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A novel electrochemical device as a disinfection system to maintain water quality during washing of ready to eat fresh produce

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ABSTRACT

Electrolyzed water (EW) is known by its bactericidal efficacy and capability to oxidize organic matter. The present research evaluated the efficacy of recently developed electrolytic cells able to generate higher concentration of reactive oxygen species using lower power and salt concentration than conventional cells. This study tested the inactivation of *Escherichia coli* O157:H7, the organic matter depletion and trihalomethane (THM) generation by EW in process wash water under dynamic conditions. To achieve this, clean tap water was continuously added up to 60 min with artificial process water with high chemical oxygen demand (COD) inoculated with *E. coli* O157:H7, in experiments performed in a pilot plant that recirculated water through one electrolytic cell. Plate counts of *E. coli* O157:H7, COD, THMs, free, combined and total chlorine, pH, temperature and oxidation–reduction potential were determined. Results indicate that the novel electrolysis system combined with minimal addition of NaCl (0.05%) was able to suppress *E. coli* O157:H7 population build-up and decreased the COD accumulation in the process wash water. THM levels in the water were relatively high but its concentration in the washed product was marginal. Highly effective electrolysis has been proven to reduce the occurrence of foodborne diseases associated to cross-contamination in produce washers without having an accumulation of THMs in the washed product.

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1. Introduction

Washing is still an essential step in the production of fresh produce to mainly remove dirt, debris, cell exudates after cutting as well as to precool the product. Additionally, for many producers, one of its alleged functions is to decrease the microbial load of the product, to which a disinfectant is usually added with the aim of improving water disinfection potential, being chlorine the most common one. More than 20 years ago, Adams and co-workers (Adams, Hartley, & Cox, 1989) already warned on the limited efficacy of chlorine to reduce the microbial load of lettuce. Further works tried to find disinfectants more efficient than chlorine (Allende, Selma, Lopez-Galvez, Villaescusa, & Gil, 2008; Gopal, Coventry, Wan, Roginski, & Ajlouni, 2010; Lopez-Galvez, Ragaert, Palermo, Eriksson, & Devlieghere, 2013). Results found so far have been unsuccessful and even though some microbial inactivation is

achieved in fresh produce immediately after washing with disinfectants, its overall effect tends to disappear during storage due to a faster growth of the epiphytic microbiota. The use of disinfectants, however, has been proven to be essential in water-based processes that still place in the postharvest operations as a strategy to prevent cross-contamination (Gil, Selma, López-Gálvez, & Allende, 2009). This idea changes the focus of disinfectant use from the produce to the process water where the produce is washed in.

Even though the use of chlorine has many advantages in comparison with its potential alternatives, specially the low price, the search for chlorine substitutes is still needed, mainly because of its drawback as generator of disinfection by-products that can be toxic for humans such as trihalomethanes (THMs) (IARC, 1999a,b). However, the bad reputation of chlorine as generator of important amounts of THM residues on washed vegetables lacks of the support of conclusive scientific reports.

Mostly due to the above mentioned drawbacks of chlorine based sanitizers associated to the presence of disinfection by-products, alternative disinfection agents have been evaluated and recent studies have highlighted electrolyzed water (EW) as one of the

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potential substitutes. EW is a solution generated by passing a dilute salt solution, generally NaCl through an electrolytic cell, which contains free chlorine as the main microbial inactivation agent. Many authors have summarized the advantages of EW over chlorine (Gil, Gómez-López, Hung, & Allende, 2015; Issa-Zacharia, Kamitani, Muhimbula, & Ndabikunze, 2010) such as: on-site and simple production, cheap and easy-to-find raw materials (water and NaCl), low operational expenses and a lower THM generation. The latter is because EW produces several disinfectants besides chlorine, therefore it can have the same disinfection effectiveness at lower free chlorine concentrations. Furthermore, cell electrodes, especially boron-doped diamond electrodes, are able to oxidize organic matter (Kapalka, Fóti, & Comminellis, 2008), decreasing in this way the environmental impact of fresh produce industry wastewater discharges. Its main disadvantage is the cost of the electrolytic cell, which has however diminished in the last years due to technological improvements to the point that can be nowadays competitive.

The way how disinfectants are tested for efficacy has progressed in the last years, from batch experiments with clean water to dynamic experiments with process wash water that better mimic industrial operating conditions (Gómez-López, Gobet, Selma, Gil, & Allende, 2013b; Gómez-López, Lannoo, Gil, & Allende, 2014; Yang, Luo, Millner, Shelton, & Nou, 2012).

The aim of this study was to test the inactivation of *Escherichia coli* O157:H7, the organic matter depletion and THM generation by EW in process water under dynamic conditions.

2. Materials and methods

2.1. Bacterial strains and inoculum preparation

A three-strain cocktail of *Escherichia coli* O157:H7 (MB 3885, CECT4972 and CECT5947) resistant to 50 µg/mL nalidixic acid (Nal), was used in the study. All strains were activated from frozen stock cultures by transferring loopful culture into 5 mL Brain Heart Infusion broth (BHI, Oxoid, Basingstoke, United Kingdom) supplemented with 50 µg/mL Nal. Nal^R *E. coli* O157:H7 cultures were consecutively grown twice in 5 mL of BHI supplemented with 50 µg/mL Nal, at 37 °C for 24 h. After the second incubation, equal volumes of cultures were combined to give approximately equal populations of each culture. Concentration of the inoculum was confirmed by plating on Chromocult coliform agar (Merck, Barcelona, Spain) supplemented with 50 µg/mL Nal.

2.2. Process wash water

Iceberg lettuce (*Lactuca sativa* L.) was purchased from a local wholesale market in Murcia (Spain). Process wash water was produced by homogenizing lettuce pieces in water using a stomacher as described by Gómez-López, Gil, Pupunat, and Allende (2015). The batch of process wash water was divided in portions and frozen at – 18 °C until use. This process gives place to process water with similar characteristics to the washing water in a commercial tank of a processing plant. The main characteristics were the high chemical oxygen demand (COD) (2379 ± 8 mg O₂/L), pH (7.39 ± 0.03), alkalinity (36 ± 1 mg equiv. CaCO₃/L), turbidity (53 ± 1 NTU) and conductivity (1265 ± 16 µS/cm).

2.3. Electrochemical equipment

Disinfection experiments were performed using a pilot plant treatment system provided by WaterDiam (Franken, France) as previously described (Gómez-López et al., 2015). In this study, two recently developed boron-doped diamond electrolytic cells with no

separation between anodic and cathodic compartments, called DiaClean[®] 201i and 401i, were independently used during the trials. In cells 201i and 401i, two and four cell compartments were connected in series, respectively. The technical characteristics of the new electrolytic cells have been improved from previously cells to increase efficiency (Gómez-López et al., 2015). The improvement consists in a hydrodynamic design that allows to actually achieving the contact of the water flow inside the cell with the whole anode surface. They have an overall effective anode surface area of 70 cm², an electrode boron doping level of ca. 1200 µmol/mol trimethylboron and a current density of 23 mA/cm² when operated at 1.6 A. Amperage was set at 1.6 A and controlled through the experiments by a power supply, which changed the polarity of electrodes every 20 min to minimize scale build-up on their surface. Water was pumped through the electrolytic cell and recirculated to the tank. Water recirculation rate was adjusted by means of a valve to 600 L/h for cell 201i and 750 L/h for cell 401i as per manufacturer indications.

2.4. Disinfection treatments

Before starting the tests, a volume of 11.4 L of tap water was placed in the treatment tank and water pH was adjusted to ca. 6.4 using citric acid (Acros Organics, Barcelona, Spain). Depending on the test, NaCl was also added in order to reach concentrations of 0, 0.015 and 0.050% (w/v). Free chlorine concentration was adjusted to ca. 0.5 mg/L before running tests by starting the electrolysis without process water input until reaching the desired condition. Concentrated process wash water (2379 ± 8 mg O₂/L) was placed in another tank and 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 treatment. Then, the inoculated and concentrated process water was continuously added by a peristaltic pump to the water of the treatment tank at a flow of 3.1 L/h. Each experiment was repeated at least twice. Temperature and pH exhibited little change through the disinfection process, temperature increased from the initial one (5 °C) to a maximum of 9 °C and pH was between 6.3 and 6.6.

2.5. Microbiological and physicochemical analyses

Changes in the levels of Nal^R *E. coli* O157:H7 were determined as previously reported (Gómez-López et al., 2015). Briefly, water samples taken from the washing tank were neutralized with sodium thiosulfate, diluted in buffered peptone water (Scharlau, Barcelona, Spain) and pour plated in Chromocult coliform agar supplemented with 50 µg/mL Nal. Microbial enumeration was carried out after incubation at 37 °C for 24 h. The detection limit of the method was 1.05 cfu/mL. Levels of *E. coli* O157:H7 in the untreated control were obtained from previous studies where the same experimental conditions were used (Gómez-López et al., 2015). Changes in COD (mg/L), oxidation-reduction potential (ORP, in mV), temperature (°C) free and total chlorine (mg/L), and pH were measured at different time intervals as described by Gómez-López, Marín, Medina-Martínez, Gil, and Allende (2013a).

2.6. Determination of trihalomethanes

Samples were taken from tap water and lettuce process water prior mixing in the washing tank (before treatments) and from the process wash water after treatments. Samples of 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. Determination of THMs was carried out as previously described (Gómez-López et al., 2013a).

2.7. Data processing

Each experiment was repeated twice. Linear regressions were obtained by using Excel (Microsoft Corporation, Redmond, WA, USA). Data of THMs were analyzed for normality by the Kolmogorov-Smirnov test and for homocedasticity by the Levene's test. Mean statistical differences were analyzed by the Mann-Whitney test. All tests had $P = 0.05$, and the software IBM SPSS version 19 (Chicago, USA) was used.

3. Results and discussion

3.1. Microbial inactivation

There was a continuous input of inoculated process water to the washing tank, hence when no residual concentration of the disinfection agent was present, a population build-up was observed. The effect of different treatments on the prevention of *E. coli* O157:H7 population build up in the washing tank is shown in Fig. 1. Accumulation of *E. coli* O157:H7 in the washing tank was clearly observed when no disinfection treatment was performed, that started from absence of *E. coli* O157:H7 at time 0, when the inoculum had not been added yet. The electrolysis using both electrolytic cells (201i and 401i) without NaCl addition was unable to prevent the accumulation *E. coli* O157:H7 in the tank, with a limited effect of 1 log cfu/mL reduction for both cells (Fig. 1A and B). A very small addition of NaCl (0.015%) increased the inactivation capability of the electrolyzed water, especially for the treatment with cell 401i (Fig. 1B), which was able to keep *E. coli* O157:H7 population below the detection limit at the beginning of the test, but it decreased the effectiveness after 20 min. Even though the treatment with 0.015% NaCl controlled the contamination build-up better than the treatment without addition of NaCl during the treatment interval 20–60 min, it was not effective enough for avoiding a continuous increase of *E. coli* O157:H7 population during the test timeframe. Holvoet et al. (2014) have shown that wash water with 2.9 log cfu/mL *E. coli* O157:H7 can increase lettuce contamination up to 1.9 log cfu/g. The treatments with 0.050% NaCl were able to keep the accumulation of *E. coli* O157:H7 below the detection limit for the entire duration of the tests (Fig. 1). The present study was inspired in our previous study (Gómez-López et al., 2015), where an electrolysis cell with only one cell compartment and a doping level of 8000 $\mu\text{mol/mol}$ (called DiaClean® 101 8000) was operated at 4 A

for a current density of 60 mA/cm² and under 750 L/h water flow and 0.050% NaCl. Although no direct comparison can be done between different electrolytic cells, it can be observed that cells 201i and 401i were able to avoid *E. coli* O157:H7 accumulation in the washing tank as efficiently as cell 101 8000 (Gómez-López et al., 2015) using less than half of the current, which implies lower energy consumption for the same antimicrobial capacity. These cells named by the manufacturer with a number plus the suffix “i” have an improved hydrodynamic design with respect to cells used in our previous studies. The main hydrodynamic improvement consists in a better diffusion of the process wash water passing through the cell, which improves the treatment action upon water.

3.2. Changes in the physicochemical characteristics of process wash water by the novel electrolytic cells

Fig. 2 shows the evolution of free chlorine levels generated in different treatments during 60 min. As expected, treatments with the highest NaCl concentration yielded the highest production of free chlorine caused by a higher availability of chloride ions. Microbial inactivation in electrolyzed water in the bulk of water was closely related to free-chlorine levels and thus, the microbial build up was arrested at the highest free chlorine levels. Treatments with 0 and 0.015% NaCl never reached 2 mg/L free chlorine, while treatments with 0.05% NaCl reached 4 mg/L free chlorine when using 201i (Fig. 2A) and even higher (23 mg/L) for the cell 401i (Fig. 2B). The results agree with the recommendation on the need of at least 7 mg/mL free chlorine to inhibit *E. coli* O157:H7 survival in wash water (Gómez-López et al., 2014) although a recent publication recommends a minimum of 3.66 mg/mL free chlorine (Zhou, Luo, Nou, Lyu, & Wang, 2015).

Some authors have considered ORP as an adequate indirect measure of active chlorine in systems where water contacts fresh produce (Tomás-Callejas et al., 2012). In the case of EW, ORP has been considered even more determinant for the microbial inactivation than free chlorine since the antimicrobial action of EW is an oxidative process (Kim, Hung, & Brackett, 2000). In this study, no *E. coli* O157:H7 presence was observed at ORPs >400 mV; the ORPs of the treatments that best prevented *E. coli* O157:H7 accumulation (0.05% NaCl) were always higher than that value (Fig. 3). This result cannot be used to fully support the generalized use of ORP as monitoring value for electrolyzed water disinfection efficacy, although ORP data was consistent with our previous study (Gómez-

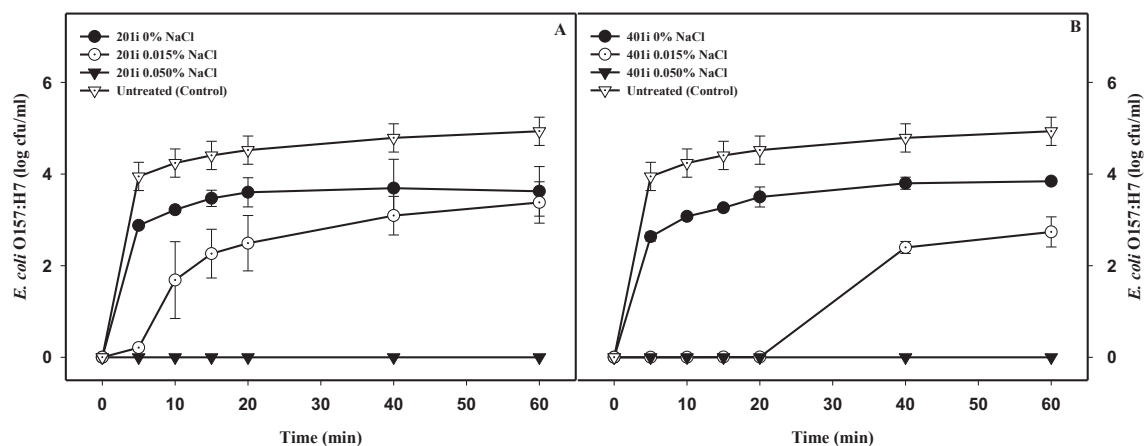


Fig. 1. Changes in *E. coli* O157:H7 populations during disinfection of process wash water using highly effective electrolysis cell 201i (A) and 401i (B) combined with different NaCl concentrations under dynamic conditions. Results are means of at least two repetitions \pm standard deviation.

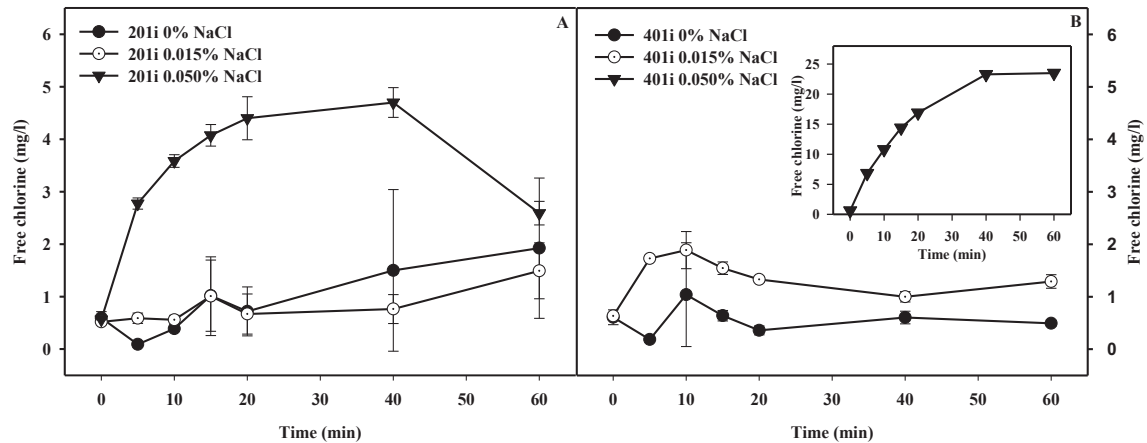


Fig. 2. Evolution of free chlorine during disinfection of process wash water using highly effective electrolysis cell 201i (A) and 401i (B) combined with different NaCl concentrations under dynamic conditions. Results are means of at least two repetitions \pm standard deviation.

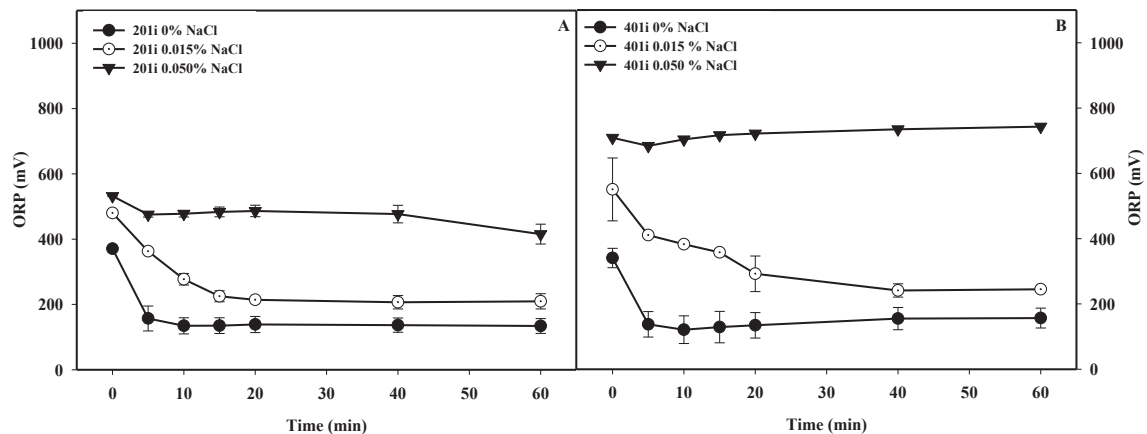


Fig. 3. Evolution of oxidation reduction potential (ORP) during disinfection of process wash water using highly effective electrolysis cell 201i (A) and 401i (B) combined with different NaCl concentrations under dynamic conditions. Results are means of at least two repetitions \pm standard deviation.

López et al., 2015). However, chlorinated water used in that study was unable to control *E. coli* O157:H7 build-up in spite of having an ORP of 600 mV. In fact, the use of ORP as a measure to verify the process control does not always describe adequately the levels of active form of sanitizers (Whitaker & Gorny, 2013).

Besides water disinfection, electrolyzed water has shown to be able to decrease the organic load of process water (Gil et al., 2015). It is known that BDD electrodes can mineralize organic matter, which occurs when reactive oxygen species generated by these electrodes oxidize the organic matter (Polcaro, Vacca, Mascia, Palmas, & Rodriguez, 2009). In the dynamic system used, there was a continuous flux of organic matter to the washing tank; consequently an increase in COD was expected whereas a slower increase of COD is related to organic matter mineralization. The increase of COD during treatments was linear with determination coefficients between 0.97 and 0.99. Cells 201i and 401i showed 14 and 25% less COD accumulation, respectively than that observed in the untreated water (Fig. 4A and B). This is a positive feature of this disinfection technology because process water with lower COD will have a lower chlorine demand, lower by-product generation (Shen, Norris, Williams, Hagan, & Li, 2016) and also from the environmental point of view because it decreases the contamination caused by the disposal of process water with high organic load.

3.3. Trihalomethane generation

The concentration of THMs generated in the process water by the treatments is shown in Table 1. Chloroform was the predominant THMs that counted for more than 90% and it was associated to a higher availability of chloride ions. Brominated THMs were not expected in process wash water as they have been found in water with bromide ions such as seawater (Díaz, Ibáñez, Gómez, Urtiaga, & Ortiz, 2011). The percentage of chloroform increased in treatments with higher NaCl concentrations, which was also expected given the higher production of THM and limited bromine amount. THM concentration produced by cell 401i was twice those produced by cell 201i at both salt concentrations. Unexpectedly, the THM concentration generated in process wash water treated with EW in the present study was much higher than the THM levels obtained using sodium hypochlorite under a similar experimental set up (Gómez-López et al., 2015). This means that the high oxidizing capacity of this novel electrolysis cells generated higher concentration of reactive oxygen species able to react with the organic matter. The recirculation system applied in the present study allowed the process wash water containing high organic matter to pass through the electrolytic cells, being probably responsible for the high THM generation.

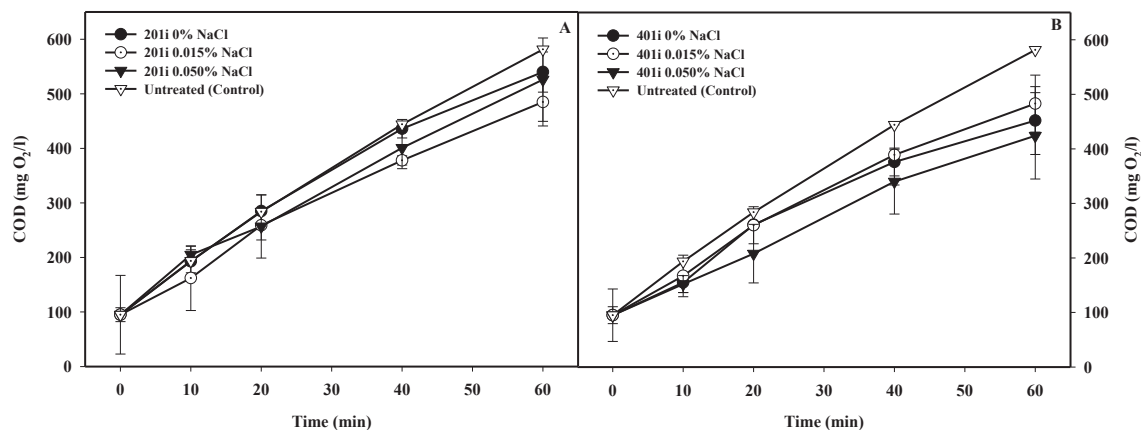


Fig. 4. Changes in chemical oxygen demand (COD) during disinfection of process wash water using highly effective electrolysis cell 201i (A) and 401i (B) combined with different NaCl concentrations and under dynamic conditions. Results are means of at least two repetitions \pm standard deviation.

Table 1

Individual and total trihalomethanes ($\mu\text{g/L}$) after electrolyzing process water under different conditions.

Compound	Treatment			
	Cell 201i		Cell 401i	
	0% NaCl	0.050% NaCl	0% NaCl	0.050% NaCl
Chloroform	97.84 \pm 12.10 ^c	329.70 \pm 93.27 ^b	205.01 \pm 69.71 ^b	664.87 \pm 56.69 ^a
Dichlorobromomethane	3.11 \pm 0.27 ^b	3.17 \pm 0.67 ^b	2.21 \pm 0.24 ^b	3.59 \pm 0.34 ^a
Chlorodibromomethane	4.15 \pm 0.42 ^a	2.26 \pm 0.92 ^b	3.29 \pm 0.22 ^{ab}	2.87 \pm 0.89 ^b
Tribromomethane	1.87 \pm 0.08 ^a	0.85 \pm 0.15 ^c	1.58 \pm 0.12 ^b	1.05 \pm 0.10 ^c
Total THMs	106.96 \pm 12.18 ^c	336.00 \pm 90.51 ^b	212.08 \pm 68.82 ^b	672.50 \pm 54.45 ^a

^{a,b,c} Within the same row, means with different superscript are statistically different ($p < 0.05$).

There is no legal limit for the concentration of THM in process water, therefore, the limits established for drinking water can be used as reference taking into account that they are much more stringent than those that could be established for process water. The concentrations detected in the process wash water were over the authorised 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). Since the tested samples simulate a washing process of a fresh-cut industry, these results should be analyzed in the context of its potential effect on the human health after consumption of vegetables washed in process water with those high THM levels.

Table 2 shows the levels of THMs in cut lettuce after washing in process wash water treated with the cell 201i and combined with 0.05% of NaCl. As previously mentioned, the THM levels in the process wash water were above 300 $\mu\text{g/L}$ (Table 1). However, THM levels in the fresh produce washed in process wash water were approximately 9 $\mu\text{g/kg}$, value which was significantly reduced after rinsing with potable water (Table 2). The concentration of THMs in

Table 2

Individual and total trihalomethanes ($\mu\text{g/Kg}$) in fresh-cut lettuce washed in process wash water treated with cell 201i combined with 0.05% NaCl and washed and rinsed in potable water.

Treatment	Washed	Washed and rinsed
Chloroform	8.1 \pm 1.2 ^a	2.9 \pm 0.1 ^b
Dichlorobromomethane	0.3 \pm 0.0 ^a	0.6 \pm 0.3 ^a
Chlorodibromomethane	0.2 \pm 0.0 ^b	1.58 \pm 0.1 ^a
Tribromomethane	0.1 \pm 0.0 ^b	1.16 \pm 0.0 ^a
Total THMs	8.7 \pm 1.6 ^a	6.2 \pm 0.4 ^b

^{a,b} Within the same row, means with different superscript are statistically different ($p < 0.05$).

fresh produce washed in process wash water treated with the novel electrochemical device was lower than that currently allowed for drinking water (100 $\mu\text{g/L}$). These results agree with previous reports showing that a rinsing step after washing decreases THM concentration below the detection limit (Gómez-López et al., 2013a; Van Haute, Sampers, Holvoet, & Uyttendaele, 2013). Therefore, although one cannot underestimate the risks associated with washing vegetables with water with high THM levels, the risk seems to be relatively low.

In conclusion, it was confirmed that the new, highly effective, electrolysis system combined with 0.05% NaCl was efficient to control the build-up of *E. coli* O157:H7 during a simulated fresh-cut washing process and simultaneously decreased the accumulation of organic matter in the washing tank. Even though the concentration of THMs generated during the process was high, accumulation in the vegetable tissue was very reduced which makes ingestion of THMs through the consumption of ready to eat vegetables irrelevant for the total daily human intake. Among the tested electrolytic cells, cell 201i was the best choice because it prevented the *E. coli* O157:H7 accumulation in the washing tank with lower chlorine generation, consequently lower THM generation as well as relatively low electrical consumption.

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