limit established in the EU legislation for leafy greens.

Keywords: fresh produce; agricultural water; disinfection by-products; chemical risk; water disinfection; food safety

INTRODUCTION

Irrigation water is an important source of contamination of fresh produce with human pathogenic microorganisms.[1,2] In primary production, the role of water quality on the safety of fresh produce has been of particular interest over the years.[3,4] Disinfection of irrigation water is a recommended mitigation strategy to reduce microbiological contamination and ensure the food production compliance with established microbial limits, particularly faecal indicator bacteria such as *Escherichia coli*.[5–7] In addition, growers can minimise the risk of exposure to plant diseases and reduce pesticide use by ensuring that irrigation water is free of plant pathogens, especially in intensive agricultural production regions.[8]

Several disinfection technologies can be used for irrigation water including chlorine derivative solutions available for use in greenhouse operations. One of these technologies is electrolysed water (EW), which has shown efficacy for the inactivation of many different microorganisms.[9] EW has some advantages such as it only needs NaCl and water as ingredients for its production.[10] However, as is the case of other chlorinated disinfectants, the use of EW can lead to the presence of disinfection by-products (DBPs) such as trihalomethanes (THMs)[10] and haloacetic acids (HAAs).[11] On the other hand, chlorates (ClO_3^-) can be formed during the manufacture and storage of EW.[12] The presence of chlorate in fresh produce has been linked to the use of chlorinated water for produce washing and disinfection of processing equipment.[7] However, the presence of chlorates in irrigation water could cause the chemical accumulation during crop production. To the best of our knowledge, there is no information that describe the dynamics of DBPs in fresh produce over time during primary production in commercial settings, and after processing, including washing and storage.

Most of the leafy vegetables used by the fresh-cut industry are cultivated in greenhouses to protect them from adverse weather conditions such as wind and rain.[13] Baby lettuce intended for use in bagged salads can be highly productive under these conditions, particularly because they have the ability to regrow after the first harvest and they can be consecutively harvested in the same substrate within 10–30 day intervals.[14]

Chlorination of irrigation water and the presence of DBPs that may occur in the produce have been pointed out in research and development actions because of the lack of data for regulation of maximum residue level (MRL).[15] In the European Union, the default MRL for chlorate in food is 0.01 mg kg^{-1}.[16] However, a limit of 0.06 mg kg^{-1} is being suggested for baby leaves.[15] The maximum concentration of THMs in water for human consumption is 100 μg L^{-1} in European Union legislation and 80 μg L^{-1} based on the US Environmental Protection Agency (EPA).[17,18]

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The use of EW for the disinfection of irrigation water could cause the presence of DBPs in the water and in the crop, and postharvest practices could also have an impact on their accumulation. The aim of the present study was to assess the presence of disinfection by-products (chlorates and trihalomethanes) in irrigation water treated with electrolysed water (EW), and in baby lettuce cultivated in a commercial greenhouse production during three consecutive harvests. The impact of postharvest practices such as washing and storage on DBP content was also assessed.

MATERIALS AND METHODS

Disinfection of irrigation water

The electrolysed water used to disinfect irrigation water was generated using the following protocol. Electrolytic cells were used to generate a concentrated solution of electrolysed water (EW) (∼1500 mg L^{-1} free chlorine, pH 2–3) using reverse osmosis water and NaCl as ingredients. The EW generator was located in a room situated at a distance of ∼100 m from the greenhouse. The concentrated EW solution was stored in a black plastic container located in the open air outside the EW generation room and was pumped as needed into the irrigation network. The water used for irrigation was surface water from a reservoir mixed with the concentrated EW at a target free chlorine concentration of ∼5 mg L^{-1} following commercial guidelines. The irrigation water treated with EW was pumped into the greenhouse and was applied by sprinkler irrigation.

Cultivation system

Two types of red baby lettuce (*Lactuca sativa* L.), Red Oak Leaf cultivar BHR-805 and Red Batavia cultivar BBR-1514, were cultivated in a commercial greenhouse with a surface area of 3 ha. Mean minimum temperature in the greenhouse ranged from 13 °C in May to 23 °C in July while mean maximum temperature ranged from 27 °C in May to 35 °C in July. Seeds were cultivated on peat as substrate in polystyrene trays of 294 alveolus placed on stainless steel tables inside the greenhouse. A boom irrigation system with nozzles moved above the trays watering the plants. The average amount of irrigation water applied per day was approximately 50 m^3 ha^{-1} and the irrigation was applied early in the morning and late in the afternoon. There was not a standard control treatment of lettuce irrigated with untreated water.

Lettuce harvest, processing and storage

For each type of lettuce, the commercial size product was harvested from the same trays three times during the growing cycle in May, June and July 2016, corresponding to the first, second and the third harvests. This is a commercial practice for baby leaves that regrow and are cut several times from the same plants. At harvest, trays were passed automatically through a harvester and cut lettuce leaves were transported by conveyor belts to crates. Trays were placed again on the same position on the tables for regrowth and further harvests. Two kilograms of each type of baby lettuce were sampled from the crates and transported under refrigeration to the laboratory in less than 2 h. Produce was kept in a cold room at 7 °C for 2 h before processing. Then, baby leaves were washed and rinsed in cold tap water (7 °C), centrifuged in an automatic salad spinner (K-50; Kronen, Kehl am Rhein, Germany) for 1 min, and packaged in polypropylene bags (230 × 320 mm, 100 g per bag). Passive modified atmosphere packaging was used (air as initial atmosphere). Lettuce bags were stored in darkness at 7 °C for 9 days. The concentration of O_2 and CO_2 in the headspace of the bags was measured after 9 days of storage at 7 °C using an O_2 analyser with a ceramic oxide–zirconia electrochemical detection cell (CG-1000, Ametek; Thermox Instruments Co., Pittsburgh, PA, USA) and an infrared CO_2 detector (Via 510; Horiba Instruments, Irvine, CA, USA). Oxygen and CO_2 gas concentrations were converted to partial pressures (kPa).

Figure 1 represents a diagram with the sampling points for water and the plant material at harvest, after processing, and after storage. In the case of water, the presence of DBPs was assessed in two replicates because of the similarities between replicates. For lettuce, quality characteristics and presence of DBPs were measured in four replicates.

Water sampling and physico-chemical analyses

Twelve water samplings were carried out during May, June and July 2016 with a weekly frequency. Water was sampled once a week before disinfection (untreated water taken at the EW generation room before treatment) and after disinfection (EW treated irrigation water from the nozzles of the boom irrigation system located inside the greenhouse). Two litres of water were taken in aseptic polyethylene bottles (2.7 L) (Deltalab, Barcelona, Spain) and transported to the laboratory in refrigerated conditions. Free chlorine, temperature, pH, and oxidation–reduction potential (ORP) were measured immediately after taking the samples. Free chlorine was analysed by means of the DPD method using a free chlorine test kit and the Spectroquant photometer (Merck, Darmstadt, Germany).[19] Temperature, pH and ORP were measured with a multimeter (pH and redox 26; Crison, Barcelona, Spain). Total organic carbon (TOC) and UV absorbance at 254 nm (UV254) measurements were performed at the CEBAS-CSIC laboratories (Murcia, Spain) using samples transported in refrigerated conditions. TOC in the water was assessed by means of a multi N/C 3100 analyser (Analytik Jena, Jena, Germany) after filtration through ashless paper filters (Albet Labscience, Dassel, Germany). Water filtered through 0.45 μm syringe nylon filters (Fisherbrand; Fisher Scientific, Waltham, MA, USA) was used for measuring the absorbance at 254 nm using a UV–visible spectrophotometer (V-630; Jasco, Tokyo, Japan) and quartz cuvettes with a path length of 1 cm (Hellma, Müllheim, Germany). Free chlorine, pH, temperature, ORP and TOC were measured in one sample per water type and sampling day. UV254 was measured in two samples per water type and sampling day.

Disinfection by-products in irrigation water

Chlorate content was analysed in the untreated and EW treated irrigation water samples, and also in the concentrated EW solution (taken from the EW storage container). For chlorates, two samples (45 mL) from each type of water were taken in Falcon tubes (50 mL) each sampling day. Trihalomethanes were analysed in untreated and EW treated irrigation water samples. For THMs, two samples (14 mL) per water type and sampling day were taken in tubes containing sodium thiosulfate to quench residual free chlorine. Water samples for chlorates and THMs were frozen at −20 °C upon arrival at the laboratory until analyses were performed. Chlorate content in water samples was analysed by LC–MS as explained in Gil *et al.*[20] Trihalomethanes were analysed by GC–MS as explained in Gómez-López *et al.*[21] Areas of the peaks detected by MS were used for the quantification of THMs and chlorates. Results were expressed in mg L^{-1} for chlorates and in μg L^{-1} for THMs.

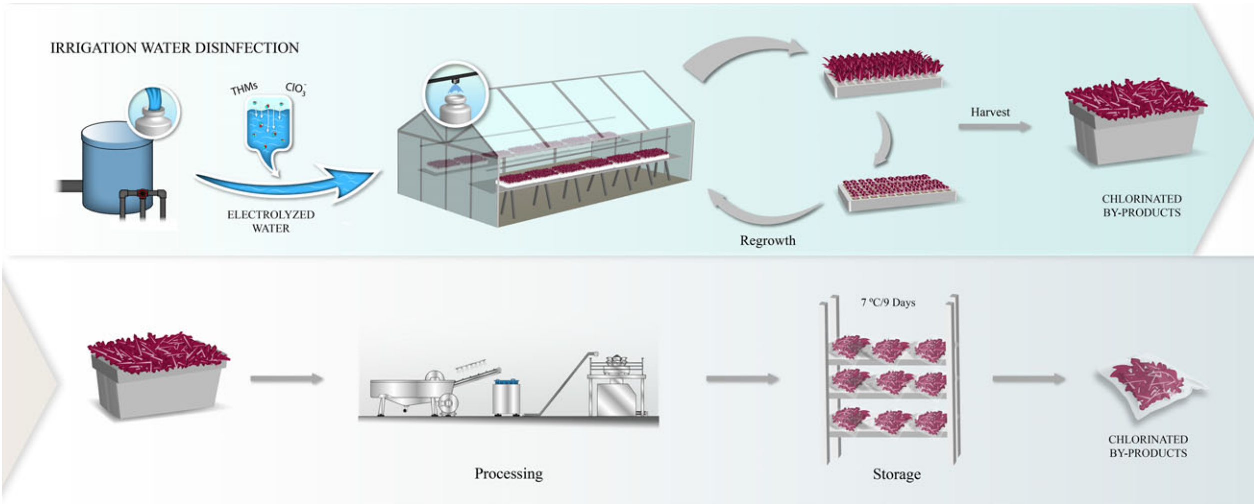

Figure 1. Diagram representing study workflow: sampling points for water (untreated water taken at the EW generation room before treatment; EW treated irrigation water from the nozzles of the boom irrigation system located inside the greenhouse), plant material (Red Oak Leaf and Red Batavia harvested from the same trays after regrowth, corresponding to the first, the second and the third harvests), and commercial samples after processing and storage.

Disinfection by-products in baby lettuce

Analyses for the content of DBPs in baby lettuce were performed just after harvest (raw material for processing), after processing (d0), and after storage (d9). For each type of sample, four replicates (50 g) of baby lettuce were taken and kept at −20 °C until they were analysed. Chlorate content of lettuce samples was analysed by LC–MS as explained in Gil *et al.*,[20] using an analytical standard of chlorate (RTC, ICS-004-100; Fluka, Sigma–Aldrich, Madrid, Spain) for quantification. Trihalomethanes were analysed by GC–MS as explained in Gómez-López *et al.*[21] Results were expressed in mg kg^{-1} fresh weight for chlorates and in μg kg^{-1} fresh weight for THMs.

Quality characteristics of baby lettuce

Assessment of quality characteristics of baby lettuce had three goals: (1) to confirm that all the product that was evaluated had acceptable quality, (2) to assess plant characteristics that could explain differences in DBP content between harvests, and (3) to evaluate if the continuous irrigation with disinfected water affected the quality characteristics among harvests. Morphological characteristics (length, width and surface area) as well as colour (hue, saturation and value) of the leaves were assessed by digital image analysis. Leaf images were taken using a digital camera (Canon 70D) mounted on the top of a photography box of 100 cm × 60 cm × 60 cm (height × length × width) with matt white translucent walls and black matt ground. The light was provided by one lead panel of 60 × 60 cm of 42 W and 6400 K mounted on the top of the box. The image acquisition was conducted in a darkroom maintained at room temperature. Acquired picture files were saved in RAW format (5472 × 3648 pixels), sRGB colour space. Images were transformed to HSV (hue, saturation, value) colour space and measured using the open source image processing software ImageJ 1.50i image processing software (NIH, Bethesda, MD, USA). Thirty-five leaves were analysed by lettuce type and harvest day. Sensory analysis of lettuce samples was performed by a panel of at least three trained members. Sensory quality was examined just after processing (d0), and after storage (d9). Each panellist evaluated one bag for d0 and another for d9, firstly for off-odours and then for the evaluation of the remaining quality parameters. These parameters were assessed by marking a line in a 10 cm hedonic scale: off-odours, off-odours 5 min after opening the bag, leaf edge browning, spoilage or decay and general visual quality. Spoilage or decay scale ranged from 'absence' (0) to 'severe' (10). General visual quality scale ranged from 'poor' (0) to 'excellent' (10). The scale for the remaining parameters ranged from 'absence' (0) to 'very strong' (10).

Statistical analysis

IBM SPSS statistics 23 was used for statistical analyses. The Shapiro–Wilk test was performed to assess the normality of the data ($P > 0.05$). Levene's test was used to test homogeneity of variance. When normality could be assumed, ANOVA was performed, with Tukey's HSD or Dunnett's as post hoc tests depending on the homogeneity of the variances. When data was not following a normal distribution, non-parametric tests (Kruskal–Wallis and Mann–Whitney) were applied. Pearson's correlation coefficient was calculated to evaluate correlation between data.

RESULTS AND DISCUSSION

Impact of EW on disinfection by-products in irrigation water

Measured free chlorine concentration in EW treated irrigation water during the study ranged between 2.1 and 5.7 mg L^{-1} (Fig. 2A), with an average ± standard deviation (SD) of 4.2 ± 1.1 mg L^{-1}. Measured concentration of free chlorine was close to the target concentration of 5 mg L^{-1} recommended by the supplier of the EW production equipment. Table 1 shows the average values of some of the physico-chemical parameters such as temperature, pH, ORP, TOC and UV254 of untreated and EW treated irrigation water samples taken throughout the study. There were no significant differences in water temperature between untreated water and EW treated irrigation water. The value of pH was significantly higher in untreated water because the concentrated EW solution had acidic pH, and its addition significantly lowered pH of the EW treated irrigation water. The pH of irrigation water treated with EW was within the range needed for an efficient antimicrobial action

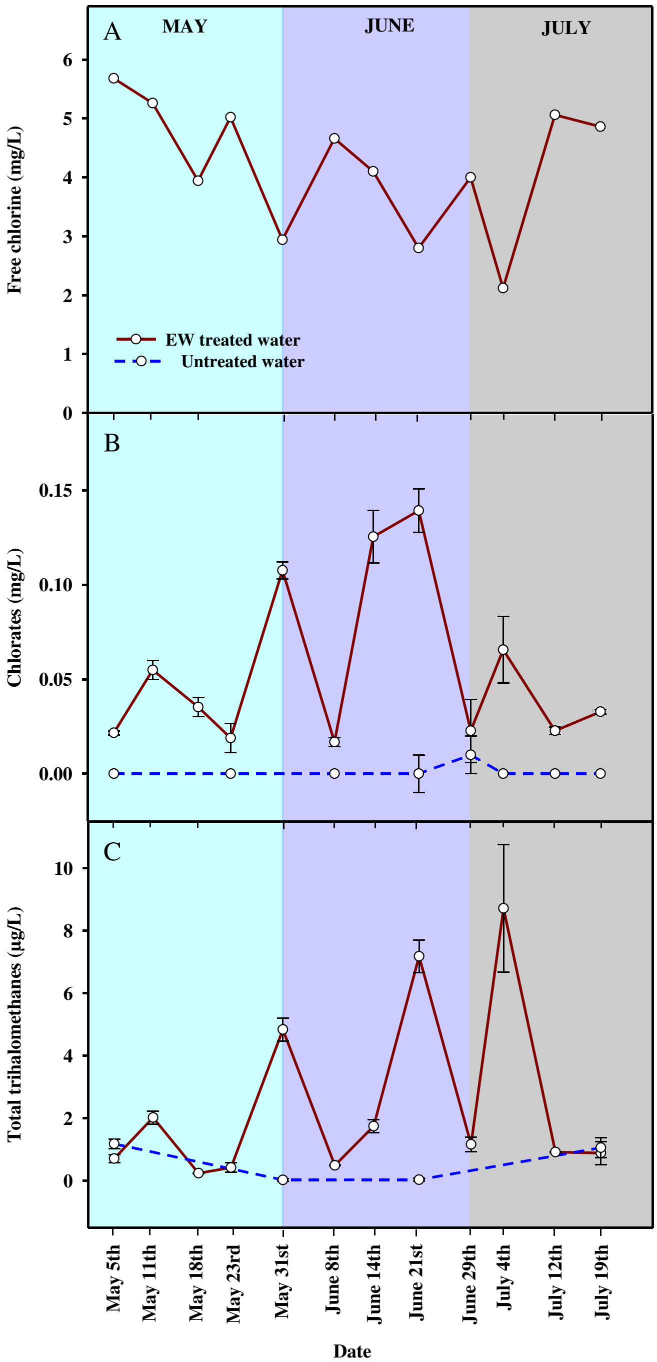

Figure 2. Concentration of free chlorine (mg L^{-1}) (A), chlorates (mg L^{-1}) (B), and total trihalomethanes (μg L^{-1}) (C) in EW treated irrigation water in the 12 water samplings performed throughout May, June and July. Values for chlorates and total trihalomethanes are the mean ± standard deviation ($n = 2$).

of free chlorine as a pH of 6.0–7.5 is desirable for the chlorine to be mostly in the more effective hypochlorous acid form.[22] On the other hand, ORP, TOC and UV254 were significantly higher in EW treated irrigation water compared with untreated water. Higher ORP and UV254 would be explained by the presence of the oxidant species.[23]

Mean concentration of chlorates in the concentrated EW solution was 17.8 ± 8.0 mg L^{-1}. In our study, chlorate/free chlorine proportion in the concentrated solution was low (0.6%) compared

Table 1. Physico-chemical parameters of untreated and EW treated irrigation water samples

Parameter	Untreated water	EW treated irrigation water
Temperature (°C)	23.5 ± 2.8	23.4 ± 3.0^{NS}
pH	8.4 ± 0.1	7.3 ± 0.4*
ORP	254 ± 77	637 ± 170*
TOC (mg L^{-1})	8.4 ± 1.9	10.7 ± 3.3*
UV254 (cm^{-1})	0.02 ± 0.00	0.09 ± 0.05*

ORP, oxidation–reduction potential; TOC, total organic carbon; UV254, absorbance of water at 254 nm.
Values are the mean ± standard deviation of the 12 samplings performed throughout May, June and July ($n = 12$).
NS, not significant. *Significant differences between untreated and EW treated irrigation water samples ($P < 0.05$).

with other electrolysis plants (2–8%).[24] Formation of chlorate by electrolysis depends on a number of factors like the material of the electrode, composition of the electrolyte, current applied, pH, and temperature.[25] Apart from the formation of chlorate during the electrolysis used to produce EW, chlorate content can increase during storage of chlorinated disinfectant solutions, due to disproportionation of hypochlorite ion to chlorate and chloride ions.[26] Such a disproportionation process is faster at higher temperatures. The container of the concentrated EW solution was located outside with no control of temperature conditions. However, higher chlorate concentration in the concentrated EW solution was detected during May (mean outside temperature 19.6 ± 1.8 °C) compared with June (22.8 ± 2.2 °C) and July (25.5 ± 1.6 °C). Taking into account the volume of EW solution consumed every day (~750 L), the residence time of the solution in the storage tank would have been too short to result in a significant increase in chlorate concentration due to the storage temperature. Chlorate concentration was significantly higher ($P < 0.05$) in EW treated irrigation water (0.06 ± 0.05 mg L^{-1}) compared with the untreated water (0.01 ± 0.00 mg L^{-1}). Figure 2B shows the changes in chlorate concentration in the untreated water and EW treated irrigation water throughout the study. In most of the samplings, chlorate level in EW treated irrigation water was below 0.05 mg L^{-1}. However, there were some sample points in which chlorate concentration reached a maximum of 0.14 mg L^{-1}. Although disinfection of irrigation water using EW significantly increased the amount of chlorates, the levels in our study were well below the World Health Organization guideline level for chlorate in potable water (0.7 mg L^{-1}).[27] Other disinfection treatments for irrigation water such as ClO_2 can also lead to the presence of chlorates in water and in the irrigated crop.[15]

Content of THMs in untreated water and EW treated irrigation water is shown in Fig. 2C. Low levels of THMs were detected in untreated water (1.19 ± 0.15 μg L^{-1} max). Concentration of THMs in EW treated irrigation water fluctuated with a maximum concentration of 8.7 ± 2.0 μg L^{-1}. The amount of THMs detected in EW treated water was well below the legal limits for water intended for human consumption (100 μg L^{-1}).[17] The higher concentrations of THMs in EW treated irrigation water were significantly correlated with lower concentration of free chlorine ($P < 0.01$; Pearson correlation coefficient of −0.84). There was no significant correlation ($P > 0.05$) between the concentration of THMs in EW treated irrigation water and indicators of organic matter content (UV254 and TOC). In general, the amount of THMs in chlorine-treated water shows a positive correlation with organic matter as measured by

Table 2. Growing cycle and morphological characteristics (length, width and area) of Oak Leaf and Red Batavia baby lettuce at different harvests

Baby lettuce	Harvest	Growing cycle (days)	Length (mm)	Width (mm)	Area (cm^2)
Oak Leaf	1	35	84.2 ± 15.4^{A}	33.5 ± 6.4^{B}	1294.9 ± 285.4^{A}
	2	24	53.2 ± 7.3^{B}	25.1 ± 4.9^{C}	644.1 ± 200.6^{B}
	3	21	79.3 ± 13.8^{A}	38.6 ± 8.7^{A}	1139.1 ± 381.0^{A}
Red Batavia	1	30	101.5 ± 21.9^{a}	37.9 ± 4.5^{b}	1816.3 ± 444.8^{b}
	2	21	83.6 ± 12.8^{b}	33.2 ± 6.5^{c}	1676.6 ± 462.4^{b}
	3	16	95.7 ± 21.4^{a}	46.0 ± 8.2^{a}	2381.4 ± 817.4^{a}

Values are the mean ± standard deviation ($n = 35$).
Different letters indicate significant differences ($P < 0.05$) between harvests for each type of lettuce.

Table 3. Colour (hue, saturation and value) and concentration of O_2 and CO_2 in the headspace of the bags of Oak Leaf and Red Batavia baby lettuce at different harvests

Baby lettuce	Harvest	Hue	Saturation	Value	O_2 (kPa)*	CO_2 (kPa)*
Oak Leaf	1	30.3 ± 6.9^{A}	68.8 ± 11.0^{C}	127.1 ± 16.9^{A}	11.1 ± 0.4^{A}	6.9 ± 0.2^{B}
	2	29.8 ± 11.6^{AB}	73.7 ± 15.5^{B}	117.3 ± 19.3^{B}	6.9 ± 0.4^{B}	8.9 ± 0.3^{A}
	3	27.5 ± 10.7^{B}	85.9 ± 12.5^{A}	108.4 ± 17.9^{B}	11.6 ± 0.8^{A}	5.8 ± 0.1^{C}
Red Batavia	1	27.4 ± 10.4^{b}	74.7 ± 7.5^{b}	113.9 ± 12.7^{NS}	12.5 ± 0.6^{a}	5.0 ± 0.5^{b}
	2	45.7 ± 27.2^{a}	81.3 ± 11.6^{a}	109.9 ± 22.9^{NS}	10.3 ± 0.5^{b}	6.2 ± 0.1^{a}
	3	31.7 ± 14.9^{b}	87.1 ± 9.7^{a}	109.3 ± 18.7^{NS}	10.6 ± 1.2^{b}	6.0 ± 0.2^{a}

Values are the mean ± standard deviation (colour: $n = 35$), (O_2 and CO_2: $n = 4$).
Different letters indicate significant differences ($P < 0.05$) between harvests for each type of lettuce.
NS, not significant.
*Determined after 9 days at 7 °C.

the total organic carbon (TOC).[28] In our study, TOC levels measured in irrigation surface water were similar to those described for rivers and lakes.[29] TOC levels in untreated water were lower than in EW treated irrigation water probably due to the biofilm formation generated in the pumping system and tubes that could have caused the increase in the content of TOC in the EW treated irrigation water.

Apart from chlorates and THMs, other DBPs can be present in water treated using EW.[11] In order to determine the relevance of other DBPs, some samples of EW treated irrigation water were analysed for haloacetic acids (HAAs) and perchlorates. No perchlorates were detected in any of the samples analysed and HAAs were present but in low concentration (<3 μg L^{-1}).

Quality characteristics of baby lettuce

Evaluation of the quality characteristics of baby lettuce focused on the differences between harvests for the same lettuce type. For both lettuce types, the growing period before the first harvest was longer than the other two regrowths and harvests. The length, width and area of the second harvest were significantly smaller, meaning there was a more immature stage for baby leaves of the second harvest (Table 2). This difference in the maturity stage between harvests was due to a decision of the grower and not due to a physiological stress caused by the disinfection treatment. Significant differences in colour, hue and saturation between harvests were observed for both lettuce types (Table 3). Changes in the headspace gas composition by the respiration of the product showed that O_2 decreased while CO_2 increased within the headspace of the bags. After 9 days of storage at 7 °C, levels of O_2 in the bags ranged between 6.9 to 12.5 kPa O_2 while CO_2 fluctuated between 5.0 to 8.9 kPa (Table 3). Differences observed in headspace gas composition were mainly due to lettuce type and not due to the different harvests. Oak Leaf had slightly higher respiration rate than Red Batavia. In agreement with these observations, Cantwell *et al.*[30] reported higher respiration rate of Red Oak Leaf than Batavia lettuce. When sensory quality was examined, no significant differences were observed between harvests, with only one exception. This exception was Red Batavia from the third harvest that after processing showed a significant ($P < 0.05$) decrease in visual quality when compared with the other two harvests. In any case, visual quality of baby lettuce after processing was always good, with scores ranging between 8.6 and 10 (very good and excellent) in both types of lettuce. After storage, lower visual quality was observed, but remained at acceptable levels (score > 5).

Presence of by-products on baby lettuce

Regarding differences in chlorate content due to processing or storage, the content of chlorate (mean ± SD) in Oak Leaf, taking into account data from the three harvests was 0.08 ± 0.03, 0.08 ± 0.04, and 0.11 ± 0.09 mg kg^{-1}, before processing, after processing (d0) and after storage (d9), respectively. Mean chlorate concentration in Red Batavia was 0.05 ± 0.01, 0.07 ± 0.02, and 0.10 ± 0.09 mg kg^{-1}, before processing, after processing (d0) and after storage (d9), respectively. Tap water used for lettuce washing contained chlorates (~0.5 mg L^{-1}), but it did not affect the chlorate content in lettuce (Fig. 3A and B). Data analysis for both types of lettuce revealed that there were only significant differences among samples of the third harvest, in which chlorate content was significantly higher after storage (Fig. 3A and B). Kaufmann-Horlacher *et al.*[31] indicated that one possible source for chlorate residues in fresh produce is from the use of chlorinated washing water. In the present study, baby leaves were washed and rinsed in

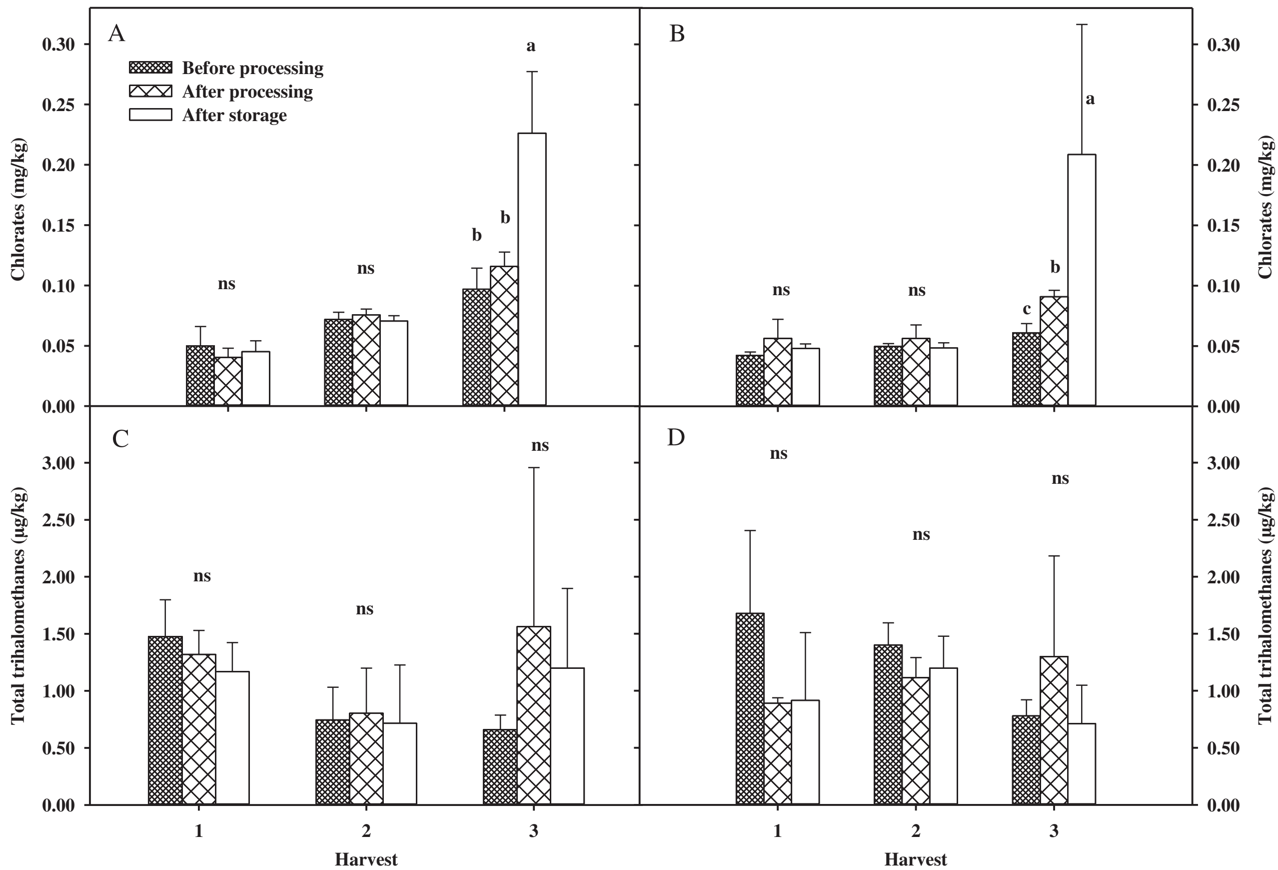

Figure 3. Content of chlorates (mg kg^{-1} fresh weight) in Oak Leaf (A) and Red Batavia (B) lettuce, and trihalomethanes (μg kg^{-1} fresh weight) in Oak Leaf (C) and Red Batavia (D) lettuce at different harvests before and after processing and storage. Different letters for the same harvest indicate statistically significant differences in the chlorate or trihalomethanes concentration ($P < 0.05$). ns, no significant difference. Values are the mean ± standard deviation ($n = 4$).

cold tap water, and consequently processing did not promote the accumulation or the degradation of chlorates. In our previous study on chlorate residues during processing of fresh-cut lettuce, we observed that process wash water disinfected with sodium hypochlorite was the source for chlorate residues in the lettuce.[20] The occurrence of chlorate has been also described in hypochlorite solutions used for drinking water disinfection.[32] In addition, the content of chlorates was not affected during storage except for baby lettuce of the third harvest, with significant increases after processing and storage in both lettuce types. This could be due to the better extractability of chlorates from the lettuce tissue which had deteriorated more, with a poor visual quality as mentioned in the sensory evaluation. On the other hand, when looking at differences between harvests, results showed that there was an accumulation of chlorates during cultivation higher in the third > second > first. These differences were observed more clearly in Oak Leaf while in Red Batavia differences were statistically significant only in the third harvest. Chlorates present in irrigation water would have accumulated in baby lettuce in two ways. First, by direct contact of irrigation water with the aerial parts of the plants because of the sprinkler irrigation, and second, by the uptake from the substrate. Chlorates can be taken up through the roots, accumulating mainly in the leaves.[15] Nitsopoulos *et al.*[33] detected systemic uptake of chlorate in lettuce irrigated with water containing low levels of chlorates (0.043 mg L^{-1}). Chlorates present in the substrate were not measured in our study, but these ions can persist in soil and would have accumulated through the successive growing cycle and through the further harvests. The accumulation in the substrate could explain the higher content of chlorates in samples of the third harvest. Our results confirm that the use of EW as a chlorine based agent for the disinfection of irrigation water can be a source for chlorate residues in food.[15,33] The limit for chlorates in the European Union is 0.01 mg kg^{-1}.[16] All the lettuce samples showed chlorate levels above this limit. New limits are being evaluated by the European Commission for each group of commodities and for baby leaves is 0.06 mg kg^{-1}.[15] Content of chlorates in our study was above this level in 51.4% of the lettuce samples (63.9% for Oak Leaf and 38.9% for Red Batavia).

Changes in the content of THMs in Oak Leaf and Red Batavia are shown in Fig. 3C and D, respectively. Total THMs ranged between 0.66 and 1.56 μg kg^{-1}, and 0.71 and 1.68 μg kg^{-1} in Oak Leaf and Red Batavia, respectively. The slight decrease at second harvest, and then the slight increase in values after processing and storage at third harvest could be due to the plant variability as the concentration of THMs detected was very low in all the cases. However, these differences observed were not statistically significant between lettuce types, harvests, or due to processing and storage. The slight decrease at second harvest, and then the slight increase in values after processing and storage at third harvest could be due to the plant variability There is some information available related to the presence of THMs in fresh produce due to the use of chlorinated water during processing.[34,35] The tap water used for lettuce washing in our study contained THMs (95.3 ± 5.1 μg L^{-1}), but it did not affect the THMs content of lettuce (Fig. 3). However, lack of data related to the presence of these compounds in the agricultural environment and the potential accumulation at the pre-harvest step has been reported.[36] Apart from the THMs present in the irrigation water, more DBPs such as haloacetic acids (HAAs) could have been formed by the reaction of free chlorine (present in irrigation water) with organic matter.[11] Sorption of THMs and HAAs present in water from washing and rinsing vegetables has been observed, suggesting potential uptake from irrigation water.[11,37] Recently, Lonigro *et al.*[38] observed accumulation of organohalogenated by-products in soil, roots and leaves of lettuce irrigated with chlorinated municipal wastewater. In our study, low amounts of organic matter were present in the water, and low quantities of THMs were detected in treated water and in baby lettuce. Trihalomethanes are volatile compounds at room temperature with a high vapour pressure, and therefore, those present on the surface of the plant due to sprinkler irrigation would partition in the surrounding air, avoiding the accumulation inside the plant tissue. If there was accumulation in substrate and absorption by the plants, accumulation of these compounds was not detected by the analysis of the lettuce tissue.

CONCLUSIONS

We observed that the disinfection of irrigation water with a concentrated solution of EW led to a significant increase in chlorate concentration. The presence of chlorates in the irrigation water caused an accumulation of chlorates in the crop reaching levels above the current maximum residual limit for leafy greens. Accumulation of chlorates in the substrate and absorption through the roots could explain the higher levels detected in the second and third harvests compared to the first one after the plants regrew. In general, processing, particularly washing with tap water, and storage did not affect the chlorate content in lettuce except for the third harvest that even increased. On the other hand, EW did not impact the presence of THMs in irrigation water and in baby lettuce as the content remained very low. Therefore, mitigation strategies that reduce the occurrence of DBPs caused by the disinfection technologies should be assessed (e.g. use of alternatives to chlorine).

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