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Water reconditioning by high power ultrasound combined with residual chemical sanitizers to inactivate foodborne pathogens associated with fresh-cut products

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ABSTRACT

The suitability of high power ultrasound (HPU, 20 kHz, 0.28 kW/l) combined with residual chemical sanitizers for water reconditioning was studied. A synergetic disinfection effect was observed when HPU was combined with peroxyacetic acid (PAA) or a commercial mix of organic acids and phenolic compounds (OA/PC). In recycled water (RW) with a chemical oxygen demand (COD) of 500 mg O_2/l, PAA inactivated 2 log units of *Escherichia coli* O157:H7 at concentrations of 3.2, 6.4, 16 mg/l after 7 min, 2 min, 29 s, respectively. The OA/PC or HPU treatments alone needed 26 min treatments to achieve the same reduction. The addition of TiO_2 (5 g/l) to HPU (sonocatalysis) did not improve *E. coli* O157:H7 inactivation. However, when HPU was combined with a residual concentration of PAA (3.2 mg/l), the total inactivation of *E. coli* O157:H7 and *Salmonella* (6 log unit reductions) occurred after 11 min, but for *Listeria monocytogenes* only 1.7 log reductions were detected after 20 min. When HPU was combined with OA/PC, a synergistic effect for the inactivation of *E. coli* O157:H7 was also observed, but this sanitizer significantly modified the physical-chemical quality characteristics of the RW. These results show that the residual PAA concentration that can be found in the wash water combined with HPU could result in an environmentally friendlier and toxicologically safer strategy for water reconditioning of the fresh-cut industry. The use of the sanitizer alone requires higher concentrations and/or longer contacts times. Even though the residual PAA in combination with HPU was adequate for water reconditioning, it is not appropriate for the process wash water because this wash water must be instantaneously disinfected.

1. Introduction

The term 'recycled water' (RW) basically refers to the water that is collected after washing the product and can be pumped back into the system for washing new produce. Disinfection technologies for process wash water (PWW) and RW are necessary to reduce wastewater and therefore the environmental impact. However, the disinfection technologies for each type of water are different because of the differences in the water quality characteristics (Luo, Nou, Yang, Abadias, & Conway, 2011). Water quality of PWW changes constantly as the product is constantly added to the washing tank (Gil, Selma, López-Gálvez, & Allende, 2009). Disinfection technology for PWW requires short contact times because microorganisms must be 'instantly' inactivated. A residual level of the sanitizer is always needed to avoid cross-contamination (Gil, Allende, & Selma, 2010; Gil et al., 2015). The sanitizer must preserve product quality as it is in direct contact with the product. However for RW, the disinfection technology must be able to treat large volumes of water but for longer contact times. The organic matter content does not change as rapidly as in PWW. The disinfectant can be used at high doses because it is not in direct contact with the product, but for environmental reasons it should be used at the lowest concentration possible (Gil, personal communication).

The use of chlorinated water for PWW has been widespread throughout the fresh produce industry over the past 30 years (Suslow, 1997). However, for RW, alternative technologies to

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chlorine must be used due to the instability of chlorine and the adverse effects of by-product formation in the presence of organic matter (Gómez-López, Marín, Medina-Martínez, Gil, & Allende, 2013; Van Haute, Sampers, Holvoet, & Uyttendaele, 2013; Waters & Hung, 2014). Among these alternatives, peroxyacetic acid (PAA) and Citrox® (a mix of organic acids and phenolic compounds, OA/PC), inactivate *Escherichia coli* in PWW without by-product formation and with lower pH dependence (Kitis, 2004; López-Gálvez, Allende, Selma, & Gil, 2009). The mix of OA/PC is also effective against *E. coli* O157:H7, *Salmonella* spp. and *Listeria* spp. inoculated on apple plugs (Abadias, Alegre, Usall, Torres, & Viñas, 2011). However, the main disadvantage is the increase in the organic matter content of the effluent (Kitis, 2004; López-Gálvez et al., 2009) and the longer time needed to reach the inactivation. According to Ölmez and Kretzschmar (2009), an efficient disinfection technology for RW is the combination of physical and chemical methods.

High power ultrasound (HPU) at low frequencies (20–100 kHz) can be considered to be an emerging and promising technology for water disinfection (Mason & Peters, 2002). This method has already been implemented by the industry to control the microbial quality of water systems (Broekman, Pohlmann, Beardwood, & Cordemans de Meulenaer, 2010). Its power is sufficient to inactivate microorganisms as opposed to low power ultrasound (McClements, 1995). In order to increase the efficacy, ultrasound has been combined with titanium dioxide (TiO_2) (Dadjour, Ogino, Matsumura, & Shimizu, 2005; Kubo, Onodera, Shibasaki-Kitakawa, Tsumoto, & Yonemoto, 2005; Shimizu, Ogino, Dadjour, & Murata, 2007) and chlorine (Drakopoulou, Terzakis, Fountoulakis, Mantzavinos, & Manios, 2009; Duckhouse, Mason, Phull, & Lorimer, 2004). Previous studies have described the effect of ultrasound in combination with PAA for the reduction of natural microbiota and *Salmonella* inoculated on tomatoes (Brilhante & Dantas, 2012) and *E. coli* O157:H7 inoculated on spinach (Zhou, Feng, & Luo, 2009). Recently, Palma, Pearlstein, Luo, and Feng (2014) showed that the quality of lettuce during the shelf-life was not negative affected by ultrasound combined with PAA. Most of the studies concerning the evaluation of sanitizers on the reduction of pathogenic microorganisms do not take into account the presence of organic matter (Beuchat, 1996). Indeed, PWW contains high organic loads with chemical oxygen demand (COD) between 500 and 3000 mg O_2/l (Selma, Allende, López-Gálvez, Conesa, & Gil, 2008). There is a gap in the knowledge of the ultrasound efficacy in combination with sanitizers at a very low concentration, for RW. In the present study the efficacy in elimination of some foodborne pathogens by HPU combined with residual concentration of the non-chlorinated sanitizers (PAA, OA/PC) and TiO_2 was investigated.

2. Material and methods

2.1. Recycled water production and characterization

Recycled water (RW) was artificially generated as previously described (López-Gálvez et al. 2012). Briefly, leaves of Romaine lettuce (*Lactuca sativa* L.) were cut in 3 cm pieces. Then, 67 g of those lettuce pieces were disposed in a sterile stomacher filter bag (Seward Limited, London, UK). Two hundred ml of potable water was added to the bag and the mixture was homogenized for 120 s in a stomacher (IUL instruments, Barcelona, Spain). This procedure was repeated until the required volume was generated. For the microbial characterization of RW, total aerobic mesophilic bacteria were enumerated by standard plate count method on plate count agar (PCA, Oxoid, Basingstoke) after incubation for 48 h at 30 °C. Total coliforms and *E. coli* were enumerated in chromocult coliform agar (Merck, Darmstadt, Germany) after incubation for 24 h at 37 °C. Yeasts and moulds were counted in rose bengal chloramphenicol agar (Scharlab, Barcelona, Spain) after incubation for 72 h at 25 °C. Lactic acid bacteria were enumerated in de Man, Rogosa, Sharpe agar (MRS) (Scharlab) after incubation for 72 h at 30 °C under microaerophilic conditions. COD was measured using a photometer (Spectroquant, NOVA 60, Merck, Darmstadt, Germany) and the standard photometric method (APHA, 1998). Turbidity was measured by a turbidity meter (Turbiquant 3000 IR, Merck, Darmstadt, Germany) following the nephelometric method (APHA, 1998) and expressed as nepholometric turbidity units (NTU).

Microbial counts (log CFU/ml) were very similar; 6.02 ± 0.38 for total aerobic mesophilic bacteria, 3.13 ± 0.33 for total coliforms, 5.08 ± 0.36 for moulds and yeasts, and 1.24 ± 0.18 for lactic acid bacteria. Reported results for mesophilic bacteria and coliforms after washing fresh-cut escarole (12 kg/5 l) were very similar (Allende, Selma, López-Gálvez, Villaescusa, & Gil, 2008a). Turbidity and pH values of RW were 179.6 ± 15.3 NTU and 7.3 ± 0.1, respectively while COD values reached 2833 ± 804 mg O_2/l. Similar values have been reported for PWW (Gómez-López, Gobet, Selma, Gil, & Allende, 2013; Gómez-López et al., 2014; Van Haute et al., 2013). Allende et al. (2008a) reported lower COD value in PWW of fresh-cut escarole (1648 ± 50 mg O_2/l) probably due to differences in the cell exudates of the different lettuce types. RW was diluted 1/15 or 1/7 (v/v) in tap water at 4 °C to achieve COD levels of 200 and 500 mg O_2/l, respectively.

2.2. Bacterial inoculation

E. coli O157:H7 CECT 5947 and *Listeria monocytogenes* strains CECT 940 and CECT 5672 were obtained from the Spanish Type Culture Collection (CECT, Valencia, Spain). *Salmonella enterica* serovar Typhimurium (NCTC 12023) was obtained from the National Collection of Type Cultures (NCTC, London, UK). Nalidixic acid-resistant (NalR) *E. coli* O157:H7, NalR *L. monocytogenes* and ampicillin-resistant *Salmonella* cultures were prepared by consecutive 24 h transfers in brain heart infusion broth (BHI, Oxoid, Basingtoke, UK), increasing the concentrations of nalidixic acid (Nal) or ampicillin (Amp) until strains were resistant to 50 μg of Nal or 80 μg Amp per ml BHI. The strains were sub-cultured twice in 5 ml of BHI supplemented with Nal (50 μg/ml) or Amp (80 μg/ml) at 37 °C for 20 h, achieving the stationary phase of growth. After the second incubation, *L. monocytogenes* cultures were vortexed, and in equal volumes, cell suspensions were combined to give approximately similar concentrations of each strain. Final concentrations of *E. coli* O157:H7, *L. monocytogenes* cocktail and *Salmonella* of approximately 10^9 CFU/ml were used to inoculate RW, reaching a final concentration of 10^6 CFU/ml.

2.3. Bacterial inactivation experiments

For HPU treatments, 200 ml of RW with a COD of 500 mg O_2/l, inoculated with *E. coli* O157:H7, *L. monocytogenes* or *Salmonella* were treated in batch with a Branson sonifier (Branson Sonifier S-450A, Branson, Dansbury, USA). The ultrasound equipment used a horn sonotrode that operates at 20 kHz and has a horn tip with a diameter of 1.3 cm. The specific acoustic energy and intensity of the sonifier was examined by calorimetric calibration as described previously (Gómez-López et al., 2014). A volume of 200 ml resulted in an exposure of the samples to an intensity of 0.28 kW/l. Ultrasound power was selected according to previous results (Gómez-López et al., 2014), where 0.28, 0.56 and 1.12 kW/l were found to have very good disinfection capacity according to Madge and Jensen (2002). Given these results, the lowest power (0.28 kW/l) was selected because of the lower energy requirements. The tip of the horn was placed in the centre of the sample and immersed for

1.5 cm in the RW. The test vessel was placed on ice to control the temperature rise due to cavitation.

For the evaluation of the disinfection efficacy of HPU (0.28 kW/l) combined with TiO_2, 5 g/l of catalyst particles (Degussa P-25 Evonik industries, Hanau-Wolfgang, Germany) with a surface area of 50 m^2/g (size 20–40 nm) were used for both RW and tap water (TW) with COD levels of 500 and 60 mg O_2/l, respectively, inoculated with *E. coli* O157:H7. To avoid any light-driven effects, the photocatalytic treatments were conducted in the dark. Therefore, the inactivation of *E. coli* O157:H7 was only due to the ultrasonic treatment with and without the activation by TiO_2. At several time intervals, 1.5 ml samples were taken for bacterial enumeration during HPU treatments alone or combined with TiO_2.

The commercial sanitizer P3-Tsunami-100 (Ecolab, Minnesota, USA) was used as a source of PAA. Concentrations of 10, 20, 40, 100 mg/l Tsunami, that can be considered as a residual concentration of the PWW, which corresponded to 1.6, 3.2, 6.4, 16 mg/l PAA, respectively, were evaluated for the inactivation of *E. coli* O157:H7 in RW (COD 500 mg O_2/l). Combination of HPU (0.28 kW/l) and PAA (3.2 g/l) was studied in RW (COD of 200 and 500 mg O_2/l) inoculated with *E. coli* O157:H7, *L. monocytogenes* or *Salmonella*. Samples of 1.5 ml were taken at various times during bacterial disinfection by PAA, and 10% sodium thiosulphate neutralizer (S-8503, Sigma) was added in a 1:1 ratio. Samples were plated by the Eddy Jet Spiral Plater (IUL instruments, Barcelona, Spain) for bacterial enumeration.

The commercial sanitizer Citrox (Middlesbrough, United Kingdom), a mix of OA/PC, at concentrations of 500 and 1000 mg/l was tested for the inactivation of *E. coli* O157:H7. Concentration of 1000 mg/l was selected for the combined treatments of HPU and OA/PC for RW with a COD of 500 mg O_2/l inoculated with *E. coli* O157:H7, *L. monocytogenes* or *Salmonella*. Samples of 1.5 ml were taken at various times during bacterial disinfection, added in a 1:1 ratio to a neutralizer containing 3% Tween 20, 3% saponine, 0.1% histidine and 0.1% cysteine and plated for bacterial enumeration. Concentration of sanitizers was selected to provide doses below manufacturer's recommendation since the goal was to reduce the use of chemicals by combining them with HPU.

2.4. Enumeration of inoculated bacteria

E. coli O157:H7 inoculated in RW was enumerated using chromocult coliform agar (Merck) containing 50 μg/ml Nal after incubation for 24 h at 37 °C. *L. monocytogenes* was counted using a chromogenic *Listeria* agar base (Oxoid, Hampshire, UK) containing 50 μg/ml Nal, after incubation for 24 h at 37 °C. *Salmonella* Amp resistant was enumerated in nutrient agar (Oxoid) containing 80 μg/ml Amp after incubation for 24 h at 37 °C.

2.5. Data modelling and statistical analysis

All experiments were repeated three times. Data obtained from the inactivation experiments of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* were log transformed (log CFU/ml). Model analysis for the inactivation of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* by HPU (log linear model), the mix of OA/PC, PAA and the combination of both HPU + PAA (biphasic with shoulder model) in RW is included in Supplementary Information (Tables S1 and S2). For each model, the Root Mean Square Error (RMSE), R^2 and adjusted R^2 were used to estimate how well the model predicts this experimental data. Analysis of variance (ANOVA), followed by Bonferroni post hoc test with a significant level of $P \leq 0.05$, was performed on the data using SPSS 19.0 for Windows.

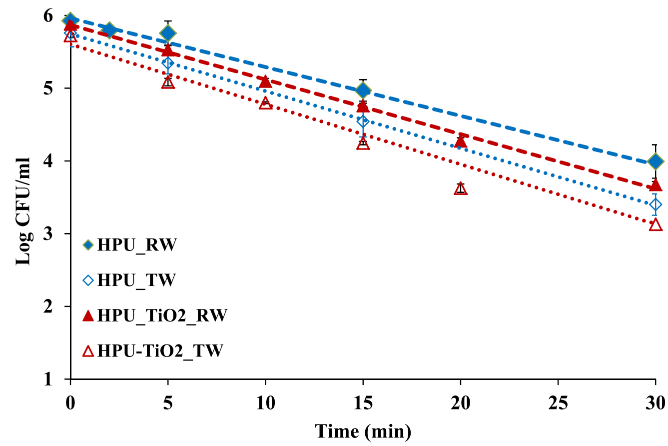

Fig. 1. Inactivation of *E. coli* O157:H7 in recycled water (RW, 500 mg O_2/l COD) and tap water (TW, 60 mg O_2/l COD) by high-power ultrasound (HPU, 20 kHz, 0.28 kW/l) combined or not with titanium dioxide (HPU–TiO_2, 5 g/l).

3. Results and discussion

3.1. HPU treatments for water reconditioning

The inactivation of *E. coli* O157:H7 inoculated in RW (COD of 500 mg O_2/l) by HPU was only 2 log units after 27.7 ± 1.9 min, similar to that observed for TW (COD of 200 mg O_2/l) (Fig. 1). These results demonstrated that the efficacy of HPU was not affected by differences in COD levels, in agreement with those reported for organic matter content between 9 and 3525 mg O_2/l (Madge & Jensen, 2002). As shown in a previous study (Gómez-López et al., 2014) the inactivation observed was not related to a temperature increase because the temperature was controlled in the range of 20–25 °C using an ice bath. Our results indicated that HPU can be applied to treat RW but improvements in the disinfection efficacy would be needed.

3.2. Improvement in the disinfection efficacy of HPU by TiO_2

Disinfection efficacy of HPU for the inactivation of *E. coli* O157:H7 in RW and TW did not significantly improve by the addition of TiO_2 (Fig. 1). After 30 min, despite a significant reduction ($P < 0.05$) of the initial inoculum, only an extra 0.1 reduction was

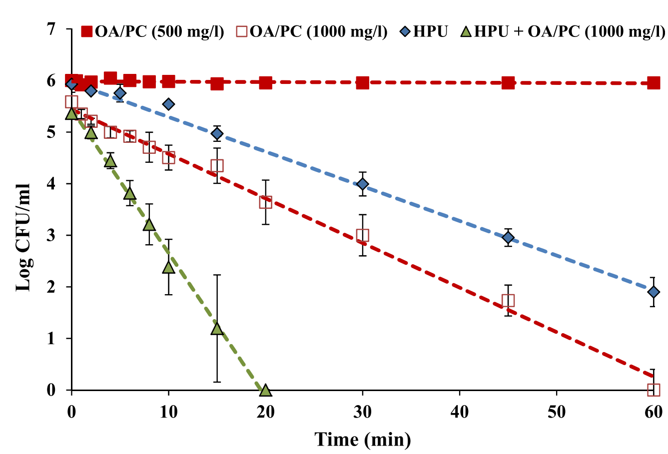

Fig. 2. Inactivation of *E. coli* O157:H7 in recycled water (RW, 500 mg O_2/l COD) by high-power ultrasound (HPU, 20 kHz, 0.28 kW/l) combined or not with a mix of organic acids and phenolic compounds (OA/PC) at different concentrations (500 and 1000 mg/l).

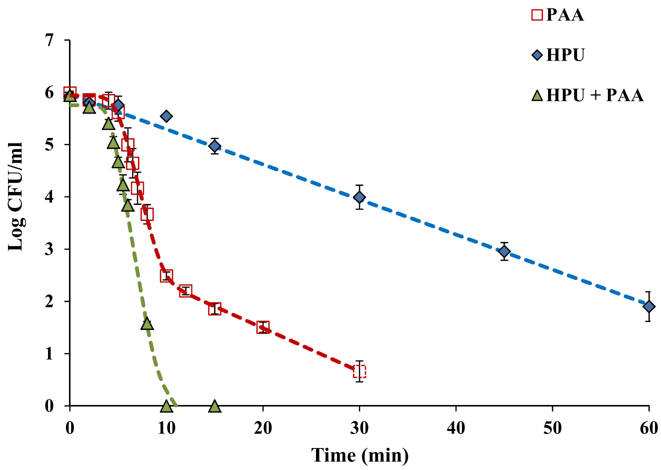

Fig. 3. Inactivation of *E. coli* O157:H7 in recycled water (RW, 500 mg O_2/l COD) by high-power ultrasound (HPU, 20 kHz, 0.28 kW/l) combined or not with peroxyacetic acid (PAA, 3.2 mg/l).

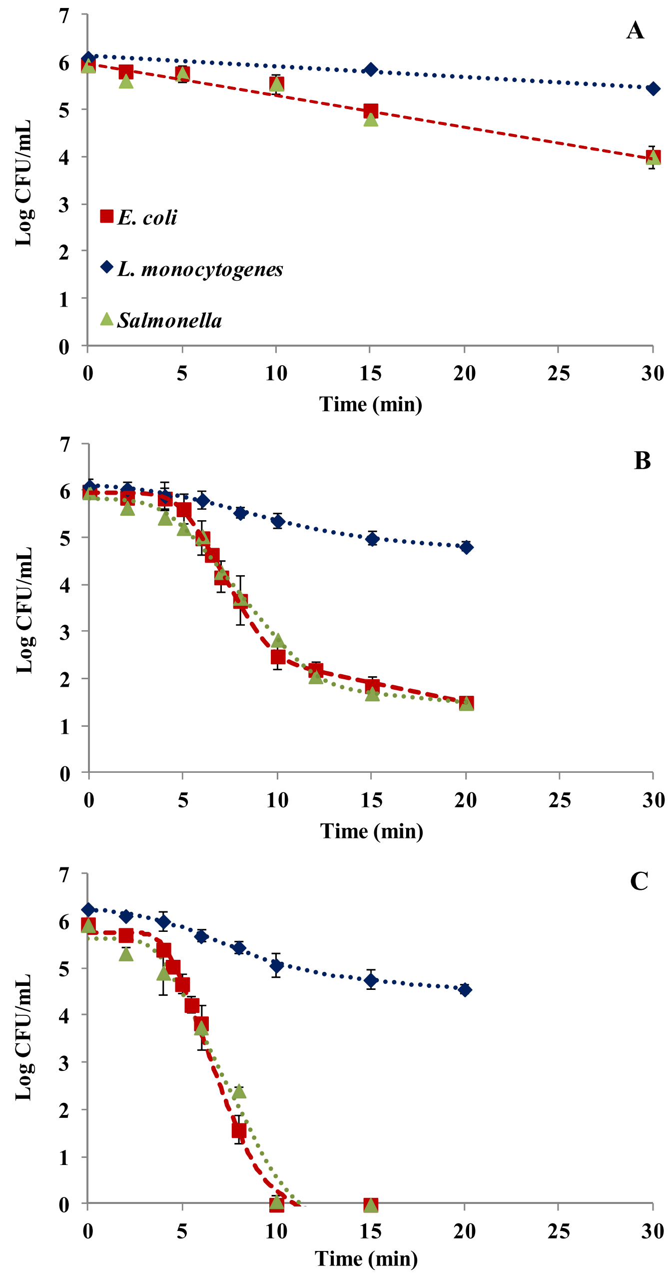

Fig. 4. Inactivation of different pathogenic bacteria in recycled water (RW, 500 mg O_2/l COD) by high-power ultrasound (HPU, 20 kHz, 0.28 kW/l) (A), peroxyacetic acid (PAA, 3.2 mg/l) (B) and the combination of HPU + PAA (C).

observed when HPU was combined with TiO_2. Previous studies reported that TiO_2 improved the disinfection of an ultrasonic process, but the concentration of TiO_2 was much higher (2000 g/l vs 5 g/l) (Dadjour et al. 2005; Kubo et al. 2005). In addition, in previous studies by Dadjour et al. (2005) and Kubo et al. (2005), the particle size was 2 mm and 5 µm, respectively, while in our study it was 20–40 nm. Similarly to our study, Drakopoulou et al. (2009) evaluated the disinfection efficacy of ultrasound (24 kHz, 300 W) combined with TiO_2 (5 g/l, 21 nm particle size) for the secondary treatment of municipal wastewater and only small differences (0.3 log CFU/ml) in the reduction of total coliforms were observed after 30 min.

3.3. Improvements in the disinfection efficacy of HPU by a mix of organic acids and phenolic compounds

Concentration of 500 mg/l of the mix of OA/PC was not effective in reducing *E. coli* O157:H7 in RW with 500 mg O_2/l COD (Fig. 2). When the concentration of OA/PC increased to 1000 mg/l, a reduction of 2 log units was observed after 24.3 ± 2.7 min (Fig. 2). The lower efficacy of this natural antimicrobial mix has been previously reported and even at higher concentrations (5000 mg/l), no more than 2.2 log reductions of generic *E. coli* were observed after 1 min (López-Gálvez et al., 2009). Concentrations higher than 1000 mg/l were not studied because we wanted to simulate residual concentrations easily found in RW and also because this sanitizer significantly modifies the physical-chemical characteristics of the RW (Allende, Selma, López-Gálvez, Villaescusa, & Gil, 2008b; López-Gálvez et al., 2009). In our study, the addition of 1000 mg/l OA/PC increased the COD from 500 to 739 mg O_2/l and decreased the pH from 7.4 to 3.6. When OA/PC (1000 mg/l) and HPU which showed similar disinfection efficacy ($P = 0.96$) were combined, *E. coli* O157:H7 (5.4 log CFU/ml) was completely inactivated in 20 min (Fig. 2). The synergistic effect of ultrasounds and chlorine for the disinfection of municipal wastewater has been described (Duckhouse et al., 2004). The present study shows for the first time the synergistic effect of OA/PC when combined with HPU. As organic acids exert their microbicidal action by penetrating the interior of bacterial cells, and HPU damages bacterial inner membrane (Cameron, McMaster, & Britz, 2008), it is possible that the observed synergistic effect was due to HPU facilitating the penetration of organic acids.

3.4. Improvements in the disinfection efficacy of HPU by peroxyacetic acid (PAA)

Different concentrations of PAA (1.6, 3.2, 6.4 and 16 mg/l) were examined in order to select a residual dose of PAA to then combine with HPU for the bacterial inactivation in the RW and 3.2 mg/l PAA was selected (Supplementary Information, Fig. S1 and Table S1). *E. coli* O157:H7 (6 log CFU/ml) was inactivated beyond the detection limit when HPU was combined with 3.2 mg/l PAA after 11 min (Fig. 3). When only PAA was used, the inoculum level of *E. coli* O157:H7 remained at 1.5 log CFU/ml after 20 min. HPU was also more effective when combined with PAA than with OA/PC and inactivation kinetic parameters confirmed this (Supplementary Information, Table S2). This is the first time that a synergetic effect of ultrasounds and PAA is described. Both, HPU and PAA, have the cellular membrane as a common target. Therefore the observed synergistic effect could be due to membrane collapse caused by the simultaneous attack of HPU and PAA.

The efficacy of HPU combined with PAA at the residual concentration (3.2 mg/l) selected for the inactivation of *E. coli* O157:H7 was evaluated for other potential foodborne pathogens such as *Salmonella* and *L. monocytogenes*. Disinfection efficacy of HPU, PAA and HPU + PAA against *Salmonella* inoculated in RW (500 mg O_2/l) was similar to that observed for *E. coli* O157:H7 (Fig. 4) and inactivation kinetic parameters of both bacteria were not significantly different (Supplementary Information, Table S2). In contrast, *L. monocytogenes* was more resistant to these water reconditioning treatments. The kinetic parameters obtained for *L. monocytogenes* inactivation confirmed the lowest efficacy of HPU and PAA compared to *E. coli* O157:H7 and *Salmonella* (Supplementary Information, Table S2). Therefore, HPU combined with a residual dose of PAA was particularly effective on Gram negative pathogens such as *E. coli* O157:H7 and *Salmonella*. However, longer contact time and/or higher doses are needed for the complete inactivation of *L. monocytogenes*. A contact time of 4.3 h would be required to obtain 6 log reductions of *L. monocytogenes* by HPU or PAA treatments alone while 2.9 h would be enough when combined HPU and PAA (Supplementary Information, Table S2). Data confirm the large differences between Gram positive and Gram negative bacteria when disinfection technologies are evaluated. These differences have to be taken into account to set up the contact time needed to eliminate both Gram positive and Gram negative pathogens.

4. Conclusions

A synergetic effect was observed when HPU was combined with a residual concentration of PAA (3.2 mg/l). The combination of HPU and a residual OA/PC concentration could be also a promising alternative but it significantly modified the physical-chemical quality characteristics of RW, including pH and COD. Therefore, HPU in combination with PAA was a more suitable and promising water reconditioning technology for RW. This could result in an environmentally friendlier and toxicologically safer strategy for the disinfection of RW in the fresh-cut produce industry. However, HPU combined with PAA would not be appropriate for the disinfection of PWW because higher doses must be used to achieve the instantaneous microbial inactivation. These laboratory findings must be validated in pilot plant tests and later on in real-life processing conditions using appropriately selected surrogates in order to draw final conclusions about its applicability.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.foodcont.2014.12.032.

References

Abadias, M., Alegre, I., Usall, J., Torres, R., & Viñas, I. (2011). Evaluation of alternative sanitizers to chlorine disinfection for reducing foodborne pathogens in fresh-cut apple. *Postharvest Biology and Technology, 59*, 289–297.

Allende, A., Selma, M. V., López-Gálvez, F., Villaescusa, R., & Gil, M. I. (2008a). Impact of wash water quality on sensory and microbial quality, including *Escherichia coli* cross-contamination, of fresh-cut escarole. *Journal of Food Protection, 71*, 2514–2518.

Allende, A., Selma, M. V., López-Gálvez, F., Villaescusa, R., & Gil, M. I. (2008b). Role of commercial sanitizers and washing systems on epiphytic microorganisms and sensory quality of fresh-cut escarole and lettuce. *Postharvest Biology and Technology, 49*, 155–163.

APHA, American Public Health Association. (1998). *Standard methods for the examination of water and wastewater* (20th ed.). Washington, DC: American Public Health Association.

Beuchat, L. R. (1996). Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection, 59*, 204–216.

Brilhante, S. J. J. F., & Dantas, V. M. C. (2012). Effect of ultrasound and commercial sanitizers in removing natural contaminants and *Salmonella enterica* Typhimurium on cherry tomatoes. *Food Control, 24*, 95–99.

Broekman, S., Pohlmann, O., Beardwood, E. S., & Cordemans de Meulenaer, E. (2010). Ultrasonic treatment for microbiological control of water systems. *Ultrasonic Sonochemistry, 17*, 1041–1048.

Cameron, M., McMaster, L. D., & Britz, T. J. (2008). Electron microscopic analysis of dairy microbes inactivated by ultrasound. *Ultrasonics Sonochemistry, 15*, 960–964.

Dadjour, M. F., Ogino, C., Matsumura, S., & Shimizu, N. (2005). Kinetics of disinfection of *Escherichia coli* by catalytic ultrasonic irradiation with TiO_2. *Biochemical Engineering Journal, 25*, 243–248.

Drakopoulou, S., Terzakis, S., Fountoulakis, M. S., Mantzavinos, D., & Manios, T. (2009). Ultrasound-induced inactivation of Gram-negative and Gram-positive bacteria in secondary treated municipal wastewater. *Ultrasonics Sonochemistry, 16*, 629–634.

Duckhouse, H., Mason, T. J., Phull, S. S., & Lorimer, J. P. (2004). The effect of sonication on microbial disinfection using hypochlorite. *Ultrasonics Sonochemistry, 11*, 173–176.

Gil, M. I., Allende, A., & Selma, M. V. (2010). Advances in fresh-cut fruits and vegetables processing. In O. Martin-Belloso, & R. S. Fortuny (Eds.), *Treatments to assure safety of fresh-cut fruits and vegetables* (pp. 211–229). USA: CRC Press.

Gil, M. I., Selma, M. V., López-Gálvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: problems and solutions. *International Journal of Food Microbiology, 134*, 37–45.

Gil, M. I., Selma, M. V., Suslow, T., Jacxsens, L., Uyttendaele, M., & Allende, A. (2015). Pre- and post-harvest preventive measures and intervention strategies to control microbial food safety hazards of fresh leafy vegetables. *Critical Reviews in Food Science and Nutrition, 55*, 453–468.

Gómez-López, V. M., Gil, M. I., Allende, A., Blancke, J., Schouteten, L., & Selma, M. V. (2014). Disinfection capacity of high-power ultrasound against *E. coli* O157:H7 in process water of the fresh-cut industry. *Food and Bioprocess Technology, 7*, 3390–3397.

Gómez-López, V. M., Gobet, J., Selma, M. V., Gil, M. I., & Allende, A. (2013). Operating conditions for the electrolytic disinfection of process wash water from the fresh-cut industry contaminated with *E. coli* O157:H7. *Food Control, 29*, 42–48.

Gómez-López, V. M., Marín, A., Medina-Martínez, M. S., Gil, M. I., & Allende, A. (2013). Generation of trihalomethanes with chlorine-based sanitizers and impact on microbial, nutritional and sensory quality of baby spinach. *Postharvest Biology and Technology, 85*, 210–217.

Kitis, M. (2004). Disinfection of wastewater with peracetic acid: a review. *Environment International, 30*, 47–55.

Kubo, M., Onodera, R., Shibasaki-Kitakawa, N., Tsumoto, K., & Yonemoto, T. (2005). Kinetics of ultrasonic disinfection of *Escherichia coli* in the presence of titanium dioxide particles. *Biotechnology Progress, 21*, 897–901.

López-Gálvez, F., Allende, A., Selma, M. V., & Gil, M. I. (2009). Prevention of *Escherichia coli* cross-contamination by different commercial sanitizers during washing of fresh-cut lettuce. *International Journal of Food Microbiology, 133*, 167–171.

López-Gálvez, F., Posada-Izquierdo, G. D., Selma, M. V., Pérez-Rodríguez, F., Gobet, J., Gil, M. I., et al. (2012). Electrochemical disinfection: an efficient treatment to inactivate *Escherichia coli* O157:H7 in process wash water containing organic matter. *Food Microbiology, 30*, 146–156.

Luo, Y., Nou, X., Yang, Y., Abadias, M., & Conway, W. (2011). Determination of free chlorine concentrations needed to prevent *Escherichia coli* O157:H7 cross-contamination during fresh-cut produce wash. *Journal of Food Protection, 74*, 352–358.

Madge, B. A., & Jensen, J. N. (2002). Disinfection of wastewater using a 20 kHz ultrasound unit. *Water Environment Research, 74*, 159–169.

Mason, T. J., & Peters, D. (2002). *Practical sonochemistry: Power ultrasound uses and applications* (2nd ed.). (pp. 12–21). Horwood Publishing.

McClements, J. (1995). Advances in the application of ultrasound in food analysis and processing. *Trends in Food Science and Technology, 6*, 293–299.

Ölmez, H., & Kretzschmar, U. (2009). Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *LWT – Food Science and Technology, 42*, 686–693.

Palma, S., Pearlstein, A. J., Luo, Y., & Feng, H. (2014). Quality of Iceberg (*Lactuca sativa* L.) and Romaine (*L. sativa* L. var. *logifolial*) treated by combinations of sanitizer, surfactant, and ultrasound. *LWT – Food Science and Technology, 56*, 261–268.

Selma, M. V., Allende, A., López-Gálvez, F., Conesa, M. A., & Gil, M. I. (2008). Disinfection potential of ozone, ultraviolet-C and their combination in wash water for the fresh-cut vegetable industry. *Food Microbiology, 25*, 809–814.

Shimizu, N., Ogino, C., Dadjour, M. F., & Murata, T. (2007). Sonocatalytic degradation of methylene blue with TiO_2 pellets in water. *Ultrasonics Sonochemistry, 14*, 184–190.

Suslow, T. V. (1997). *Postharvest chlorination. Basic properties and key points for effective disinfection*. Publication 8003 by the Regents of the University of California.

Van Haute, S., Sampers, I., Holvoet, K., & Uyttendaele, M. (2013). Physicochemical quality and chemical safety of chlorine as a reconditioning agent and wash water disinfectant for fresh-cut lettuce washing. *Applied and Environmental Microbiology, 79*, 2850–2861.

Waters, B. W., & Hung, Y. C. (2014). The effect of organic matter on stability of various chlorine- based sanitizers. *International Journal of Food Science & Technology, 49*, 867–875.

Zhou, B., Feng, H., & Luo, Y. G. (2009). Ultrasound enhanced sanitizer efficacy in reduction of *Escherichia coli* O157:H7 population on spinach leaves. *Journal of Food Science, 74*, M308–M313.