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Credit Author Statement

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Effectiveness of short exposure times to electrolyzed water in reducing *Salmonella spp* and Imidacloprid in lettuce.

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Abstract

EW has been proposed as a sanitization method for home use to reduce chemical and biological hazards in fresh products. Most studies have evaluated exposure times of 1 to 10 minutes which may be too long for processing fresh produce. The aim of this work was to evaluate if short exposure times (15, 30 or 45 s) to electrolyzed water (EW – 50 ppm of free chlorine) were enough to significantly reduce *Salmonella spp* counts and Imidacloprid concentrations in lettuce. Results showed that EW treatment of 45 s achieved a reduction of 4 log CFU/g in the *Salmonella spp* counts and a reduction of 48,57% in Imidacloprid concentrations. As to quality parameters, neither texture profile nor flavor were affected by the treatment. The fact that only 45 s were enough to effectively reduced *Salmonella spp* and Imidacloprid makes the EW treatment an ideal sanitization method for lettuce in both the industry and the household.

Keywords: *Salmonella*, lettuce, electrolyzed water, Imidacloprid

1. Introduction

Lettuce (*Lactuca sativa*) is one of the most popular leafy vegetables in the world. The high consumption levels are associated with the fact that it is low in calories and fat, a good source of vitamins, protein, dietary fiber and minerals (including iron, calcium, and nitrates), and it is rich in phytochemicals (Kim, Moon, Tou, Mou, & Waterland, 2016). However, the consumption of raw lettuce can pose a risk to consumers in terms of food safety, since it can transport chemical and/or biological contaminants (Pang & Hung, 2016).

The most important chemical contaminants are pesticide residues (Carozza, Li, Wang, Horel, & Cooper, 2009). The effects of pesticides on human health range from minor disorders such as nausea, allergies and headaches, to chronic disorders such as neurological ailments, cancer and reproductive malfunction (Farina, Abdullah, Bibi, & Khalik, 2017; Li, Tai, Liu, Gai, & Ding, 2014; Qi, Huang, & Hung, 2018). Imidacloprid is a neonicotinoid insecticide that has been widely used to control pests, particularly in vegetables (Lu, Chang, Palmer, Zhao, & Zhang, 2018). Even though it is considered to be only mildly toxic to humans, numerous reports indicate it has adverse effects in mammals such as teratogenic, mutagenic, neurotoxic and immunotoxic ones (Mikolić & Karačonji, 2018).

Regarding biological contaminants, one of the most important pathogens responsible for foodborne diseases is *Salmonella spp* (Olaimat & Holley, 2012). This pathogen has been isolated from lettuce and salads prepared with fresh vegetable products and it has been reported as the main cause of outbreaks of foodborne diseases (Jeddi et al., 2014; Mattia & Manikonda, 2018; Sagoo, Little, Ward, Gillespie, & Mitchell, 2003). *Salmonella spp* usually causes self-limited enterocolitis with diarrhea. Bloodstream infection (which is a severe manifestation of the disease), occurs in approximately 6% of patients with diarrheal enterocolitis (Vugia et al., 2004). Home processing can reduce pesticide residues and biological contaminants in lettuce by washing. In this regard, several sanitization methods have been proven to be effective to eliminate both, pathogenic microorganisms and pesticide residues (Warriner & Namvar, 2013; Wu, An, Li, Wu, & Pan, 2019). Electrolyzed water (EW) is a promising alternative for food decontamination due to its cost efficiency, easy of application, effective decontamination and has no detrimental effects neither on public health nor on the environment (Rahman, Ding, & Oh, 2010). It can be produced with tap water with no added chemicals, other than sodium chloride. EW is generated by the electrolysis of water containing an electrolyte, such as sodium chloride. After onset of electrolysis, negatively charged ions (OH- and Cl-) move towards the anode where electrons are released and hypochlorous acid (HOCl), hypochlorite ion (−OCl), hydrochloric acid (HCl), oxygen gas (O2), and chlorine gas (Cl2) are generated (Hricova, Stephan, & Zweifel, 2008). At a near-neutral pH (pH 6.3–6.5), the predominant chemical species is the highly biocidal hypochlorous acid species (HOCl) with the oxidation reduction potential (ORP) of the solution ranging from 800 to 900 mV. The germicidal activity is believed to be due to the inhibition of enzyme activity essential for microbial growth, damage to the membrane and DNA, and disturbance of membrane transport functions (S. Rahman, Khan, & Oh, 2016). Regarding Imidacloprid, it is believed that reactive oxygen species may help in the oxidation of pesticide residues leading to their reduction (Bhilwadikar, Pounraj, Manivannan, Rastogi, & Negi, 2019). Most studies have evaluated exposure times of 1 to 10 minutes which may be too long for processing fresh produce, especially for household washing techniques (Bhilwadikar et al., 2019; Izumi, 1999; Koseki, Yoshida, Kamitani, & Itoh, 2003; Park, Alexander, Taylor, Costa, & Kang, 2008; Venkitanarayanan, Ezeike, Hung, & Doyle, 1999). The aim of this work was to assess the efficacy of short exposure times to EW in reducing *Salmonella* counts and Imidacloprid concentrations in artificially inoculated iceberg lettuce. Likewise, the effects on lettuce quality parameters and sensory attributes were evaluated.

2. Materials and methods

2.1. *Lettuce samples*

Head iceberg lettuce was purchased at a local supermarket and transported refrigerated (4°C) to the microbiology lab at the INTA Food Technology Institute. Lettuce leaves were cut aseptically into pieces of 10 g and kept at 4°C until the assays were performed.

2.2. EW treatment

The EW (Envirolife, Argentina) was prepared following manufacturer's recommendations. Treatments were performed by immersing 10 g of sample in 100 ml of working solution of EW with a concentration of 50 ppm of free available chlorine. This concentration was selected based on previous studies (Guentzel, Liang Lam, Callan, Emmons, & Dunham, 2008; Izumi, 1999). Three exposure times were evaluated: 15, 30 and 45 s. Control samples were treated with tap water. After treatment, samples were individually packed in stomacher bags and kept at 4 ºC until analysis.

2.3. Experiment 1: Efficacy of short exposure times to EW in reducing Salmonella counts inoculated on lettuce

2.3.1. Bacterial strains and inoculum preparation

Salmonella strains used in this study were kindly provided by Dr. Pablo Chacana from the Pathobiology Institute, INTA Castelar, Argentina. The strains were originally isolated at different stages of the poultry food chain and were identified as *S.* Enteritidis, *S.* Typhimurium, *S.* Thompson, *S.* Heidelberg, and *S.* Schwarzengrund. The strains were kept in frozen culture at -80ºC until subcultures were prepared by inoculating a test tube with 10 ml of Tryptic Soy Broth (TSB, Oxoid, UK) with a single colony growth in Xylose-Lysine-Desoxycholate agar (XLD, Oxoid, UK), and individually incubated at 37 °C overnight. Cells were harvested by centrifugation at 4000 xg for 5 minutes and the pellets were washed twice with phosphate-buffered saline (PBS, pH 7.2, Oxoid), to reach a concentration of approximately 8 log CFU/ml. Equal volumes of each strain were mixed in order to obtain a pool of *Salmonella* strains.

2.3.2. Artificial microbial contamination and treatment procedure

Each sample was spot-inoculated with 100 µl of a mixed-strain suspension to obtain a final concentration of 7 log CFU/g and left to dry for 30 minutes at room temperature. Non-inoculated samples were included as raw material control. The procedure was performed in a biological safety cabinet, under sterile conditions.

2.3.3. *Microbiological analysis*

Samples were transferred into sterile stomacher bags and 90 ml of 0.1% peptone water (PW, Biokar, France) were added. Immediately after, samples were stomached (easy Mix, AES, France) for 60 s and serial dilutions were prepared. *Salmonella* counts were performed in Tryptic Soy agar (TSA, Biokar, France). All plates (in duplicate) were incubated overnight at 37°C.

2.4. Experiment 2: Efficacy of short exposure times to EW in reducing Imidacloprid concentrations added on lettuce

2.4.1. Preparation of Imidacloprid working solution and calibration standards

Imidacloprid standard was purchased from Sigma-Aldrich (St. Louis, MN). A stock solution was prepared in acetonitrile at 1 mg/ml. Standards at a concentration of 50, 100, 250, 500, 1000 and 2000 ng/ml were prepared in the matrix blank extract. All standard solutions were stored at -20 ºC.

2.4.2. Artificial chemical contamination and treatment procedure

An aqueous solution of Imidacloprid was added to lettuce samples by spraying and left to dry for 15 minutes at room temperature. The final concentration was of 0.7 mg/kg. Samples without Imidacloprid were included as raw material control. The procedure was performed in a chemical fume hood.

2.4.3. Sample extraction

The QuEChERS extraction procedure described in AOAC Official Method 2007.01 was used for sample extraction and cleanup (Anastassiades, Lehotay, Štajnbaher, & Schenk, 2003). Briefly, a volume of 10 mL of extraction solvent (acetonitrile), 1 g of sodium acetate and 2 g of magnesium sulfate were added to 5 g of lettuce. The samples were homogenized with an Ultraturrax (25 basic IKALabor technick, USA) for 3 min, sonicated for 30 min and centrifuged for 5 min at 1000 xg. A volume of 4 mL of extract was transferred to glass flasks and evaporated to dryness at 45 °C under a constant current of N2. The samples were suspended in 1 mL of acetonitrile containing 3% magnesium sulfate, 3% sodium acetate and 1.5% sodium chloride and were sonicated for 10 min before being centrifuged at 3000 xg for 5 min. The resulting supernatant was analyzed for pesticides through liquid chromatography/mass spectrometry as described below.

2.4.4. Liquid chromatography

Analyses were performed using a Waters Acquity ultra-performance liquid chromatography (UPLC) apparatus equipped with a single quadrupole mass detector using XBridge BEH C18 2.5 μm 2.1 × 150 mm column, 0.1% acetic acid in water: methanol at the following gradient; (95:5) -(95:5) 0–2 min, (95:5) -(80:20) 2–5 min, (80:20) -(20:80) 5–10 min, (20:80) -(0:100) 10–11 min, (0:100) -(0:100) 11–13 min, (0:100) -(95:5) 13–14 min, (95:5) 14–20 min as the mobile phase. The single ion recording (SIR) model was used in quantification analysis with the mass-spectrometer ESI positive mode, retention time and abundance of the confirmation ion (Ion C) m/z: 256 relatives to that of quantification ion (Ion Q) m/z: 175 were used as identification criteria.

2.5. Experiment 3: Effect of EW on lettuce quality parameters

Quality parameters were assessed on non-contaminated lettuce treated with EW (50ppm) during 45s, contact time that guaranteed a significant reduction of microbial and chemical contamination. Chromatic parameters and texture profile were determined by instrumental and sensory analysis. Flavor also was evaluated by sensory analysis. All determinations were carried out after 24h of storage and compared with lettuce samples treated with tap water.

2.5.1. Chromatic parameters analysis

The analysis of chromatic parameters was carried out using a Minolta CR-400 colorimeter (Konica Minolta Sensing, Inc. Osaka, Japan) with D–65 light source and a 2° standard observer angle. A standard white tile was used for the calibration process. Measurement of lettuce leaf was performed at 5 random locations. Results were expressed as lightness (L*), intensity of red (+a*)/green (-a*) and intensity of yellow (+b*)/ blue (-b*). The hue angle (h), Chroma (C*) and the color difference (ΔE) were calculated using the software of the colorimeter. All measurements were performed three times.

2.5.2. Texture profile analysis

The analysis of texture profile was carried out using a texture analyzer Stable Micro Systems model TA. XT plus (Stable Micro Systems, UK). The puncture test was performed at a constant speed of 1 mm/s and using a needle probe (P/2N) with a load cell of 5kg. Three stacked samples (5 by 5 cm) were placed onto the press holder and were measured at 5 random locations on each sample, obtaining a total measurement of 15 for each replicate. The peak force, defined hardness (g) and the area under the curve, defined cut resistance (g.s), were recorded using the software of the texture analyzer. All experiments were performed three times and compared with control.

2.5.3. Sensory analysis

Sensory attributes (color, texture and flavor) were evaluated by difference test front control with blind control. Twenty consumers selected at random evaluated color and texture (crispness) following the scales presented in Table 1. All samples were assigned random three-digit codes. The color test was carried out in a cabinet with standardized light (Verivide, CAC 120, UK) with D65 illuminant and the visual angle was kept constant during all tests. Samples were collocated in transparent plastic containers, simulating the presentation of commercial salads. The texture test was carried out in individual booths with a green light filter. Each evaluator received 2 circular portions of lettuce of 5 cm in diameter per sample. To determine the similarity of flavor between the treated and control samples, a triangular similarity test was carried out with forty-two consumers selected at random. Each evaluator received 3 circular portions of lettuce of 5 cm in diameter and identified which was the different sample.

2.6. Experimental design and statistical analysis

Three replicate experiments were conducted with three samples per test in each replicate. An analysis of variance (One factor-ANOVA) was carried out using the SPSS software package, version 21 (SPSS Inc., Chicago, Ill., U.S.A.). Sensory analysis data were analyzed by a variance analysis of two factors (sample and evaluator) that were performed to determine significant differences between the samples. Significant differences were analyzed by Tukey test (Rogers, 2017). The result of the triangular test was analyzed by comparison with the table made based on the binomial distribution: "maximum number of correct answers necessary to conclude that two samples are similarly based on the triangular test".

3. Results

3.1. *Experiment 1: Efficacy of short exposure times to EW in reducing Salmonella counts inoculated on lettuce*

Salmonella average count in untreated samples was 7.19 log CFU/g. *Salmonella* average counts in samples treated with tap water after 15 s was 5.63 log CFU/g, after 30 s was 5.47 log CFU/g and after 45 s was 5.65 log CFU/g. These results were statistically different from untreated samples but equal among the different contact times. The average log reduction after treatment with tap water was 1.6 log CFU/g. *Salmonella* average counts in samples treated with 50 ppm of EW after 15 s was 4.13 log CFU/g, after 30 s was 4.46 log CFU/g and after 45 s was 3.13 log CFU/g. These results were different from untreated samples and from samples treated with tap water (P<0.05). Among different exposure times, 15 and 30 s were equal and 45 s was different (P< 0.05). The average log reduction after EW treatment for 15 and 30 s was 2.9 logs CFU/g and after 45 s was 4.06 log CFU/g (Table 2).

3.2. Experiment 2. Efficacy of short exposure times to EW in reducing Imidacloprid concentrations adde d on lettuce

Imidacloprid average concentration in untreated samples was 0.70 mg/kg. Imidacloprid average concentration in samples treated with tap water was 0.50 mg/kg after 15 s, 0.46 mg/kg after 30 s and 0.51 mg/kg after 45 s of exposure. The average reduction after tap water treatment was 30%. Imidacloprid average concentration in samples treated with 50 ppm of EW was 0.53 mg/kg after 15 s, 0.47 mg/kg after 30s and 0.36 mg/kg after 45 s of exposure. The average reduction after EW treatment for 15 or 30 s was 29% while after for 45 s was 48.57%. The only treatment that differed from untreated samples and the rest of the treatments analyzed was EW treatment with an exposure time of 45 s (P<0.05) (Table 3).

3.3. Experiment 3. Effect of EW on lettuce quality parameters

Figures 1 and 2 show the experimental data of the chromatic parameters and the texture profile. Lettuce samples treated with EW and tap water did not present significant differences (P>0.05) on these parameters. The color difference (ΔE) found was 2.46. As to the sensory attributes, the panelists perceived a slightly lighter color (p<0.05) in samples treated with EW compared to samples treated with tap water. No significant differences (P>0.05) were found neither in the texture profile nor in the flavor.

4. Discussion

Based on our results, short exposure times of EW were enough to significantly reduce *Salmonella* counts. The average *Salmonella* log reduction after an EW treatment for exposure times of 15 and 30 s was of 2.90 log CFU/g and, after 45 s it was of 4.06 log CFU/g. Park, Alexander, Taylor, Costa, & Kang (2008) evaluated the ability of EW (37.5 ppm) to inactivate *S.* Typhimurium in lettuce after 15, 30 s, and 1, 3, and 5 min of exposure time and reported log reductions of 2.90 CFU/g after 30 s and more than 3.41 CFU/g for treatments above 1 min. The log reductions after 30 s were the same as those reported in the present study but the log reduction for treatments above 1 min were lower, the difference may be due to the concentration of free chlorine used in each study (37.5 vs 50 ppm) as well as variability in strain resistance. We used a pool of 5 native different *Salmonella* serovars while Park et al. (2008) used 3 reference strains of *S.* Typhimurium. Other authors evaluated the effectiveness of EW for longer exposure times and reported similar or even lower bacterial reductions (Abadias, Usall, Oliveira, Alegre, & Viñas, 2008; Koseki et al., 2003; Stopforth, Mai, Kottapalli, & Samadpour, 2016). Abadias et al. (2008) evaluated the bactericidal activity of EW (containing approximately 50 ppm of free chlorine) against *Salmonella* on lettuce and reported that exposure times of 1 and 3 min caused reductions of 1–2 log CFU/g with no significant differences between the exposure times analyzed. Stopforth et al (2016) evaluated EW (50 ppm) in leafy greens (organic baby lettuces, organic red and green chard, organic mizuna, organic arugula, organic friseé, and organic radicchio) and demonstrated that after 60 or 90 s of exposure *Salmonell*a reductions were 2.0 to 2.5 log CFU/g, with no significant difference between the two exposure times analyzed. Koseki et al. (2003) examined the influence of the inoculation method, spot inoculation site, and inoculation size on the efficacy of EW (40 ppm) against *Salmonella* inoculated on lettuce and reported that the inoculation method and the site of inoculation affected EW effectiveness. After

1 min of exposure time, the samples inoculated with the dip method resulted in a 1 log CFU/g reduction of *Salmonella* populations, samples inoculated by spot inoculation of the inner surface of the lettuce leaf reduced approximately 2.5 log CFU/g while the spot inoculation of the outer surface of the lettuce leaf resulted in approximately 4.6 log CFU/g. We used spot inoculation, Abadias et al. (2008) used dip inoculation and Stopforth et al. (2016) used spray inoculation, none made distinctions between leaf surface sites method. Based on Koseki et al. (2003) findings, the lower reduction reported by Abadias et al. (2008) could be due to the inoculation method. The lower reduction reported by Stopforth et al. (2016) could be related with the food matrix evaluated as well as the inoculation method. Regardless of the specific log reductions estimated by each one of the authors, in general it was observed that only slight differences were observed with longer treatment times.

As to the effectiveness of short exposure times to EW (50 ppm) in reducing Imidacloprid concentration in lettuce, we demonstrated that 45 s were enough to reduce its concentration in 48%. To the best of our knowledge, there are no other studies that had assessed the effectiveness of EW in reducing Imidacloprid in lettuce. However, there is a study that evaluated the effectiveness of EW in reducing the presence of other pesticides. Qi et al. (2018) demonstrated that after 15 min of exposure to EW (120 ppm) it was removed up to 59.2, 66.5 and 37.1% of diazinon; 43.8, 50.0 and 31.5% of cyprodinil; 85.7 73.0 and 49.4% of phosmet from spinach, snap beans and grapes, respectively. The EW treatment evaluated in the present study not only achieved similar reductions than those achieved for other pesticides but also achieved them in a shorter period of time and with a lower concentration of free available chlorine (50 ppm/45 s vs 120 ppm/15 min). In another study it has been assessed the efficacy of different strategies to reduce the contamination with Imidacloprid from fresh products. Abdullah et al. (2016)

measured the residual levels of Imidacloprid in spinach after applying different washing treatments and reported that a reduction of 47-50% was observed after dipping the sample for 10 min at 30±5 ∘C in the following solutions: 4% of acetic acid, 4% of citric acid and 6% of hydrogen peroxide. Based on these findings, the EW treatment that was the object of the present study, not only achieved reductions in the concentration of Imidacloprid similar to other washing solutions, but also achieved them in a shorter period of time (45 s vs 10 min). The fact that short exposure times were as effective as longer exposure times in reducing not only a biological contaminant, such as *Salmonella*, but also a chemical contaminant, such as Imidacloprid, in lettuce makes the EW treatment an ideal sanitization method for both the industry and the household, which are always pressed for time. Likewise, short exposure times are essential to preserve the nutritional content and general appearance of lettuce.

As to quality parameters, the experimental data of the instrumental measurements of the chromatic parameters and the texture profile showed that EW treatment did not cause a negative impact on the lettuce quality. Several authors reported similar results (Izumi, 1999; Qi et al., 2018; Yang, Swem, & Li, 2003). Regarding the color difference, depending on its value can be estimated as not noticeable (0 to 0.5), slightly noticeable (0.5 to 1.5), noticeable (1.5 to 3.0), well visible (3.0 to 6.0), and great (6.0 to 12.0) (González-Cebrino, Durán, Delgado-Adámez, Contador, & Ramírez, 2013; Kaushik, Kaur, Rao, & Mishra, 2014). In our work, the color difference between samples treated with EW and tap water was 2.46, so the color change could be considered noticeable. In the sensory analysis, the panelists perceived this color change as a slightly lighter color. This suggested that EW treatment for 45 s did not cause an important discoloration on lettuce. As to the texture profile and flavor, they were not significantly affected

(P>0.05) by EW treatment. Therefore, we considered that EW treatment for 45 s would not affect the acceptability of the final product.

5. Conclusions

Results showed that at least 45 s of exposure time to EW are required to significantly reduce both *Salmonella spp* counts and Imidacloprid concentrations, achieving a reduction of 4 log CFU/g in the *Salmonella spp* counts and a reduction of 48.57% in Imidacloprid concentrations. As to quality parameters, neither texture profile nor flavor were affected by the treatment. Regarding the sensory analysis of color, although a slight difference was found between the treated and control samples, we considered that the acceptability of the product would not be affected. Therefore, the use of EW for 45 s is an effective alternative to reduce *Salmonella spp* and Imidacloprid, improving product safety without negatively affecting the quality of the lettuce.

REFERENCES

Abadias, M., Usall, J., Oliveira, M., Alegre, I., & Viñas, I. (2008). Efficacy of neutral electrolyzed water (NEW) for reducing microbial contamination on minimally-processed vegetables. *International Journal of Food Microbiology*, *123*(1–2), 151–158. https://doi.org/10.1016/j.ijfoodmicro.2007.12.008

Abdullah, Randhawa, M. A., Akhtar, S., Mansoor-ul-Hassan, Asghar, A., Sohaib, M., Aadil, R. M. & Jahangir, M. A. (2016). Assessment of different washing treatments to mitigate imidacloprid and acetamaprid residues in spinach. *Journal of the Science of Food and Agriculture*, *96*(11), 3749–3754. https://doi.org/10.1002/jsfa.7563

Bhilwadikar, T., Pounraj, S., Manivannan, S., Rastogi, N. K., & Negi, P. S. (2019). Decontamination of Microorganisms and Pesticides from Fresh Fruits and Vegetables: A Comprehensive Review from Common Household Processes to Modern Techniques. *Comprehensive Reviews in Food Science and Food Safety*, *18*. https://doi.org/10.1111/1541-4337.12453

Carozza, S. E., Li, B., Wang, Q., Horel, S., & Cooper, S. (2009). Agricultural pesticides and risk of childhood cancers. *International Journal of Hygiene and Environmental Health*, *212*(2), 186–195. https://doi.org/10.1016/j.ijheh.2008.06.002

Farina, Y., Abdullah, M. P., Bibi, N., & Khalik, W. M. A. W. M. (2017). Determination of pesticide residues in leafy vegetables at parts per billion levels by a chemometric study using GC-ECD in Cameron Highlands, Malaysia. *Food Chemistry*, *224*, 186–192. https://doi.org/10.1016/j.foodchem.2016.11.113

González-Cebrino, F., Durán, R., Delgado-Adámez, J., Contador, R., & Ramírez, R. (2013). Changes after high-pressure processing on physicochemical parameters, bioactive compounds, and polyphenol oxidase activity of red flesh and peel plum purée. *Innovative Food Science and Emerging Technologies*, *20*, 34–41. https://doi.org/10.1016/j.ifset.2013.07.008

Guentzel, J. L., Liang Lam, K., Callan, M. A., Emmons, S. A., & Dunham, V. L. (2008). Reduction of bacteria on spinach, lettuce, and surfaces in food service areas using neutral electrolyzed oxidizing water. *Food Microbiology*, *25*(1), 36–41. https://doi.org/10.1016/j.fm.2007.08.003

Hricova, D., Stephan, R., & Zweifel, C. (2008). Electrolyzed water and its application in the food industry. *Journal of Food Protection*, *71*(9), 1934–1947. https://doi.org/10.4315/0362-028X-71.9.1934

Izumi, H. (1999). Electrolyzed Water as a Disinfectant for Fresh-cut Vegetables. *Journal of Food Science*, *64*(3), 536–539. https://doi.org/10.1111/j.1365-2621.1999.tb15079.x

Jeddi, M. Z., Yunesian, M., Gorji, M. E. haghi, Noori, N., Pourmand, M. R., & Khaniki, G. R. J. (2014). Microbial evaluation of fresh, minimally-processed vegetables and bagged sprouts from chain supermarkets. *Journal of Health, Population and Nutrition*, *32*(3), 391–399.

Kaushik, N., Kaur, B. P., Rao, P. S., & Mishra, H. N. (2014). Effect of high pressure processing on color, biochemical and microbiological characteristics of mango pulp (Mangifera indica cv. Amrapali). *Innovative Food Science and Emerging Technologies*, *22*, 40–50. https://doi.org/10.1016/j.ifset.2013.12.011

Kim, M. J., Moon, Y., Tou, J. C., Mou, B., & Waterland, N. L. (2016). Nutritional value, bioactive compounds and health benefits of lettuce (Lactuca sativa L.). *Journal of Food Composition and Analysis*, *49*, 19–34. https://doi.org/10.1016/j.jfca.2016.03.004

Koseki, S., Yoshida, K., Kamitani, Y., & Itoh, K. (2003). Influence of Inoculation Method, Spot Inoculation Site, and Inoculation Size on the Efficacy of Acidic Electrolyzed Water against Pathogens on Lettuce. *Journal of Food Protection*, *66*(11), 2010–2016. https://doi.org/10.4315/0362-028X-66.11.2010

Li, W., Tai, L., Liu, J., Gai, Z., & Ding, G. (2014). Monitoring of pesticide residues levels in fresh vegetable form Heibei Province, North China. *Environmental Monitoring and Assessment*, *186*(10), 6341–6349. https://doi.org/10.1007/s10661-014-3858-7

Lu, C., Chang, C. H., Palmer, C., Zhao, M., & Zhang, Q. (2018). Neonicotinoid Residues in Fruits and Vegetables: An Integrated Dietary Exposure Assessment Approach. *Environmental Science and Technology*, *52*(5), 3175–3184. https://doi.org/10.1021/acs.est.7b05596

Mattia, D. D., & Manikonda, K. (2018). Morbidity and Mortality Weekly Report Surveillance for Foodborne Disease Outbreaks — United States Centers for Disease Control and Prevention MMWR Editorial and Production Staff MMWR Editorial Board. *Surveillance Summaries MMWR*, *6262*(2), 2009–2015. Retrieved from https://www.cdc.gov/mmwr/pdf/ss/ss6202.pdf

Mikolić, A., & Karačonji, I. B. (2018). Imidacloprid as reproductive toxicant and endocrine disruptor: Investigations in laboratory animals. *Arhiv Za Higijenu Rada i Toksikologiju*, *69*(2), 103–108. https://doi.org/10.2478/aiht-2018-69-3144

Olaimat, A. N., & Holley, R. A. (2012). Factors influencing the microbial safety of fresh produce: A review. *Food Microbiology*, *32*(1), 1–19. https://doi.org/10.1016/j.fm.2012.04.016

Pang, Y.-H., & Hung, Y.-C. (2016). Efficacy of Slightly Acidic Electrolyzed Water and UV-Ozonated Water Combination for Inactivating *Escherichia coli* O157:H7 on Romaine and Iceberg Lettuce during Spray Washing Process. *Journal of Food Science*, *81*(7), M1743–M1748. https://doi.org/10.1111/1750-3841.13364

Park, E. J., Alexander, E., Taylor, G. A., Costa, R., & Kang, D. H. (2008). Effect of electrolyzed water for reduction of foodborne pathogens on lettuce and spinach. *Journal of Food Science*, *73*(6). https://doi.org/10.1111/j.1750-3841.2008.00809.x

Qi, H., Huang, Q., & Hung, Y.-C. (2018). Effectiveness of electrolyzed oxidizing water treatment in removing pesticide residues and its effect on produce quality. *Food Chemistry*, *239*(11), 561–568. https://doi.org/10.1016/j.foodchem.2017.06.144

Rahman, S., Khan, I., & Oh, D. H. (2016). Electrolyzed Water as a Novel Sanitizer in the Food Industry: Current Trends and Future Perspectives. *Comprehensive Reviews in Food Science and Food Safety*, *15*(3), 471–490. https://doi.org/10.1111/1541-4337.12200

Rahman, S. M. E., Ding, T., & Oh, D.-H. (2010). Effectiveness of low concentration electrolyzed water to inactivate foodborne pathogens under different environmental conditions. *International Journal of Food Microbiology*, *139*, 147–157.

Sagoo, S. K., Little, C. L., Ward, L., Gillespie, I. a., & Mitchell, R. T. (2003). Microbiological Study of Ready-to-Eat Salad Vegetables from. *Journal of Food Protection*, *66*(3), 403–409.

Stopforth, J. D., Mai, T., Kottapalli, B., & Samadpour, M. (2016). Effect of Acidified Sodium Chlorite, Chlorine, and Acidic Electrolyzed Water on *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* Inoculated onto Leafy Greens. *Journal of Food Protection*, *71*(3), 625–628. https://doi.org/10.4315/0362-028x-71.3.625

Venkitanarayanan, K. S., Ezeike, G. O., Hung, Y. C., & Doyle, M. P. (1999). Efficacy of electrolyzed oxidizing water for inactivating *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Listeria monocytogenes*. *Applied and Environmental Microbiology*, *65*(9), 4276–4279. https://doi.org/10.1128/aem.65.9.4276-4279.1999

Vugia, D. J., Samuel, M., Shallow, S., Farley, M. M., Angulo, F. J., Marcus, R., Shiferaw, B. & Smith, K. (2004). Invasive *Salmonella* Infections in the United States, FoodNet, 1996–1999: Incidence, Serotype Distribution, and Outcome. *Clinical Infectious Diseases*, *38*(Supplement_3), S149–S156. https://doi.org/10.1086/381581

Warriner, K., & Namvar, A. (2013). *Postharvest washing as a critical control point in fresh produce processing: Alternative sanitizers and wash technologies*. *Global Safety of Fresh Produce: A Handbook of Best Practice, Innovative Commercial Solutions and Case Studies*. Woodhead Publishing Limited. https://doi.org/10.1533/9781782420279.2.71

Wu, Y., An, Q., Li, D., Wu, J., & Pan, C. (2019). Comparison of different home/commercial washing strategies for ten typical pesticide residue removal effects in kumquat, spinach and cucumber. *International Journal of Environmental Research and Public Health*, *16*(3), 1–20. https://doi.org/10.3390/ijerph16030472

Yang, H., Swem, B. L., & Li, Y. (2003). The effect of pH on inactivation of pathogenic bacteria of fresh-cut lettuce by dipping treatment with electrolyzed water.pdf. *Journal of Food*

Science, *68*(3), 1013–1017.

Table 1. Scale used for the sensory panel in order to evaluate the sensory attributes.

Attribute	Scale	
Color	-3	much clearer
	-2	pretty clear
	-1	slightly clearer
	0	Same color as the reference
	+1	slightly darker
	+2	pretty darker
	+3	much darker
Crispness	-3	much less crispy
	-2	rather less crispy
	-1	slightly less crispy
	0	just as crunchy as the reference
	+1	slightly more crispy
	+2	quite crunchy
	+3	much more crispy

Table 2. *Salmonella spp* counts in Tryptic Soy agar (TSA) observed in samples treated with tap water and electrolyzed water (EW) for 15, 30 and 45 s.

EW treatment (ppm)	Exposure times (s)	TSA count (log CFU/g)	Logarithmic reduction (log CFU/g)
0	0	7.19 (0.29) a	-
0	15	5.63 (0.46) b	1,56
0	30	5.47 (0.40) b	1,72
0	45	5.65 (0.60) b	1,54
50	15	4.13 (0.60) c	3,06
50	30	4.46 (0.47) c	2,73
50	45	3.13 (0.61) d	4,06

Results are expressed as mean (SD); n=9 per treatment. a, b, c Interventions with no common letter differed significantly ($p < 0.05$; one-way ANOVA).

EW concentration was 50 ppm of free available chlorine

Table 3. Imidacloprid concentrations observed in samples treated with tap water and electrolyzed water (EW) for 15, 30 and 45 s.

EW treatment (ppm)	**Exposure time (s)**	**Imidacloprid Concentration (mg/kg)**	**Reduction (%)**
0	0	0.70 (0.05) a	-
0	15	0.50 (0.10) ab	28.57
0	30	0.46 (0.07) ab	34.29
0	45	0.51 (0.04) b	27.14
50	15	0.53 (0.05) b	24.28
50	30	0.47 (0.09) ab	32.85
50	45	0.36 (0.11) c	48.57

Results are expressed as mean; N=9 per treatment. a, b, c Interventions with no common letter differed significantly ($p < 0.05$; one-way ANOVA).

EW concentration was 50 ppm of free available chlorine

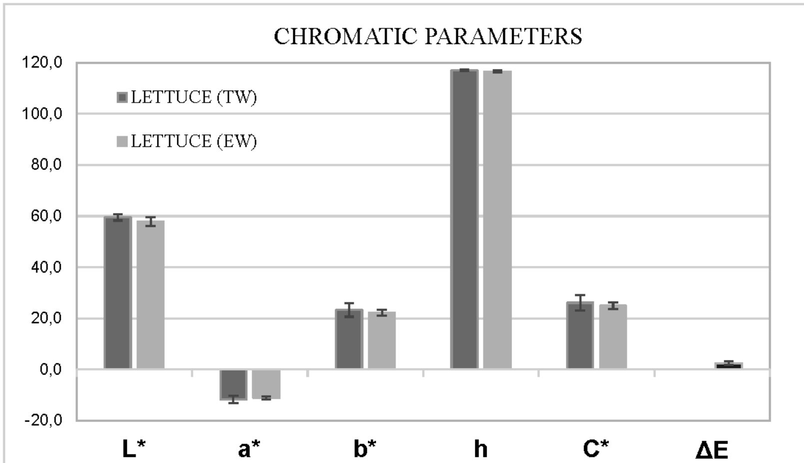

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Figure 1 Chromatic parameters of the lettuce treated with tap water (TW) and electrolyzed water (EW) for 45 s.

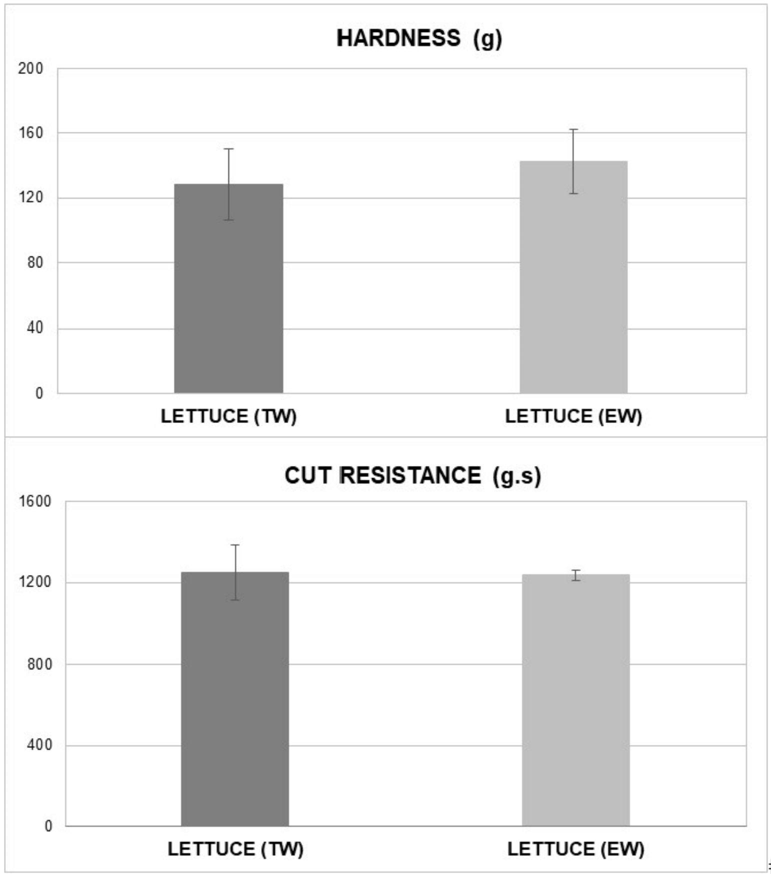

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Figure 2 Texture profile of the lettuce treated with tap water (TW) and electrolyzed water (EW) for 45 s.

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HIGHLIGHTS

1. *Salmonella spp* counts in lettuce were reduced after 45 s of EW treatment.
2. Imidacloprid concentrations in lettuce were reduced after 45 s of EW treatment.
3. Lettuce quality parameters were not affected after 45 s of EW treatment.
4. EW treatment for 45 s significantly improved lettuce safety.

Conflict of Interest and Authorship Conformation Form

Please check the following as appropriate:

- All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

- This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

- The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript

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