



Cross-contamination of *Escherichia coli* O157:H7 is inhibited by electrolyzed water combined with salt under dynamic conditions of increasing organic matter



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ABSTRACT

Water can be a vector for foodborne pathogen cross-contamination during washing of vegetables if an efficient method of water disinfection is not used. Chlorination is the disinfection method most widely used, but it generates disinfection by-products such as trihalomethanes (THMs). Therefore, alternative disinfection methods are sought. In this study, a dynamic system was used to simulate the commercial conditions of a washing tank. Organic matter and the inoculum of *Escherichia coli* O157:H7 were progressively added to the wash water in the washing tank. We evaluated the effectiveness of the electrolyzed water (EW) when combining with the addition of salt (1, 0.5 and 0.15 g/L NaCl) on the pathogenic inactivation, organic matter depletion and THM generation. Results indicated that electrolysis of vegetable wash water with addition of salt (0.5 g/L NaCl) was able to eliminate *E. coli* O157:H7 population build-up and decrease COD accumulation while low levels of THMs were produced.

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1. Introduction

Washing is one of the most important steps in the processing operations of fresh-cut produce. It is usually carried out in the presence of disinfectant solutions to clean vegetable pieces with unwanted residues such as soil and debris and decrease microbial load. However, washing can also serve as a vector for cross-contamination of enteric pathogens that could be eventually present. Therefore, it is important not only to use wash water with high quality but also to protect it from the continuous entrance of organic matter and microorganisms transported by the permanent incoming flow of vegetable pieces into the washing tank. A deeper discussion about problems and solutions of water disinfection for the fresh-cut industry has already been published (Gil et al., 2009).

Water chlorination is the most used disinfection method for wash water. However, the potential formation of dangerous concentrations of disinfection by-products, such as trihalomethanes

(THMs), has promoted the search for other water disinfection technologies of similar or better efficacy. Electrolyzed water (EW) is produced by passing water through an electrolytic cell, where diverse disinfectant species including free chlorine are generated. An external source of chloride such as small amounts of salt (i.e. NaCl) can be added to increase the generation of free chlorine (Fallanaj et al., 2013; Gómez-López et al., 2008). Besides free chlorine, reactive oxygen species (ROS) such as $\cdot\text{OH}^-$ and O_3 , have been identified as disinfection agents in EW (Jeong et al., 2006; Hao et al., 2012). EW inactivates bacteria by increasing membrane permeability and leakage of intracellular content as well as by decreasing dehydrogenase and nitrate reductase activities (Kiura et al., 2002; Zeng et al., 2010). The bactericidal effects of EW on foodborne pathogens are well documented by numerous studies (Izumi, 1999; Gómez-López et al., 2008; Park et al., 2008; Ding et al., 2010). EW has multiple advantages over the use of chlorinated water: 1) it can be produced *on-site*, 2) the raw materials (water and NaCl) are found everywhere, 3) it is environmentally friendly, 4) it reduces cost and hazards associated to chlorine handling, 5) it has low cytotoxicity and 6) the development of resistant strains has not been reported yet (Huang et al., 2008; Al-Haq and Gómez-López,

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2012). *Escherichia coli* O157:H7 is one of foodborne pathogens of great concern during vegetable washing as it has been associated with outbreaks in fresh-cut produce (Erickson, 2012). Electrolyzed water (EW) has been described as effective in inactivating *E. coli* O157:H7 and oxidizing the organic matter in process wash water, allowing water reuse and reducing the environmental pollution caused by water discharges (López-Gálvez et al., 2012). The electrolytic oxidation of organic pollutants has been reviewed (Anglada et al., 2009; Panizza and Cerisola, 2009). However, the increase in the chlorine formation by EW in combination with salt and the formation of THMs is not well known.

Trihalomethanes (THM) have been the main disinfection by-products of concern when chlorine is present in contact with organic matter. Two THMs, chloroform and bromodichloromethane have been classified by the WHO's International Agency for Research on Cancer as possibly carcinogenic to humans (IARC, 1999a,b). Drinking water is the classical exposure route of concern, but others routes are possible and could cause an accumulative effect in addition to drinking water, such as the dermal/inhalation exposure due to showering/bathing/swimming (Richardson et al., 2007). Huang and Batterman (2010) have described that vegetables can absorb THMs from the washing water. The generation of THMs in both process wash water and fresh-cut produce by different disinfection agents have been studied in our group (López-Gálvez et al., 2010).

Most of the studies about fresh produce washing have been performed at laboratory scale with potable water, but it has been recognized that significant differences are found when assessments are performed at a pilot or factory scale (Gil et al., 2009). Moreover, most of the studies have described bacterial inactivation in clean water and only some studies have been performed using wash water with organic matter but in almost all the cases, they were carried out in one-time-event experiment. In this regard, we have previously shown that organic matter decreases the efficacy of EW to inactivate *E. coli* O157:H7 in process water (López-Gálvez et al., 2012). To evaluate the impact of different operating factors on the efficacy of EW to inactivate *E. coli* O157:H7 and the organic matter depletion, experiments were carried out in a one-time-event. This means that free chlorine was added once the washing process has been initiated and that the washing process was simulated at a single moment with the addition of a specific COD (725 mg O₂/L) (Gómez-López et al., 2013a) or different CODs (60, 300, 500 or 750 mg O₂/L) (López-Gálvez et al., 2012). Recently Van Haute et al. (2013) published the results of a dynamic washing process for addition of chlorine but with a fixed COD value for wash water disinfection. However, in our opinion, the real situation in a washing tank, at least during the first hours, is better exemplified by a continuous addition of organic matter and microorganisms to the process water. **The aim of this study was to evaluate the efficacy of EW combined with salt on *E. coli* O157:H7 inactivation in vegetable wash water simulating the process wash water from leafy vegetable processing companies. The chemical safety of the wash water during the washing process was also evaluated by measuring the organic matter depletion and THM generation.**

2. Materials and methods

2.1. Bacterial strains and inoculum preparation

A five-strain cocktail of *E. coli* O157:H7 (CECT 4267, 4076, 4782, 4783, and 5947), provided by the Hibro Group from the University of Cordoba (Spain), was used in the study. Cultures were rehydrated in Brain Heart Infusion broth (BHI, Oxoid, Basingtoke, United Kingdom). Nalidixic acid-resistant (Nal^R) *E. coli* O157:H7 cultures were obtained by consecutive 24-h transfers of BHI cultures to BHI

with increasing concentrations of nalidixic acid (Nal) (Merck, Darmstadt, Germany) until strains were resistant to 50 µg Nal/mL. Nal^R *E. coli* O157:H7 cultures were consecutively sub-cultured twice in 5 mL of BHI supplemented with Nal (50 µg/mL) at 37 °C for 20 h. After the second incubation, cultures were mixed, equal volumes of cell suspensions were combined to give approximately equal populations of each culture. Final concentrations of the inoculum solutions were confirmed by plating on Chromocult coliform agar (Merck, Barcelona, Spain) Nal⁺ (50 µg/mL).

2.2. Wash water

The process wash water was obtained following the protocol described by López-Gálvez et al. (2010). Iceberg lettuce (*Lactuca sativa* L.) was purchased from a local wholesale market in Murcia (Spain) at the day of harvest and transported within 15 min under refrigerated conditions to the laboratory. Outer leaves were manually removed and discarded while internal leaves were cut into 3 cm pieces. Afterward, batches of 67 g of lettuce each were placed into stomacher bags (Seward Limited, London, UK). Two hundred mL of water were added and the mixture was homogenized for 2 min in a stomacher (AES Chemunex, Bruz, France). The obtained process wash water was filtered through a nylon mesh with gaps of 0.5 mm, in order to avoid obstruction of the electrolytic cell. The batch of process wash water was divided in portions and frozen at –20 °C until use. The obtained process water had a very high COD (>2000 mg/L) that needed to be diluted to attain specific COD levels. Process water was thawed overnight the day before each test, and then diluted with tap water. Thawed vegetable wash water had the following characteristics: alkalinity (150 ± 25 mg equiv. CaCO₃/L), turbidity (123 ± 58 NTU), and conductivity (1786 ± 126 µS/cm).

2.3. Electrochemical equipment

Disinfection experiments were performed using a bench top treatment system defined as “dynamic system” where organic matter was constantly added to the washing tank (Fig. 1A). The dynamic system included: a power supply, control board, centrifuge pump for water recirculation, two peristaltic pumps for dosing, 30 L stainless steel tank (treatment tank), flow-meter of water recirculation, two reservoir polypropylene tanks (one process water reservoir used in all experiments and one chlorine reservoir for chlorination experiments), pipes, and electrolytic cells (for electrochemical disinfection). Different configurations of the dynamic system were used in order to test different disinfection treatments. Fig. 1B shows the configuration when sodium hypochlorite was tested and Fig. 1C corresponds to the configuration for the electrochemical disinfection. The electrolytic cells were kindly provided by WaterDiam France SAS (Franken, France). Process temperature was controlled by pumping cold water through stainless steel heat exchangers immersed into the process water. The electrolysis cell DiaClean[®] 101 8000 with no separation between anodic and cathodic compartment was used. This cell has one boron-doped diamond (BDD) cathode and one BDD anode with an overall effective anode surface area of 67 cm², an electrode boron doping level (known as B/C ratio) of 8000 ppm and a current density of 60 mA/cm² when operated at 4 amperes (A). Amperage was set and controlled through the experiments by the power supply, which changed the polarity of electrodes every 20 min to minimize scale build-up on their surface. The vegetable wash water in the tank was pumped through the electrolytic cell and recycled back to the tank (Fig. 1C). Water recirculation rate was adjusted by means of a valve to 750 L/h. The system was assumed to be a perfectly mixed reactor; at that recirculation rate the whole volume

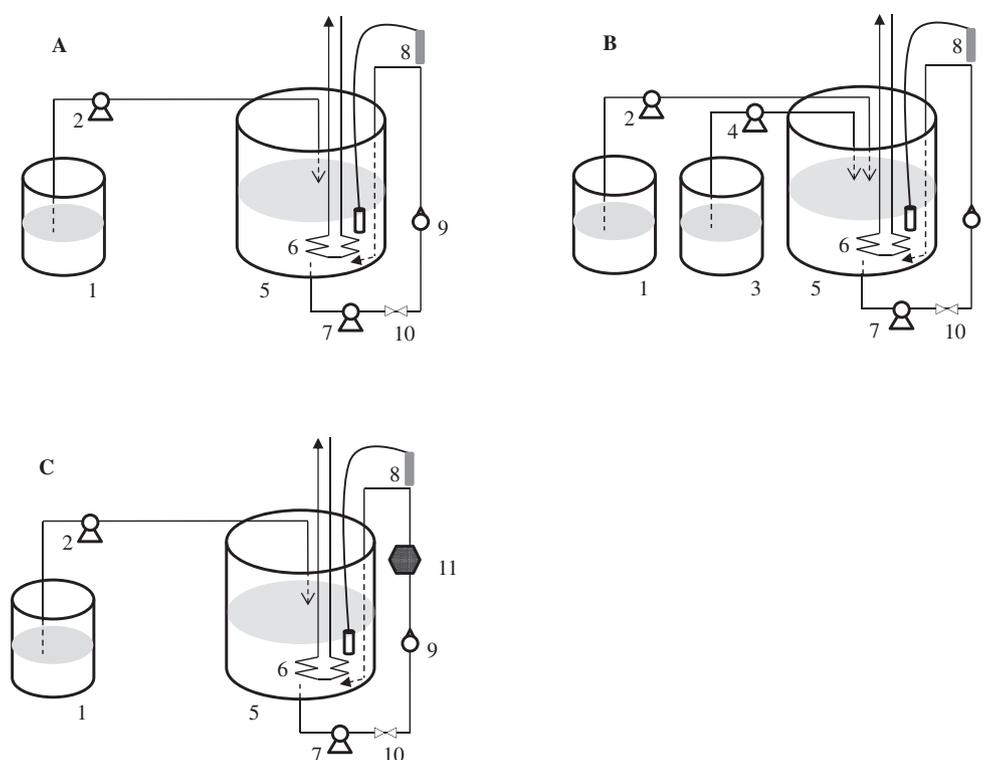


Fig. 1. Schematic representation of dynamic system used for disinfection treatments simulating a washing tank from the fresh-cut industry. A: untreated system, B: chlorination system, C: electrolysis system. 1: reservoir polypropylene tank for inoculated process water; 2, 4: peristaltic pumps for dosing; 3: reservoir polypropylene tank for sodium hypochlorite; 5: 30 L stainless steel tank (washing tank); 6: cooling system; 7: centrifuge pump for water recirculation; 8: sensor for pH, redox potential and temperature; 9: rotameter; 10: flow control valve; 11: electrolytic cell. Figures lacking of any indicative number lack of the respective part.

of water of the tank was re-circulated nearly every minute. Results of microbial counts from experiments with no inactivation (controls) were highly correlated ($r > 0.98$) with those expected for a perfectly mixed reactor estimated by simple mass balances.

2.4. Disinfection treatments

Different disinfection experiments were carried out in the washing tank during 60 min. Before treatments, water pH was adjusted to ca. 6.4 using citric acid to improve chlorine disinfection efficacy. A volume of 11.4 L of tap water was placed in the washing tank before starting the tests with electrochemical disinfection. When sodium hypochlorite was tested, the initial volume was 10.25 L. The concentrated process wash water (>2000 mg/L COD) was inoculated with the *Nal^R E. coli* O157:H7 cocktail at an inoculum level of approximately 5 log cfu/mL just before the beginning of the treatments. The inoculum and the concentrated process wash water solution were continuously added to the washing tank at a flow of 3.6 L/h during the whole duration of the test by a peristaltic pump. Control tests were carried out under the same procedures but without any disinfection technology. For chlorinated water (CW), a second peristaltic pump dosed a ca. 2 mg/L free chlorine solution into the washing tank during the whole duration of the test at a variable flow to maintain a stable free chlorine concentration. This treatment was used as a 'control treatment'. Before running the tests, free chlorine concentration was adjusted to ca. 0.5 mg/L by 1) addition of sodium hypochlorite or 2) starting the electrochemical system without the addition of vegetable wash water. Sodium chloride (NaCl) was added at concentrations of 0.15, 0.5 and 1.0 g/L to the wash water that was pumped through the electrolytic cell to get the disinfection treatments of EW combined with salt. At the start of the treatments, water temperature was

about 6 °C and the highest temperature reached during the experiments was 13 °C.

2.5. Microbiological and physicochemical analyses

Changes in levels of *Nal^R E. coli* O157:H7 were measured at different time intervals during 60 min, with shorter intervals at the beginning of the experiments. Microbial enumeration was carried out as previously described (López-Gálvez et al., 2012). Additionally pour plate method was used to enumerate low number of survivors with a detection limit of 1 cfu/mL.

Changes in the levels of free and total chlorine (mg/L), pH, oxidation-reduction potential (ORP, in mV), temperature (°C), and chemical oxygen demand (COD, in mg/L) were measured at different time intervals. Temperature, ORP, and pH were measured using a multimeter pH and redox 26 (Crison, Barcelona, Spain). Oxidants expressed as free, combined and total chlorine were determined based on the N,N-diethyl-p-phenyldiamine (DPD) method (APHA, 1998) using a commercial kit (Merck, Darmstadt, Germany) and the Spectroquant NOVA 60 photometer (Merck). Chemical oxygen demand (COD) was determined by the standard photometric method (APHA, 1998) using the same Spectroquant photometer. Vegetable wash water was characterized for alkalinity by potentiometric titration with HCl until pH 4.3, the turbidity by a nephelometer (Turbiquant 3000 IR, Merck), and the conductivity by a conductimeter (CM 35, Crison).

2.6. Analysis of trihalomethanes

The extraction protocol and the analytic procedures for THMs quantification were already described by López-Gálvez et al. (2010). Samples were taken from tap water and vegetable wash water

before and after treatments. Summarizing, 20 mL of water were collected in plastic flasks containing sodium thiosulfate (10 g/L) to quench residual chlorine and stored at -20°C until analysis. An internal standard solution of 1-bromo-3-chloropropane was used. Headspace THM's were collected using a solid phase micro-extraction (SPME) device (Supelco, JVA Analytical Ltd, Ireland). Separation was carried out on a gas chromatograph (GC) (Agilent 7890A GC) equipped with an MS detector (Agilent 5975C). Trihalomethanes were separated on an HP-5MS $30\text{ m} \times 0.25\text{ mm}$ (i.d.) capillary column (Agilent) with helium as a carrier gas. Data were collected using the Agilent software and mass spectra of detected compounds were analyzed by library searching in the National Institute of Standards and Technology (NIST) databases. Estimation of THMs was based on the areas of the peaks detected by MS.

2.7. Experimental design

Each experiment was repeated at least twice. Data are the mean of two repetitions. For THMs, data were analyzed by Kruskal–Wallis and Mann–Whitney. Microbial loads were log-transformed and introduced in Excel spreadsheet along with time (raw microbial data). Results were compiled and graphs were made using Sigma Plot 12.0 Systat Software, Inc. (Addilink Software Científico, S.L. Barcelona). Mean statistical differences were analyzed by the Mann–Whitney test. The confidence intervals were established at 95%. IBM SPSS statistics 19 was used for statistical analysis.

3. Results and discussion

3.1. Increase in COD in the washing tank by a dynamic system

By means of the dynamic system, the increase in COD in the washing tank was monitored in a continuous way as the system was fed with concentrated vegetable wash water simulating the industrial conditions of a processing line (Fig. 2). This original approach differed from a previous publication in which they compared EW disinfection in one-time-event experiments with different organic loads (López-Gálvez et al., 2012). In the present study, untreated tap water was used as a control which consisted of

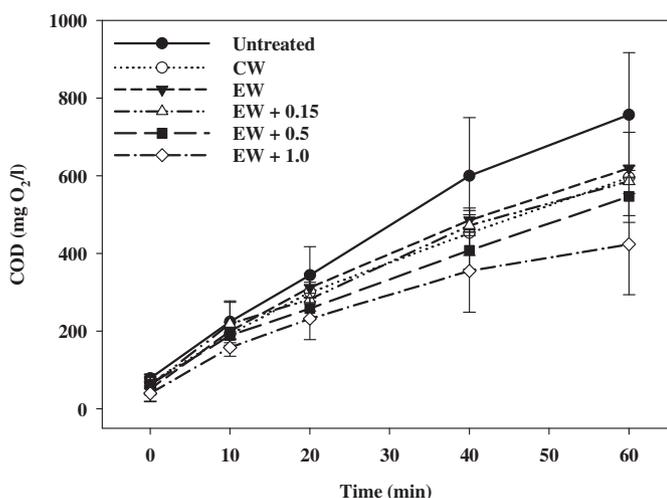


Fig. 2. Changes in chemical oxygen demand (COD) under dynamic conditions for untreated and treated vegetable wash water with chlorine (CW), electrolysis (EW) combined or not with salt (EW + 0.15, EW + 0.5 and EW + 1.0 g/L NaCl). Results are means of at least two repetitions of 3 replicates each \pm standard deviation.

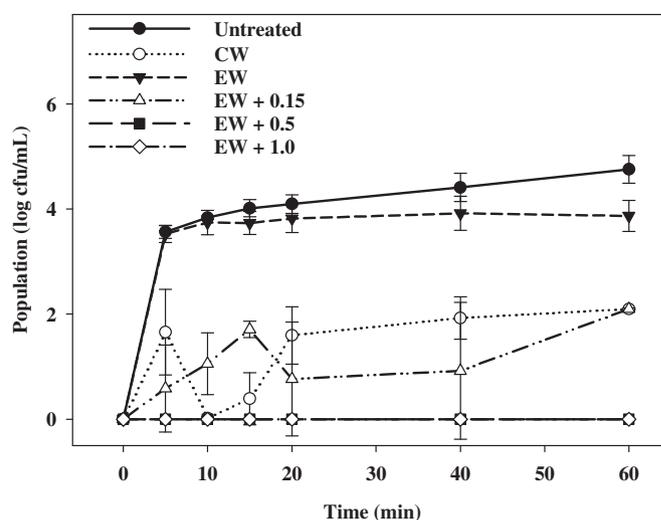


Fig. 3. Changes in *E. coli* O157:H7 populations under dynamic conditions for untreated and treated vegetable wash water with chlorine (CW), electrolysis (EW) combined or not with salt (EW + 0.15, EW + 0.5 and EW + 1.0 g/L NaCl). Results are means of at least two repetitions of 3 replicates each \pm standard deviation.

the constant addition of inoculated and concentrated vegetable wash water to clean tap water in the washing tank without the application of any disinfection treatment. Thus, accumulation of COD in the untreated water was the highest when compared to the rest of the treatments, reaching a COD level approx. 800 mg/L after 60 min. However, depending on the disinfection technology used, we observed differences in the accumulation of COD (Fig. 2). In all cases, the accumulation of COD approached to linearity showing a small deflection, with R^2 between 0.96 and 0.99. The slopes were 76, 81, 75 and 68% smoother than the untreated water in CW, EW, EW + 0.15 and EW + 0.5 treated water respectively. However, the lowest increase in COD corresponded to EW + 1.0, with a slope of 55% smoother than the untreated water, which was used as control treatment for COD accumulation (Fig. 2). This reduction in COD was expressed as percentage of slope respect to the untreated water. There was a linear relation with the concentration of salt added to the EW as: % slope reduction = $81 - 255\% \text{ NaCl}$, $R^2 = 0.99$.

Previous study described that organic matter reduces the efficacy of EW (Park et al., 2008). At the same time, it is known that COD can be degraded during the electrochemical disinfection. The degradation of COD could be related to two phenomena: 1) electrochemical mineralization of organic matter and 2) electroflocculation–electroflotation. In the first process, mineralization occurs when reactive oxygen species generated by BDD electrodes oxidize organic matter (Polcaro et al., 2009) and in the case of electroflocculation–electroflotation, a layer of foam at the top of the vegetable wash water was formed during the experiments (Ghernaout et al., 2011). These authors reported that when foam occurs during electrolysis by hydrogen bubbles, it can carry attached coagulated particles. The bubbles are transported to the top of the liquid where they could be separated in the washing facilities.

3.2. Inactivation of *E. coli* O157:H7 in the dynamic system

Changes in *E. coli* O157:H7 populations during the disinfection of the vegetable wash water under the dynamic conditions are shown in Fig. 3. As expected, bacteria counts increased in the untreated water during 60 min of the tests because *E. coli* O157:H7 cells were entering into the treatment tank and no disinfection treatment was

applied. The highest counts corresponded to the untreated water and it served as a reference for the rest of the tests. When no salt was added to the EW, pathogen count increased in the washing tank reaching similar counts to those of the untreated water. Results showed that the levels of *E. coli* O157:H7 were reduced more than 2 log units in CW compared to EW. When salt was added to EW, there was a reduction in the counts, indicating the electrolytic inactivation of *E. coli* O157:H7 cells by EW in the presence of salt (Fig. 3). The minimum concentration of salt needed to reach the complete inactivation was 0.5 g/L NaCl. This shows that EW can be very effective when combined with salt for the inactivation of pathogenic bacteria in vegetable wash water even at high COD. These results also showed that EW combined with salt ≥ 0.5 g/L was more effective than CW in controlling the contamination of the vegetable wash water by *E. coli* O157:H7. In our previous study (López-Gálvez et al., 2012), the operating conditions in batches for the electrochemical disinfection of process water contaminated with *E. coli* O157:H7 needed the recirculation throughout the electrolytic cell for 1 h. The efficacy of the cited treatment without salt added to reduce the level of the pathogen to the limit of detection was also due to the high electrical charge (190 mA/cm²) which was 3 times higher than the one used in the experiments reported hereby (60 mA/cm²). In the present study, we achieved the complete inactivation of the pathogenic bacteria entering into the washing tank with lower electrical charge and therefore a higher efficiency process of the disinfection treatment. Conditions including high current density (180 mA/cm²), high flow rate (750 L/h) and high doping level (8000 μ mol/mol) were described to provide a disinfection efficiency suitable to decrease the chance of bacterial cross contamination of process wash water (Gómez-López et al., 2013a). However, from a practical standpoint, this system was more efficient than the one previously described as it was capable of inactivating *E. coli* O157:H7 cells in the wash water even in the presence of organic matter but only when salt was added.

3.3. Changes in wash water quality in the dynamic system

Chlorinated water was designed to simulate a typical scenario where free chlorine concentration in the washing water is maintained around 0.5 mg/L by the continuous addition of bleach. Accumulation of free chlorine in CW did not occur because of the continuous increase in the amount of organic matter supplied by the dynamic system that reacted very fast with free chlorine (Fig. 4A). Free chlorine was not detected in EW and when 0.15 g/L of salt was added it reached approx. 0.3 mg/L (Fig. 4B, C). Total chlorine and combined chlorine matched the same concentration in CW, EW and EW + 0.15. However, when the concentration of salt was increased to 0.5 and 1.0 g/L NaCl, and free chlorine was accumulated up to 17 and 67 mg/L, respectively, the values of total and combined chlorine differed (Fig. 4C, D, E). Because of the complexity of these experimental conditions simulating a vegetable processing line, the inflow of bacterial contaminants was counteracted by the addition of disinfectants and the generation of oxidizer species. These results illustrate the practical difficulty of maintaining very low (i.e., in the range of 0.3 mg/L) free chlorine concentrations in wash water with high organic matter. As expected, the highest inactivation occurred in the disinfection treatments that generated the highest free chlorine, EW + 0.5 and EW + 1.0 (Fig. 3). Oomori et al. (2000) reported that the bactericidal activity of acidic EW declines in the presence of organic materials due to the conversion of free available chlorine content to the combined form. COD load had a detrimental effect on disinfection efficiency. Higher organic loads lead to faster chlorine consumption, allowing less free chlorine to be in contact with the target microorganisms (Van Haute et al., 2013).

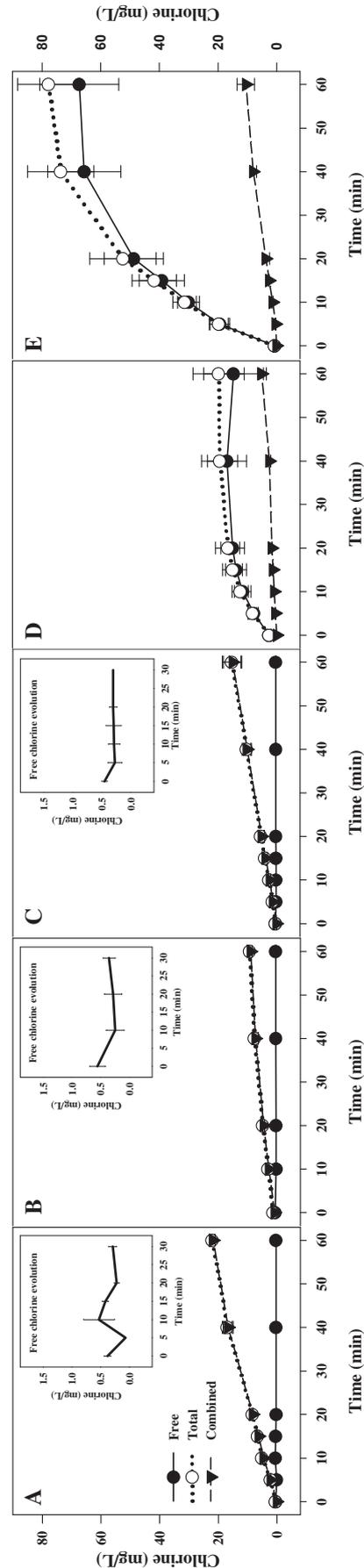


Fig. 4. Changes in the concentration of free, total and combined chlorine under dynamic conditions for treated vegetable wash water with chlorine (CW) (A), electrolysis (EW) (B) and EW + 0.15 g/L NaCl (C), EW + 0.5 g/L NaCl (D) and EW + 1.0 g/L NaCl (E). Results are means of at least two repetitions of 3 replicates each \pm standard deviation.

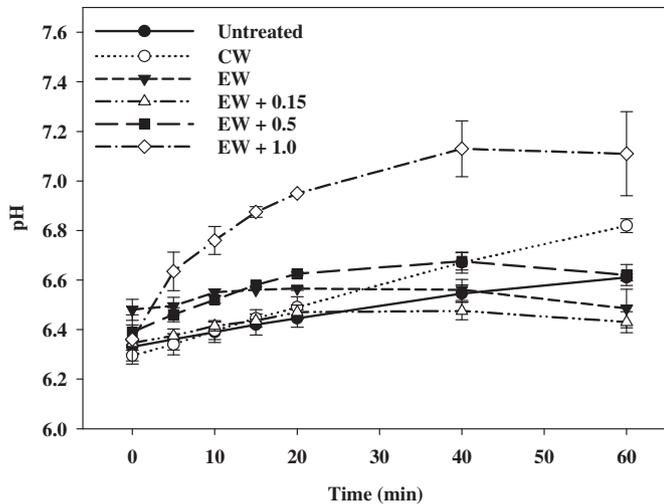


Fig. 5. Evolution of pH under dynamic conditions for untreated and treated vegetable wash water with chlorine (CW), electrolysis (EW) combined or not with salt (EW + 0.15, EW + 0.5 and EW + 1.0 g/L NaCl). Results are means of at least two repetitions of 3 replicates each \pm standard deviation.

The pH was monitored during the disinfection treatments. The pH was always in the range of 6.2–6.8, except for EW + 1.0 g/L NaCl, in which the pH increased up to 7.1 (Fig. 5). This means that the addition of citric acid for pH adjustment worked well. It has been described that the efficacy of the disinfection treatments depends on pH (Park et al., 2004) although there are great variations (from 6.7 to 8.1) in different processing facilities (Barrera et al., 2012). Increasing the pH of the water used to prepare EW has been described to decrease the bactericidal activity (Pangoli and Hung, 2013).

Oxidation reduction potential (ORP) was another parameter that differentiated the disinfection technologies tested (Fig. 6). The ORP for untreated water and CW was fairly stable but as expected lower values for untreated water than for CW (260 vs 650 mV, respectively), reflecting the stronger antimicrobial status of the CW. The electrolysis changed ORP to different levels depending on the disinfection conditions. The ORP decreased after 5 min in EW when

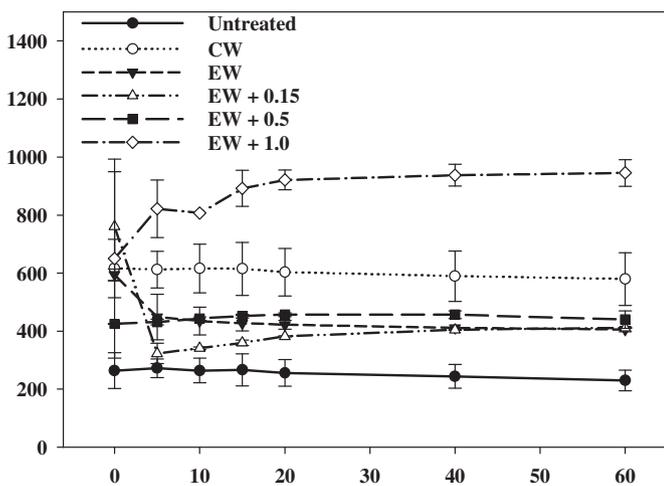


Fig. 6. Changes in oxidation reduction potential (ORP) under dynamic conditions for untreated and treated vegetable wash water with chlorine (CW), electrolysis (EW) combined or not with salt (EW + 0.15, EW + 0.5 and EW + 1.0 g/L NaCl). Results are means of at least two repetitions of 3 replicates each \pm standard deviation.

no salt or low concentration of salt was added to process water and then kept relatively constant at positive (oxidant) levels of about 400 mV during the rest of the tests (Fig. 6). However, when EW was added with 1.0 g/L of salt, ORP increased during the first 20 min of treatment and then stabilized to levels of about 900 mV. Some authors have considered ORP more determinant for microbial inactivation than free chlorine (Kim et al., 2000). In processing lines, chlorine concentration of the wash water has been controlled via ORP system at a set point. However, there are always significant variations in the readings directly taken from the biocide washing tank (Barrera et al., 2012). Therefore, ORP does not adequately describe levels of active chlorine or the associated reduction in microbial loads in the wash water. We observed that ORP decreased in EW with no salt addition while the oxidant potential of EW increased with salt.

3.4. Generation of trihalomethanes in the dynamic system

The concentration of individual and total THMs was measured in the wash waters before starting the experiments and after 60 min of the disinfection (Table 1). In general, significant changes in the concentration of THMs were observed in chloroform and total THMs while the concentration of bromodichloromethane, dibromochloromethane and tribromomethane generated by the different disinfection did not differ from tap water or untreated water. As expected, the concentration of individual and total THM was the same in tap water as in untreated vegetable water. After chlorine and electrolyzed treatments, the concentration of individual and total THMs did not significantly vary from the untreated water. However, when ≥ 0.5 g/L salt was added to the EW, the concentrations of chloroform and total THM increased significantly. Chloroform accounted for 44, 71, 79, 93 and 94% of the total THM generated for CW, EW, EW + 0.15, EW + 0.5 and EW + 1.0 treatments respectively, compared to only 21% for untreated water. It is possible that there was a higher availability of chlorine than of bromine due to the addition of either NaHOCl or NaCl, which could have allowed the higher generation of all-chlorinated THMs instead of brominated ones. Brominated THMs have been described as being found in water with bromide ions such as seawater (Díaz et al., 2011). As expected, the content of THMs found in this study was lower than those reported by López-Gálvez et al. (2010) probably because of the lower levels of free chlorine and COD. They reported 217 and 3618 $\mu\text{g/L}$ of THMs in the process water with 700 mg/L of COD and 100 mg/L of free chlorine, and 1800 mg/L of COD and 700 mg/L of free chlorine, respectively. Van Haute et al. (2013) also reported considerable amounts of THMs formed in the water containing COD of 1000 mg/L due to prolonged chlorination. Currently, there is no legislation for THM limits in the wash water of the processing industry. THM concentration formed by EW + 1.0 treatment was the only one over the authorized limit (100 $\mu\text{g/L}$) fixed by the European legislation for water intended for human consumption (EU, 1998) and the American legislation (80 $\mu\text{g/L}$) (US EPA, 2012). Electrolysis of water with 0.5 g/L NaCl produced levels of THMs lower than these limits and reduced *E. coli* O157:H7 accumulation below the detection limit. The same antimicrobial efficacy was achieved using 1.0 g/L NaCl but THM levels exceeded those authorized limits due to the high production of chlorine, similar to the risks of hyperchlorination. The treatments tested in this study were focused on wash water from the fresh-cut industry. Therefore, it could be hypothesized that the concentration of THMs in the vegetables washed in this water would not represent a risk. After washing the spinach in process water, the rinsing step was enough to decrease from 200 $\mu\text{g/L}$ THM on the product to undetectable limits (Gómez-López et al., 2013b).

Table 1

Concentration of individual (chloroform, CHCl₃; bromodichloromethane, CHBrCl₂; dibromochloromethane, CHBr₂Cl and tribromomethane, CHBr₃) and total trihalomethanes (µg/L) in tap water and untreated vegetable wash water, and wash water treated with chlorine (CW), electrolysis (EW) combined or not with salt (0.15, 0.5 and 1.0 g/L NaCl).

	CHCl ₃	CHBrCl ₂	CHBr ₂ Cl	CHBr ₃	Total
Tap water	2.0 ± 1.3 c	2.2 ± 1.0	3.1 ± 1.4	2.8 ± 0.9 a	10.1 ± 4.6 c
Untreated	2.1 ± 0.3 c	2.0 ± 0.4	2.8 ± 0.6	2.8 ± 0.3 a	9.7 ± 1.6 c
CW	5.7 ± 2.1 c	1.9 ± 0.6	2.7 ± 0.7	2.7 ± 0.6 a	12.9 ± 3.8 c
EW	14.4 ± 1.6 c	1.7 ± 0.1	2.1 ± 0.0	2.1 ± 0.0a	20.3 ± 1.7 c
EW + 0.15	25.3 ± 8.4 c	1.8 ± 0.4	2.4 ± 0.3	2.4 ± 0.1 a	31.9 ± 8.5 c
EW + 0.5	70.9 ± 16.1 b	2.4 ± 0.4	2.0 ± 0.7	0.9 ± 0.1 b	76.3 ± 15.2 b
EW + 1.0	115.3 ± 28.4 a	2.6 ± 0.2 ns	2.6 ± 0.4 ns	2.3 ± 0.2 a	122.8 ± 27.9 a

Values are means of six replicates ± standard deviation. Different letters within the same column are significantly different ($p < 0.05$).

In conclusion, the electrolysis of water with a suitable amount of salt (≥ 0.5 g/L NaCl in the current case) is able to avoid *E. coli* O157:H7 population build-up in the wash water with high organic matter content due to the generation of free chlorine. The concentration of free chlorine generated is high enough to be lethal to the bacteria but low enough to avoid chemical concerns regarding the generation of high THM levels and reduces organic matter accumulation. Based on the current results using a dynamic system in which the organic matter and the inoculum are progressively added into the wash water, we believe that this experimental design is the most adequate method to simulate the washing conditions of a processing line.

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