



Should chlorate residues be of concern in fresh-cut salads?



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ABSTRACT

Chlorine remains the most popular method used by the fresh produce industry for decontamination. However, the occurrence of disinfection by-products (DBP) derived from chlorine-based disinfectants has been highlighted as a problem. After recent reports, chlorate residues in fresh produce are of concern in Europe. This study evaluated the chlorate accumulation in process wash water and the residues in fresh-cut lettuce when sodium hypochlorite was used as a wash aid. At a commercial processing facility, total chlorine was continually added to achieve a free chlorine level of 1–80 mg L⁻¹ for water disinfection as the organic load measured as chemical oxygen demand (COD) increased over time (1000–1500 mg O₂ L⁻¹). This resulted in chlorate accumulation (19–45 mg L⁻¹) in the process water. When fresh-cut lettuce was washed in that water, chlorate residues were detected in the lettuce and the concentrations increased linearly with the repeated use of the same process water, reaching concentrations of 4.5–5.0 mg kg⁻¹. To understand the chlorate accumulation in the process wash water, several experiments were performed at a pilot plant scale with different levels of COD and free chlorine. There was a significant ($p < 0.001$) correlation ($R = 0.91$) between the total added chlorine and the chlorate accumulation in the process water. We demonstrated that the added chlorine needed to maintain a free chlorine level in the process water was the contributing factor to chlorate accumulation. Chlorate residues in the washed fresh-cut lettuce after rinsing for 1 min in tap water and in commercial bags were below the limit of quantification. This study contributes to the knowledge of chlorate accumulation in the process water when sodium hypochlorite was used as a sanitizer.

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1. Introduction

The role of water during food production and processing has been recognized as an important contributing factor to foodborne disease (EFSA, 2013; FAO/WHO 2008). This is particularly relevant for the fresh-cut produce industry in which the process wash water accumulates debris, dirt, organic matter and microorganisms from the surface of the product as it is washed. If contaminated product is introduced in the washing tank, there is a potential risk for cross-contamination (López-Gálvez et al., 2010; Luo et al., 2011; Van Haute, Sampers, Holvoet, & Uyttendaele, 2013). Water disinfection has been described as an essential activity to reduce cross-contamination during washing and inadequately or improperly disinfected wash water can itself become a major source of contamination (Gil, Selma, López-Gálvez, & Allende, 2009). The risk of cross-contamination is not removed by using large quantities of

water; a water disinfection agent is needed (Whitaker & Gorny, 2013).

Chlorine is the most commonly used water disinfectant in the fresh produce industry. This prevalence of commercial chlorine usage is attributable to its established ability to kill pathogens in solution, minimal impact on product quality and low cost (Allende, Selma, López-Gálvez, Villaescusa, & Gil, 2008; Luo, 2007). Chlorine forms several compounds in water such as hypochlorous acid (HOCl), chlorine gas (Cl₂) and hypochlorite ion (OCl⁻) in amounts that vary with the water pH. The terms free chlorine or available chlorine are used to describe the amount of chlorine in any form available for oxidative reaction and disinfection (Suslow, 1997). Extensive study has revealed that hypochlorous acid is the most effective antimicrobial form of chlorine (Zhao, Doyle, & Zhao, 2001). The inhibitory or lethal activity depends on the concentration of free chlorine and contact time (Luo et al., 2011; Gómez-López, Lannoo, Gil, & Allende, 2014a). Free chlorine should be remained at a residual dose in the water as a measure of disinfection protection. Consequently, effective chlorination depends upon frequent monitoring of the free chlorine and other water

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parameters such as pH and oxidation-reduction potential (ORP).

Cleaning and rinsing designs of a processing facility for fresh-cut produce vary greatly. Different steps include the wash tanks or flume where the product can be exposed to various washes and rinses before de-watering via centrifugation and pack (Pao, Long, Kim, & Kelsey, 2012). The main characteristic of process water is that it contains a large amount of organic matter from the exudates of the cut tissues (Shen et al., 2013). A common practice in fresh produce washing is that large volumes of chlorinated water are continuously replenished into the wash water tanks throughout the processing activity (Barrera, Blenkinsop, & Warriner, 2012). Water replenishing is used to replace water loss during washing, maintain the water temperature at about 4 °C and reduce the accumulation of organic matter. Chlorine is normally applied to process water as chlorine gas, sodium hypochlorite solution or dry calcium hypochlorite to produce free chlorine in water (Suslow, 1997). Sodium hypochlorite is the chlorine source frequently used in small and medium-scale operations because it can be handled without high expertise and with a minimal hazard as compared to chlorine gas. During the last decades, increasing number of large processing facilities has changed to hypochlorite reagents too due to the more stringent norms of security. However, it is difficult to maintain a relatively consistent level of free chlorine during commercial fresh-cut wash operations because chlorine is very reactive and readily combines with almost any substrate capable of being oxidized to form DBP in the water such as trihalometanes (THM) (IFPA, 2001; Luo et al., 2011; Van Haute et al., 2013). In the case of prepared salads, the Committee on Toxicity of Chemicals (COT, 2006) reported that the occurrence of THMs complies with the WHO Guidelines for Drinking Water Quality (López-Gálvez et al., 2010; Van Haute et al., 2013; WHO., 2004). However, a new alarm blew up by a study carried out by the Chemisches und Veterinäruntersuchungsamt (CVUA, Stuttgart) on chlorate residues in plant-based food (Kaufmann-Horlacher, Scherbaum, Stroher-Kolberg, & Wildgrube, 2014a). In this survey, 600 samples of products of plant origin were analyzed. In 19.8% of them, residue levels were found between 0.01 and 0.92 mg kg⁻¹. Chlorate (ClO₃⁻) is a substance that is no longer approved as a pesticide according to Commission Decision No 2008/865/EC (EC, 2008). No specific maximum residue levels (MRLs) have been established and therefore, a default MRL of 0.01 mg kg⁻¹ was applicable. However, in many fruit and vegetable commodities chlorate levels exceeding the default MRL were found. This limit was temporally increased to 0.25 mg kg⁻¹ for all vegetable commodities (EC., 2014). Recently, EFSA was asked by the European Commission to consider the impact on dietary exposure of applying the WHO guidance level for chlorate in drinking water of 0.7 mg per kilogram (mg kg⁻¹) to all foods covered by EU legislation (EFSA, 2015). The EFSA Panel on Contaminants in the Food Chain concluded that based on the current practices in food industry, application of a hypothetical maximum residue limit (MRL) of 0.7 mg/kg for all foodstuffs and drinking water would only minimally reduce acute/chronic exposures and related risks. The Panel reported that the source of the chlorate residues detected in food could arise from the use of chlorinated water for food processing and the disinfection of food-processing equipment. Chlorate levels of up to the level of 0.7 mg/L can be found depending on the extent of chlorination, which varies amongst Member States. Accordingly, in a study carried out in carrots, chlorate residues were traced to chlorinated water used in post-harvest treatment (Kaufmann-Horlacher, Scherbaum, Stroher-Kolberg, & Wildgrube, 2014b). In the case of the water industry, chlorate is considered to come from hypochlorite reagents (García-Villanova, Oliveira Dantas Leite, Hernández Hierra, de Castro Alfame, & García Hernández, 2010). Chlorine disproportionates slowly but given enough time, it may be almost completely

converted to chloride and chlorate. Thus, one potential source of chlorate residues is the use of chlorine-based sanitizers as water disinfectant during washing of fresh produce. The aim of the present study was to gain insight regarding the potential risk of chlorinated water as a source of chlorate residues in the process water and therefore in the washed product.

2. Materials and methods

2.1. Wash process description

Two types of experiments were carried out. First, a sampling was carried out at a commercial processing facility and secondly, a pilot plant was used to understand the different factors responsible for the chlorate accumulation. At the commercial processing plant, lettuce heads were loaded onto a conveyor and passed through a shredder for cutting into 30 × 30 mm slices. Cut lettuce was emptied into a wash tank containing 1000 L of water dosed with sodium hypochlorite (Centaurio, Fab. Ind. Gamer, Murcia, Spain) with very little water replenishment during the processing activity (500–700 L h⁻¹ approx.). The resident time of the lettuce within the wash tank was approx. 1 min. Then, the washed lettuce was rinse by shower and de-watered via centrifugation prior to being packed. Sodium hypochlorite and cooling water were added into the washing tank as necessary based on the pH and free chlorine.

The experiments performed at a pilot plant were carried out using a 'dynamic system' previously described (Gómez-López, Gil, Pupunat, & Allende, 2014b). A washing tank was filled with a volume of 10 L of tap water before starting the tests. Concentrated process water containing a high concentration of organic load (>2000 mg O₂ L⁻¹, chemical oxygen demand, COD) was generated from the homogenization of Iceberg lettuce. During the experiments, concentrated process water with high organic load was constantly pumped into the washing tank simulating the washing operation in a commercial processing line. The system was assumed to be a perfectly mixed reactor. A concentrated chlorine solution of active chlorine of 40 g L⁻¹ was continuously dosed to the washing tank at a flow rate necessary to achieve the desired free chlorine. This chlorine dose represented the total amount of added chlorine and this dose minus the residual is what reacted with the organic matter.

2.2. Water quality parameters

Samples of process water from the washing tank were taken at different time intervals for the evaluation of water characteristics such as pH, oxidation reduction potential (ORP), electrical conductivity (Ec) and chemical oxygen demand (COD). The temperature of process water was also monitored and maintained within 4 ± 1 °C for the product to cool down and the chlorine dissociation at this temperature was considered to be minor. The oxidation reduction potential (ORP) is an indicator of the ability of a solution to oxidize and is related to the concentration of oxidizers and their activity or strength. Temperature, pH and ORP were measured using a multimeter pH & Redox 26 (Crison, Barcelona, Spain). Water pH was adjusted to 6.4 using citric acid to ensure the presence of chlorine in the hypochlorous acid form. Between pH 6.5 and 7.0, hypochlorous acid exists as 95–80% of the free chlorine concentration. Free chlorine was measured by the N,N-diethyl-*p*-phenylendiamine (DPD) colorimetric method (APHA, 1998) using the Spectroquant NOVA 60 photometer (Merck, Darmstadt, Germany). Chemical Oxygen Demand (COD) was measured by the standard photometric method (APHA, 1998) using the Spectroquant NOVA 60 photometer.

2.3. Fresh produce sample collection

At the commercial processing plant, samples (100 g) of washed cut lettuce and washed and rinsed cut lettuce were taken at different time periods throughout the processing activity. Dewatering was carried out by centrifugation at 2400 g for 20 s. Five commercial bags of fresh-cut Iceberg lettuce from 3 different processors were purchased from a local supermarket and analyzed. At the pilot plant, samples of 100 g of shredded Iceberg lettuce washed for 1 min in chlorinated-process water and rinsed for 1 min in tap water were taken at different time intervals. A manual salad spinner was used for 1 min to remove the excess of water.

2.4. Chlorate analyses

The Quick Polar Pesticides method (QuPpe) was used (Anastassiades et al., 2013). Ten grams of homogenized lettuce were extracted with 10 mL of acidified methanol, centrifuged at 2500 rpm for 5 min and then filtered through a 0.22 μm filter. For the analysis of water, samples were filtered and ten-fold diluted with acidified methanol. Analysis was carried out in a LC-tandem MS chromatograph (Agilent 1290 infinity, Agilent Technologies, Santa Clara, CA) coupled to a Triple Quad mass spectrometer (Agilent 6460) equipped with an interface Jet Stream ESI source operating in negative ion mode. Separations were performed using a Thermo Hypercarb column of 2.1 \times 100 mm, 5 μm particle size (Thermo Fisher Scientific, Waltham, MA). The flow rate was set from 0.2 to 0.4 mL min⁻¹. The mobile phase consisted of two solvents: water with acetic acid (1%) and methanol (5%) (A) and methanol with acetic acid (1%) (B). The method for chlorate analysis monitored two transitions: ³⁵ClO₃⁻ (*m/z* 83) to ³⁵ClO₂⁻ (*m/z* 67) for identification and quantification and the transition ³⁷ClO₃⁻ (*m/z* 85) to ³⁷ClO₂⁻ (*m/z* 69) for confirmation. Chlorate accumulation was quantified with an analytical standard of chlorate (RTC, ICS-004-100, Fluka, Sigma–Aldrich, Spain). The method was validated for water and lettuce samples and the matrix effect was evaluated by comparing the response of the ClO₃⁻ ion standard in pure water with the response of that compound in lettuce matrix diluted 10 fold with acidified methanol. No significant differences were observed between the slopes of the calibration curves for the standard in water and in the matrix. Sodium chlorate was analyzed and the results expressed as mg L⁻¹ for water samples and mg kg⁻¹ fresh weight for lettuce samples. The detection limit for chlorates in water and in produce was 0.0007 mg L⁻¹/mg kg⁻¹ and the quantitation limit was 0.0024 mg L⁻¹/mg kg⁻¹, respectively.

2.5. Statistical analysis

The pilot plant experiments were repeated three times and the test carried out at the commercial processing facility was repeated twice. Each repetition was conducted on different days. In the case of chlorate measurements, at least 4 replicates were taken for each sampling point including the analysis of commercial bags while two replicates were taken for the physicochemical analyses (COD, free chlorine). Data from one repetition was selected for the graphical representation as similar results were obtained in all the repetitions.

3. Results and discussion

3.1. Potential risk of chlorinated water as a source of chlorate residues under commercial conditions

At the commercial processing plant, water characteristics of process water such as pH, ORP, conductivity, COD and chlorine

levels at different time periods through the processing activity were evaluated (Table 1). The effect of NaOCl increasing the pH values of the process water was clearly observed. As the pH increased from 6.95 to 8.10, around 80% of the free chlorine changed from the hypochlorous acid (HOCl) form to the hypochlorous ion (OCl⁻) form which is less effective as an oxidizer. To maintain the free chlorine in its most active form (HOCl), the pH should have been maintained between 6.5 and 7.0. The ORP of the process water changed from 460 to 710 mV, indicating the ability or potential of the chlorine to oxidize (Suslow, 1997). Potable water has an ORP value between 200 and 300 mV. This range is considered the 'zero' point and the addition of oxidizing chemicals will result in values greater than 300 mV (McPherson, 1993). In 1972, the standard for drinking water issued by the World Health Organization recognized that an ORP value of 650 mV provided instantaneous disinfection. In our case, there was an important increase in the conductivity (Ec) of the process water from 670 to 1100 $\mu\text{S cm}^{-1}$ (Table 1). Process water was characterized by high organic matter content (COD value between 1000 and 1500 mg O₂ L⁻¹). Under these conditions, the consumption of total added chlorine occurred rapidly and the initial free chlorine (120 mg L⁻¹) was completely depleted after the reaction with the organic matter from the lettuce juice within the first 4 h. Chlorine replenishing was performed to maintain the desired level of free chlorine in the process water. Greater variation in free chlorine concentration occurred mostly due to the presence of larger amounts of COD. Six hours after the processing activity started, a concentration of 5 mg L⁻¹ free chlorine was maintained in the process water with a COD value of 1500 mg O₂ L⁻¹. After that, the replenishment of total added chlorine resulted in higher free chlorine. High amounts of chlorate were measured in the process water since the beginning of the washing process (Table 1). Therefore, the concentration of chlorate formed increased progressively with the increase in total added chlorine. Several studies reported the difficulties of maintaining a relatively consistent level of free chlorine because chlorine is very reactive and readily combines with the organic matter (Luo et al., 2011; Van Haute et al., 2013). Higher organic loads lead to faster chlorine consumption due to its reactivity with certain organic species (Waters & Hung, 2014). In our case, as the total added chlorine in the process water increased to some degree by the repeated addition of chlorine to the process water, the concentration of chlorate increased. The replenishment of water was not enough to dilute the chlorate concentration that increased over-time reaching values close to 45 mg L⁻¹ after 8 h (Table 1). These data indicate that due to the high levels of organic matter from the lettuce exudate when the cell content of the cut tissue leaked out into the chlorinated-wash water, higher chlorine dose was needed to be added to maintain a desired free chlorine concentration and high chlorate levels were accumulated.

When fresh-cut lettuce was washed, chlorate residues increased linearly ($R^2 = 0.994$) with time because of the repeated use of the same process water with very little water replenishment (Fig. 1). As the processing activity advanced in time, chlorate residues in the washed lettuce increased from 2.0 to 4.5–5.0 mg kg⁻¹ approx. after 4 and 8 h, respectively (Fig. 1). The rinsing step applied in this industry caused a reduction in the chlorate residues of less than 0.5 mg kg⁻¹. These results highlight the potential risk of chlorate residues in the washed product when sodium hypochlorite is used to disinfect process water with high organic loads. The results obtained also showed that chlorate residues in lettuce corresponded to approx. 10% of the levels contained in the process water. This agrees with a previous report by Nitsopoulos, Glaumer, and Friedle (2014) who showed that lettuce washed in process water containing 0.7 mg L⁻¹ of chlorates absorbed 10% of the chlorates (0.07 mg kg⁻¹). According to this, the Health and Consumers

Table 1

Quality characteristics of the process water of a commercial processing plant including pH, oxidation reduction potential (ORP), electrical conductivity (Ec), chemical oxygen demand (COD), free chlorine and chlorate values.

Time (h)	pH	ORP (mV)	Ec ($\mu\text{S cm}^{-1}$)	COD (mg L^{-1})	Free chlorine (mg L^{-1})	Chlorate (mg L^{-1})
4	6.95 \pm 0.04	463 \pm 7	672 \pm 3	1096 \pm 6	1	19.4 \pm 0.4
6	6.97 \pm 0.05	564 \pm 10	846 \pm 9	1500 \pm 100	5	27.3 \pm 0.3
8	8.10 \pm 0.04	709 \pm 10	1105 \pm 7	1205 \pm 25	80	44.8 \pm 0.4

Values are the mean of 4 replicates \pm standard deviation.

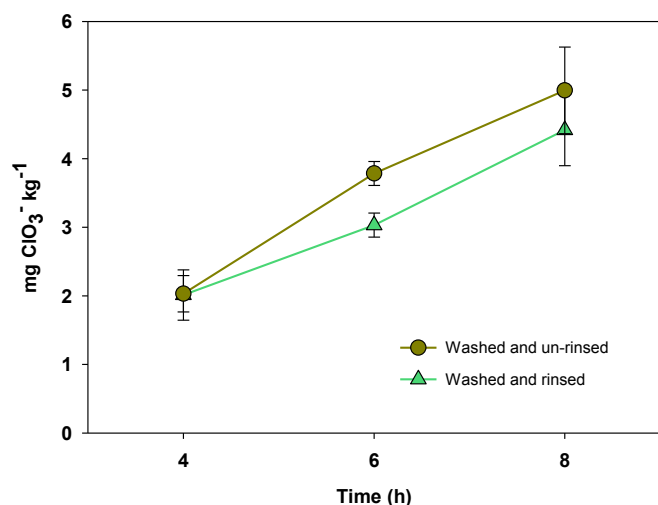


Fig. 1. Chlorate in the washed iceberg lettuce before and after the rinsing step in an industrial washing tank at different intervals. Values are the mean of 4 replicates \pm standard deviation.

Directorate-General of the European Commission (DG SANCO) in a recent statement reported that chlorates levels in fresh produce and in frozen vegetables appear to be inversely related to the replenishing rate of the washing water in which chlorate residues have a tendency to concentrate (EC., 2014).

3.2. Elucidation of contributing factors for chlorate accumulation

The accumulation of chlorate was evaluated using a pilot plant system equipped with a dynamic system in which different COD and free chlorine levels were constantly added. The first experiment started using tap water in which a continuous addition of organic matter increased the COD up to 700–750 $\text{mg O}_2 \text{L}^{-1}$ of the process water while maintaining a constant free chlorine concentration of 3 mg L^{-1} (Fig. 2). These conditions represented situations similar to those found in processing lines. Four hours after the process started, chlorate concentration in the process water was 8.3 \pm 0.3 mg L^{-1} (Fig. 2). The chlorate accumulated showed a similar trend to the total added chlorine except at 3 and 4 h where the total added chlorine in the process water increased but the chlorate concentration did not increase to the same degree.

It is well documented that hypochlorite solutions disproportionate to produce chloride and chlorate (Bolyard, Fair, & Hautman, 1992; White, 1986). Chlorate concentrations arising from the use of sodium hypochlorite may vary widely from 0.2 to 50 g L^{-1} in the stock solutions probably due to the holding time during the manufacture and storage (Bolyard, Fair, & Hautman, 1993) as well as the concentration (Stanford, Pisarenko, Snyder, & Gordon, 2011). In the case of drinking waters, García-Villanova et al. (2010) hypothesized that a higher holding time of hypochlorite solutions result in a loss of active chlorine and the need to add larger volumes of chlorine. Thus, large amounts of chlorite and chlorate would be

added to the finished water. In addition, the most stable stock solutions are those of low hypochlorite concentration (<10% available chlorine) with a pH of 11 and stored in the dark at temperatures below 20 °C (Bolyard et al., 1992; White, 1986). In the case of processing plants, due to the high consumption of chlorine, the need of concentrated stock solution (~40 available chlorine) is a reality, which difficulties the control of chlorate accumulation.

A second trial was carried out starting also with tap water and increasing the COD of the process water up to 700–750 $\text{mg O}_2 \text{L}^{-1}$. However, in this case the free chlorine was constantly increased up to 20 mg L^{-1} (Fig. 3). It was observed that, chlorate in process water was progressively formed as the total chlorine was added to increase progressively the free chlorine, confirming previous hypothesis. The concentration of chlorate was influenced by the total concentration of chlorine, which depended of the organic matter.

To investigate the factors affecting chlorate in the process water and in the product, a third trial was performed using tap water (COD = 50 $\text{mg O}_2 \text{L}^{-1}$) in which chlorine was constantly added to increase the concentration of free chlorine from 0 to up to 23.4 mg L^{-1} (Fig. 4). In this case, chlorate increased from 0 to 3 mg L^{-1} approx. after 1 h and remained constant over time. In this case, the COD of the water was very low and less chlorine was needed to maintain the free chlorine concentration. The concentration of added chlorine was very low (10–20 mg L^{-1}) because there was no chlorine demand by the organic matter. Therefore, as previously demonstrated, the most critical contributing factor to the chlorate accumulation was again the total added chlorine needed to maintain free chlorine level of the process water. Chlorate accumulation was 4 times higher in process water with high organic matter (Fig. 3) than in tap water (Fig. 4).

Our results showed that there was a significant ($p < 0.001$) correlation (Pearson's correlation factor of $R = 0.91$, $n = 28$) between the added chlorine and the chlorate accumulation. In the case of poultry chiller water the accumulation of chlorate was dependent on both chlorine dose and treatment time (Tsai, Higby, & Schade, 1995). The decomposition of chlorine and subsequent formation of chlorate are dependent on the concentration and ionic strength of the stock solution (White, 1986). Higher concentration and ionic strength will drive the reaction towards greater production of chlorate and the rate of decomposition (Stanford et al., 2011).

3.3. Potential risk of chlorate residues in commercial salad bags

A survey of chlorate residues in commercial fresh-cut lettuce bags was carried out by the analysis of commercial fresh-cut iceberg ($n = 5$) from 3 different fresh-cut producers. Surprisingly, chlorate residues were very low (0.06 \pm 0.03 mg kg^{-1} max) after the analysis of 4 replicates per producer and among the different producers the variation was very low. These levels were much lower than the 0.7 mg kg^{-1} established by EFSA for all foodstuffs to minimally reduce acute/chronic exposures and related risks. The differences observed between the chlorate residues in the commercial product and those obtained in the product tested at the commercial processing facility indicate that there must be

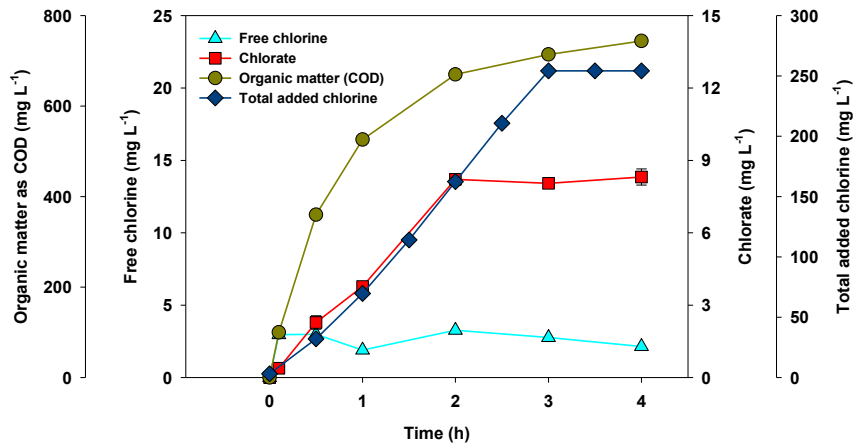


Fig. 2. Chlorate in process water where organic load and chlorine were constantly added to reach levels of chemical oxygen demand (COD) of 700–750 mg O₂ L⁻¹ and free chlorine of 3 mg L⁻¹. Values for chlorate are the mean of 4 replicates ± standard deviation.

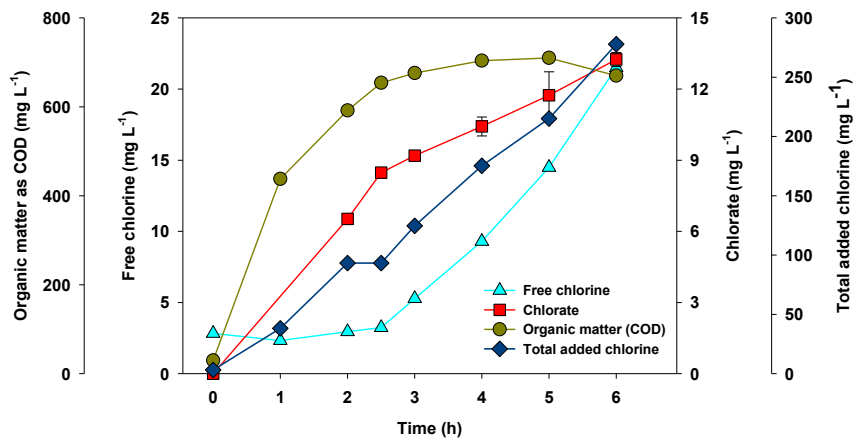


Fig. 3. Chlorate in process water where organic load and chlorine were constantly added to reach levels of chemical oxygen demand (COD) of 700–750 mg O₂ L⁻¹ and free chlorine up to 20–25 mg L⁻¹. Values for chlorate are the mean of 4 replicates ± standard deviation.

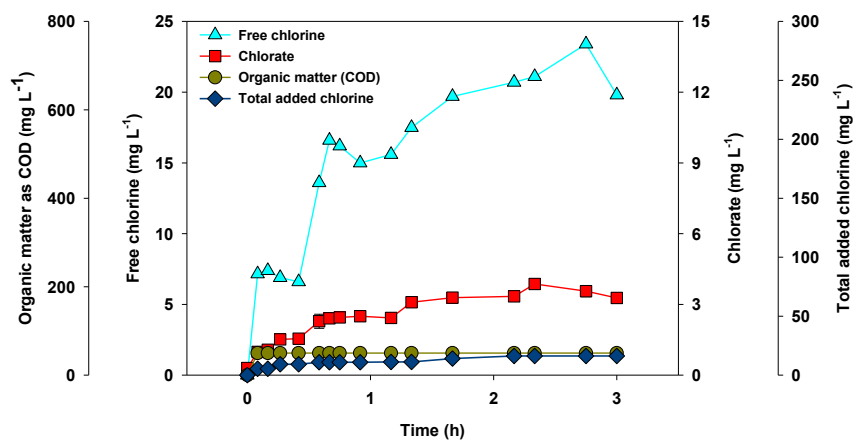


Fig. 4. Chlorate in tap water (COD = 50 mg O₂ L⁻¹) where chlorine was constantly added to reach levels of free chlorine up to 20–25 mg L⁻¹. Values for chlorate are the mean of 4 replicates ± standard deviation.

differences in some processing steps such as water replenishment and spray rinse that have a strong impact on the chlorate residues in the final product.

These results were confirmed at a laboratory scale when lettuce was washed in process water where organic load and chlorine were constantly added (Fig. 3). After rinsing for 1 min with tap water,

chlorate residues were below the limit of quantification at any time despite chlorate accumulation in process water increasing up to 13 mg L⁻¹ after 6 h. Consequently, when the chlorate accumulation in process wash water was ≤13 mg L⁻¹ together with a good rinsing step the risk of chlorate residues in the cut lettuce was low.

Once chlorate is introduced in the treated water, there is no

economically feasible technology for removing or reducing its concentration. Control strategies to minimize the accumulation of chlorate and introduction into process water must be carried out such as the use of diluting stock solutions in which the rate of chlorate accumulation decreases. Such solutions should be used as soon as possible after manufacture and should not be stored for more than one to two days (Stanford et al., 2011). Contents of chlorate in calcium hypochlorite pellets have been shown to be much lower than those of sodium hypochlorite solutions (García-Villanova et al., 2010).

4. Conclusions

Our study investigated the contributing factors of chlorate residues when chlorine was used as a disinfectant aid in process water and fresh-cut lettuce. Chlorate was already present in the sodium hypochlorite solution formed during production/storage. It concludes that sodium hypochlorite was the source of chlorate when added to the process wash water. A 22% of the chlorate was incorporated from the stock solution of sodium hypochlorite. When the organic matter increased, a higher chlorine dose was needed and higher chlorate accumulation occurred. When concentrations of chlorates were present in process water ($<13 \text{ mg L}^{-1}$), the potential risk of chlorate residues in the product was very low, particularly after a water rinse. Adequate control measures must be taken to minimize chlorate such as to have a more diluted chlorine solution and shorter storage times that help minimizing the accumulation of chlorate. The maintenance of low organic load from the exudates of the cut tissues together with a proper water replenishing are preventing actions needed to reduce the amount of chlorine for water disinfection. A proper rinse is needed in order to reduce chlorate residues in the lettuce. The fresh-cut industry needs to move to best manufacturing practices to urgently implement these mitigation options.

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References

- Allende, A., Selma, M. V., López-Gálvez, F., Villaescusa, R., & Gil, M. I. (2008). Impact of wash water quality on sensory and microbial quality, including *Escherichia coli* cross-contamination, of fresh-cut escarole. *Journal of Food Protection*, 71, 2514–2518.
- Anastassiades, M., Kolberg, D. I., Mack, D., Wildgrube, C., Sigalov, I., & Dörk, D. (2013). *Quick method for the analysis of residues of numerous highly polar pesticides in foods of plant origin involving simultaneous extraction with methanol and LC-MS/MS determination (QuPpe-Method)*. Stuttgart, Germany: Laboratory for Chemical and Veterinary Analysis of Food CVUA. Version 7.1. November.
- APHA, American Public Health Association. (1998). *Standard methods for the examination of water and wastewater* (20th ed.). Washington, D.C: American Public Health Association.
- Barrera, M. J., Blenkinsop, R., & Warriner, K. (2012). The effect of different processing parameters on the efficacy of commercial post-harvest washing of minimally processed spinach and shredded lettuce. *Food Control*, 25, 745–751.
- Bolyard, M., Fair, P. S., & Hautman, D. P. (1992). Occurrence of chlorate in hypochlorite solutions used for drinking water disinfection. *Environmental Science & Technology*, 26, 1663–1665.
- Bolyard, M., Fair, P. S., & Hautman, D. P. (1993). Sources of chlorate ion in U.S. drinking water. *Journal American Water Works Association*, 85, 81–88.
- COT, Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment. (2006). *Statement on a commercial survey investigating the occurrence of disinfectants and disinfection by-products in prepared salads* (pp. 1–7). Available at <http://cot.food.gov.uk/sites/default/files/cot/cotstatementwashhids200614.pdf>.
- EC. (2008). European commission decision of 10 November 2008 concerning the non-inclusion of chlorate in Annex I to Council directive 91/414/EEC and the withdrawal of authorizations for plant protection products containing that substance (2008/865/EC). *Official Journal of the European Union*, L 307(7), 18.11.
- EC.. (2014). *European commission, health and consumers directorate-general (DG SANCO)*. Statement as regards the presence of chlorate in food and feed. SANCO-11180-Rev0. Available at https://www.wko.at/Content.Node/branchen/sbg/Lebensmittelgewerbe/RS065_2014_Clorat-Rueckstaende_B1.pdf.
- EFSA Panel on Biological Hazards (BIOHAZ). (2013). Scientific opinion on the risk posed by pathogens in food of non-animal origin. Part 1 (outbreak data analysis and risk ranking of food/pathogen combinations). *EFSA Journal*, 11, 3025–3163.
- EFSA Panel on Contaminants in the Food Chain (CONTAM). (2015). Risks for public health related to the presence of chlorate in food. *EFSA Journal*, 13, 4135–4238.
- FAO/WHO Food and Agriculture Organization of the United Nations/World Health Organization. (2008). *Microbiological hazards in fresh leafy vegetables and herbs*. Meeting report. *Microbial Risk Assessment Series*, 14, 151.
- García-Villanova, R. J., Oliveita Dantas Leite, M. V., Hernández Hierra, J. M., de Castro Alfageme, S., & García Hernández, C. (2010). Occurrence of bromate, chlorite and chlorate in drinking waters disinfected with hypochlorite reagents. Tracing their origins. *Science of the Total Environment*, 408, 2616–2620.
- Gil, M. I., Selma, M. V., López-Gálvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: problems and solutions. *International Journal of Food Microbiology*, 134, 37–45.
- Gómez-López, V. M., Gil, M. I., Pupunat, L., & Allende, A. (2014b). Cross-contamination of *E. coli* O157:H7 is inhibited by electrolyzed water combined with salt under dynamic conditions of increasing organic matter. *Food Microbiology*, 46, 471–478.
- Gómez-López, V. M., Lannoo, A. S., Gil, M. I., & Allende, A. (2014a). Minimum free chlorine residual level required for the inactivation of *Escherichia coli* O157:H7 and trihalomethane generation during dynamic washing of fresh-cut spinach. *Food Control*, 42, 132–138.
- IFPA, International Fresh-Cut Produce Association. (2001). *Wash water sanitation*. In J. R. Gorny (Ed.), *Food safety guidelines for the fresh-cut produce industry* (pp. 121–136). Alexandria, Va: International Fresh-Cut Produce Association.
- Kaufmann-Horlacher, I., Scherbaum, E., Stroher-Kolberg, D., & Wildgrube, C. (2014a). *Chlorate residues in plant-based food: Origin unknown*. Stuttgart: CVUA. Available at http://www.cvuua.de/pub/beitrag.asp?subid=1&Thema_ID=5&ID=1854&lang=EN&Pdf=No.
- Kaufmann-Horlacher, I., Scherbaum, E., Stroher-Kolberg, D., & Wildgrube, C. (2014b). *Chlorate residues in carrots traced to chlorinated water used in post-harvest treatment*. Stuttgart: CVUA. Available at http://www.cvuua.de/pub/beitrag.asp?subid=1&Thema_ID=5&ID=1853&lang=EN&Pdf=No.
- López-Gálvez, F., Allende, A., Truchado, P., Martínez-Sánchez, A., Tudela, J. A., Selma, M. V., et al. (2010). Suitability of aqueous chlorine dioxide vs sodium hypochlorite as an effective sanitizer preserving quality of fresh-cut lettuce while avoiding by-product formation. *Postharvest Biology and Technology*, 55, 53–60.
- Luo, Y. (2007). Fresh-cut produce wash water reuse affects water quality and packaged product quality and microbial growth in romaine lettuce. *HortScience*, 42, 1413–1419.
- Luo, Y., Nou, X., Yang, Y., Alegre, I., Turner, E., Feng, H., et al. (2011). Determination of free chlorine concentrations needed to prevent *Escherichia coli* O157:H7 cross-contamination during freshcut produce wash. *Journal of Food Protection*, 74, 352–358.
- McPherson, L. (1993). Understanding ORP's role in the disinfection process. *Water Engineering & Management*, 140, 29–31.
- Nitsopoulos, A., Glaumer, T., & Friedle, A. (2014). *Chlorate- a contaminant in foodstuff and drinking water*. Agilent technologies Labor Friedle GMBH. Available at http://www.chem.agilent.com/Library/posters/Public/EPRW_Poster_Chlorat_2014_V6.pdf.
- Pao, S., Long, W., III, Kim, C., & Kelsey, F. (2012). Produce washers. In Gómez-López (Ed.), *Decontamination of fresh and minimally processed produce* (1st ed., pp. 90–103). John Wiley & Sons, Inc. (Chapter 5).
- Stanford, B. D., Pisarenko, N., Snyder, S. A., & Gordon, G. (2011). Perchlorate, bromate and chlorate in hypochlorite solutions: guidelines for utilities. *Journal American Water Works Association*, 103, 1–13.
- Suslow, T. V. (1997). *Postharvest chlorination. Basic properties and key points for effective disinfection*. Publication 8003. University of California, Division of Agriculture and Natural Resources. Available at <http://anrcatalog.ucdavis.edu/pdf/8003.pdf> (accessed March 2014).
- Tsai, L.-S., Higby, R., & Schade, J. (1995). Disinfection of poultry chiller water with chlorine dioxide: consumption and by product formation. *Journal of Agricultural and Food Chemistry*, 43, 2768–2773.
- Van Haute, S., Sampers, I., Holvoet, K., & Uyttendaele, M. (2013). Physicochemical quality and chemical safety of chlorine as a reconditioning agent and wash water disinfectant for fresh-cut lettuce washing. *Applied and Environmental Microbiology*, 79, 2850–2861.
- Waters, B. W., & Hung, Y.-C. (2014). The effect of organic loads on stability of various chlorine-based sanitizers. *International Journal of Food Science & Technology*, 49, 867–875.
- Whitaker, B., & Gorny, J. (2013). Wash water: do not harm. In *CPS symposium: 10 key learnings*. <https://cps.ucdavis.edu/>.
- White, G. C. (1986). *Handbook of chlorination* (2nd ed., pp. 68–71). New York: Van Nostrand Reinhold.
- WHO.. (2004). *Guidelines for drinking water quality* (3rd ed., Vol. 1) Recommendations. Available at http://www.who.int/water_sanitation_health/dwq/GDWQ2004web.pdf.
- Zhao, T., Doyle, M. P., & Zhao, P. (2001). Chlorine inactivation of *Escherichia coli* O157:H7 in water. *Journal of Food Protection*, 64, 1607–1609.