

Disinfection Capacity of High-Power Ultrasound Against *E. coli* O157:H7 in Process Water of the Fresh-Cut Industry

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Received: 24 March 2014 / Accepted: 26 May 2014
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Abstract The fresh-cut industry must treat process water to guarantee its microbial quality before reuse or recirculation back into the processing line. In the present study, the suitability of high-power ultrasound (HPU) for disinfecting and recycling process water was evaluated. An ultrasonic horn (20 kHz) was used to inactivate *Escherichia coli* O157:H7 inoculated in five types of process water which showed different physical and chemical characteristics. Differences in the inactivation level of *E. coli* O157:H7 at different HPU densities (0.14, 0.28, 0.56, and 1.12 kW/L) with controlled (20–25 °C) and uncontrolled (15–72 °C, 3.6 °C/min) temperature increase were studied. Results showed that the higher the power density and temperature, the higher the efficiency, reaching up to 6 log reductions of *E. coli* O157:H7. Alkalinity (between 0 and 253 mg HCO₃⁻/L) and organic matter concentration (between 9 and 3,525 mg O₂/L) in water did not reduce ultrasonic efficacy against *E. coli* O157:H7. Agglomerates >90 µm, which represented 34 % of those present in the process water, were reduced to only 11 % by HPU. Results indicate that HPU can be successfully applied to treat process water of the fresh produce industry because the antimicrobial efficacy was not affected by the continuous variation of the

process water quality. HPU can be a suitable technology for the fresh produce industry to be able to reduce consumption of water and decrease wastewater and the generation of disinfection by-products.

Keywords Food safety · Disinfection · Sanitation · Water reuse · Water saving

Introduction

Most postharvest processes in the fresh produce industry reuse process water to conserve water and energy (Suslow 1997; Ölmez and Kretzschmar 2009). However, reuse of process water can lead to the accumulation of dirt, organic matter, and disease-causing pathogens, allowing the cross-contamination of the produce (Gil et al. 2009). Furthermore, the water used for washing and chilling the produce after harvest has to be free of any chemical risks (CDC 2009). Therefore, there is a trend within the water treatment industry to develop and employ more environmentally responsible technologies to help reduce the impact of chemicals in effluent waters and reduce water consumption in the process (Broekman et al. 2010). As a result, nonchemical treatments have been increasingly demanded as disinfectant treatments for the process water, mostly because their disinfection against foodborne pathogens is independent of the process water quality characteristics, while they are safe for workers and consumers.

High-power ultrasound (HPU) at low frequencies (from 20 to 100 kHz) is a chemical-free method with the power to cause cavitation, which can inactivate microorganisms (Piyasena et al. 2003). During cavitation and subsequent collapse of microbubbles, large amounts of energy are released, generating high temperatures (of the order of 700–4,700 °C) and pressures (100–5,000 bar) as well as release of free radicals due to pyrolysis of water (Mason and Lorimer 2002; Gogate

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et al. 2003; Mason et al. 2005; de Brilhante São José et al. 2014). Several reviews have described the application of ultrasound to treat fluids alone or in combination with other technologies to degrade pollutants in wastewater (Gogate and Pandit 2004; Gogate and Kabadi 2009), in physical food transformation and enzyme inactivation (Patist and Bates 2008), wastewater disinfection (Gibson et al. 2008), and fruit and vegetable decontamination (Bilek and Turantaş 2013). As revised by Gogate (2007), many different bacteria can be inactivated by HPU such as *Escherichia coli*, *Salmonella*, and even soil spore-forming bacteria like *Bacillus subtilis* in which the thick cell wall does not offer any special protection (Scherba et al. 1991; Gao et al. 2014). This technology alone or in combination with other technologies is used already at an industrial scale (Patist and Bates 2008; Broekman et al. 2010; Gogate et al. 2014). However, there is very little information about the application of HPU for the disinfection of process water of the fresh produce industry. In our preliminary study, we demonstrated that HPU effectively eliminates *E. coli* O157:H7 in process water of a specific physical and chemical quality (Elizaquível et al. 2012). However, how the physical and chemical quality of the process water affects the efficacy of HPU was not addressed. Hence, the present study was designed to gain insight on the suitability of HPU as a disinfection treatment to allow the reuse of process water in the fresh-cut industry. To achieve this goal, the inactivation of *E. coli* O157:H7 in process water with different physical and chemical characteristics and under different HPU conditions has been evaluated.

Materials and Methods

Process Water

Five types of water were used during the experiments: (1) distilled water (DW), (2) tap potable water (TW), and three types of process water (PW)—(3) process water 1 (PW1), (4) process water 2 (PW2), and (5) process water 3 (PW3). The characteristics of the different water types are shown in Table 1. PW 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 milliliters 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 of PW3 was generated. The PW1 and PW2 were obtained by diluting 1/15 and 1/7 (v/v) of PW3 in potable water at 4 °C, respectively.

High-Power Ultrasound (HPU) Equipment and Treatments

Calorimetry was applied to establish the actual ultrasonic power density of the sonication system according to standard procedures (Gogate et al. 2001). The calorimetric calibration was executed on a horn sonotrode (Branson Sonifier S-450A, Emerson Electric, CT, USA) with a tip of 1.3 cm in diameter and a fixed frequency of 20 kHz. Calibration lines using volumes of 400 and 500 mL and a duration time of 150 s were obtained by linear regression analysis, and the Pearson's correlation coefficient was calculated. With a nominal power output of 400 W corresponding to a calorimetrically determined power of 0.056 kW, different volumes (400, 200, 100, and 50 mL) were treated continuously during different times. During HPU treatments, process water samples were placed on ice to avoid temperature increase. The tip of the horn was placed in the center of the sample and immersed 1 cm below the level of the water.

Bacterial Strains and Inoculum Preparation

A five-strain cocktail of *E. coli* O157:H7 isolated from human and foods associated with hemorrhagic colitis and hemolytic uremic syndrome (HSU) (CECT 4076, 4267, 4782, 4783 and 5947) obtained from the Spanish Type Culture Collection (CECT, Valencia, Spain) was used in the study. Cultures were rehydrated in brain heart infusion (BHI) broth (Oxoid, Basingstoke, UK). Nalidixic acid-resistant (Nal^R) *E. coli* O157:H7 cultures were obtained by consecutive 24 h transfers in BHI broth with increasing concentrations of nalidixic acid (Nal) (Merck, Darmstadt, Germany) until strains were resistant to 50 µg of Nal per mL BHI. The strains were subcultured twice in 5 mL of BHI supplemented with Nal (50 µg/mL) at 37 °C for 20 h. After the second incubation, cultures were vortexed, and equal volumes of cell suspensions were combined to give approximately the same population for each culture. The final concentration of the *E. coli* O157:H7 cocktail was approximately 10⁹ colony-forming unit (CFU)/mL and was used to inoculate the different types of water obtaining a final concentration of 10⁴–10⁶ CFU/mL. Process water was inoculated with *E. coli* O157:H7 at two inoculum levels (10³ and 10⁵ CFU/mL).

Microbial Analysis

During the HPU treatments, aliquots of 1.5 mL were taken at different times (0, 15, 30, 45, and 60 min) for microbial analysis. To obtain the appropriate 10-fold sample dilution, peptone water (Oxoid) was used. The Eddy Jet Spiral Plater (IUL Instruments, Barcelona, Spain) was used to spread the aliquots on the plates. The pathogenic Nal^R *E. coli* O157:H7 strains were enumerated using Chromocult coliform agar (Oxoid) containing 50 µg/mL

Table 1 Microbial and physical and chemical quality characteristics of the different types of water—distilled water (DW), tap water (TW), and three types of process water (PW): process water 1 (PW1), process water 2 (PW2), and process water 3 (PW3)

Types of wash water	Parameters							
	Total plate counts (log CFU/mL)	Total coliforms (log CFU/mL)	Molds and yeast (log CFU/mL)	Lactic acid bacteria (log CFU/mL)	COD (mg O ₂ /L)	Alkalinity (mg HCO ₃ ⁻ /L)	pH	Turbidity (NTU)
DW	ND	ND	ND	ND	9±7	0	5.90±0.10	0.07±0.02
TW	ND	ND	ND	ND	18±16	253±8	8.23±0.12	0.08±0.03
PW1	4.89±0.28 ^a	2.58±0.48 ^a	3.39±0.50 ^a	<1 ^a	244±55	78±25	7.09±0.19	45.10±10.70
PW2	5.23±0.28 ^a	2.91±0.48 ^a	3.72±0.50 ^a	<1 ^a	466±25	111±17	7.32±0.06	80.50±0.90
PW3	6.07±0.28	3.76±0.48	4.55±0.50	1.33±1.16	3,525±7	382±30	6.49±0.05	516.50±83.90

ND: counts below the detection limit of 1 log CFU/mL

^aMicrobial values of PW1 and PW2 were estimated according to dilution (1/15 and 1/7, v/v) of the PW3 in potable water at 4 °C

of nalidixic acid and incubated at 37 °C for 24 h. Aliquots were taken from the wash water of lettuce and spread-plated on different agar media. Total aerobic mesophilic bacteria were enumerated by the standard plate count method on plate count agar (PCA) (Scharlau, Barcelona, Spain) after incubation at 30 °C for 48 h. Total coliforms were enumerated using Chromocult coliform agar after incubation at 37 °C for 24 h. Molds and yeasts were enumerated on Rose Bengal chloramphenicol agar (Oxoid) after incubation at 25 °C for 3 days. Lactic acid bacteria were enumerated on de Man Rogosa Sharpe (MRS) agar (Scharlau, Barcelona, Spain) after incubation at 30 °C for 72 h under microaerophilic conditions. Each experiment was repeated twice and each replicate was prepared in duplicate. The amount of viable bacteria in the different samples was counted and expressed as log colony-forming unit per milliliter.

Physical and Chemical Analyses

Alkalinity was determined by potentiometric titration until pH 4.3 with HCl and a pH meter (Crison, Barcelona, Spain). Turbidity was measured by a turbidity meter (Turbiquant 3000 IR, Merck, Darmstadt, Germany) following the nephelometric method (APHA 1998) and expressed as nephelometric turbidity units (NTU). Chemical oxygen demand (COD) was used as an estimation of the organic matter content of the process water, determined by the standard photometric method (APHA 1998) using a photometer (Spectroquant NOVA 60, Merck). During the tests, the temperature of the process water was also recorded. Particle size distribution in the different types of process water was determined by laser diffraction using a Beckman Coulter LS200 (Brea, USA). Particle counts and size distributions were calculated and displayed automatically.

Data Analysis

Statistical analysis was done by analysis of variance (ANOVA) followed by Tukey's multiple range test with a significant level of $P \leq 0.05$, using the software IBM SPSS version 19 (Chicago, USA). Data for the effect of COD on HPU disinfection efficacy at high inoculums levels were fit to the model of Bigelow and Esty by using GlnaFit (Geeraerd et al. 2005).

Results and Discussion

High-Power Ultrasound Treatments and Calorimetric Calibration

In the present study, the power output was measured indirectly by following the increase in temperature due to sonication in two water volumes (400 and 500 mL). The calorimetrically determined power was similar for both water volumes. This is in consonance with Mancier and Leclercq (2008), who did not observe differences in the linearity when using different volumes ($V_{\max}/4$ and $V_{\max}/2$). A linear relation between calorimetrically determined power (actual power) and nominal power was obtained ($R^2 > 0.98$) (Fig. 1). A maximum actual power of 56 W was obtained when nominal power was 400 W. Thus, actual power displayed was seven times lower than the nominal power output. Schmidt et al. (1999) also found that the actual energy output can differ widely from the nominal output and even proved that two models of the same type of instrument can show differences in the actual energy. Therefore, the calorimetric calibration of the applied energy of the equipment was crucial to ensure reproducibility and allow comparison between results. The maximal actual power for the test volume of 400 mL gives a power density of 0.14 kW/L, which corresponded to a specific acoustic energy of 504 kJ/

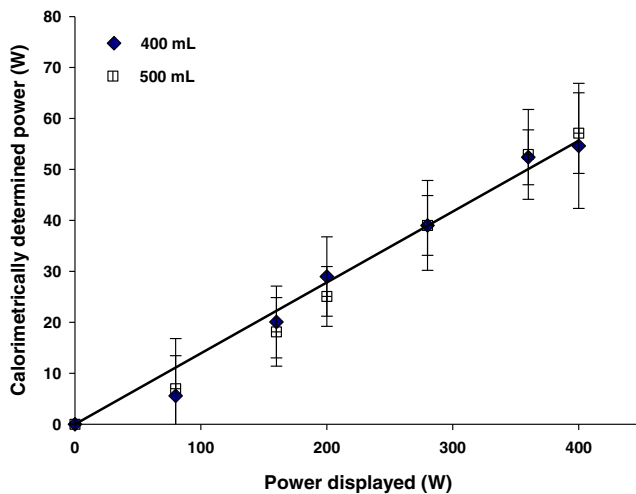


Fig. 1 Calorimetric calibration of the Branson Sonifier S450-A using a fixed frequency of 20 kHz. Correlation between nominal power displayed and calorimetrically determined power using water volumes of 400 and 500 mL

L for a 60-min test time. Power densities of 0.28, 0.56, and 1.12 kW/L were obtained when the maximal actual power was applied on water volumes of 200, 100, and 50 mL, respectively. Intensities displayed were constant during the treatments.

Effect of Power Density on *E. coli* O157:H7 Inactivation by HPU

The disinfection capacity of HPU at different power densities (0.14, 0.28, 0.56, and 1.12 kW/L) and contact times was determined (Fig. 2). Potable water was inoculated with 10^5 CFU/mL of *E. coli* O157:H7 and log reductions of 4.77, 4.29, and 4.18 log CFU/mL were obtained after 60 min of treatment for densities of 1.12, 0.56, and 0.28 kW/L, respectively. A lower log reduction (1.96 log CFU/mL) was observed at the lowest power density tested (0.14 kW/L). Therefore, power density increases resulted in faster disinfection of *E. coli* O157:H7. Madge and Jensen (2002) also reported that the disinfection efficiency on fecal coliforms increases with increased ultrasound power input, changing from 0.003 log CFU/min at 0.07 kW/L to 1.8 log CFU/min at 1.25 kW/L. However, an unlimited increase of disinfection with increasing power density is not expected, an optimal power density will exist beyond which cavitation yield decreases (Gogate et al. 2003), and consequently, bacterial inactivation will be lower. According to the classification proposed by Madge and Jensen (2002) to evaluate the disinfection performance of a proposed method, disinfection capacity is classified as poor if a total log reduction is <1 , intermediate for a total log reduction between 1 and 2, good for total log reduction between 2 and 3, and very good if the reduction is >3 . HPU application at intensities of 0.28, 0.56, and 1.12 kW/L can be considered very good and suitable for process water disinfection of *E. coli*

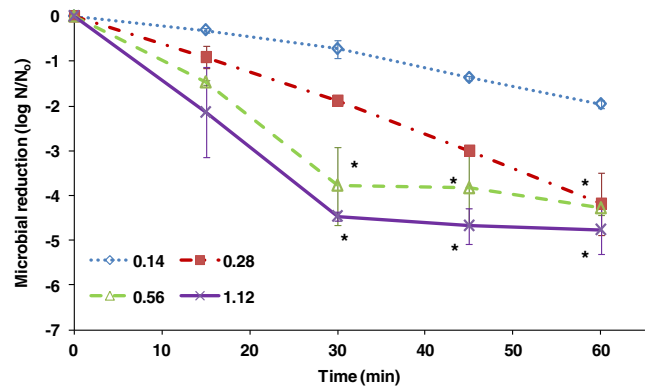


Fig. 2 Effect of power density (kW/L) on *Escherichia coli* O157:H7 inactivation by high-power ultrasound (HPU). Bars represent standard deviation. Asterisks, counts below the detection limit of 1 log CFU/mL. Intensities of 0.14, 0.28, 0.56, and 1.12 kW/mL were obtained in water volumes of 400, 200, 100, and 50 mL, respectively

O157:H7. Trying to reduce as much as possible the operating cost, the power density of 0.28 kW/L was selected for further experiments on *E. coli* O157:H7 inactivation. However, future studies should be done in order to know HPU efficacy on other pathogenic microbial forms and viruses that can be potentially present in process water.

Effect of Water Temperature on *E. coli* O157:H7 Inactivation by HPU

The inactivation of *E. coli* O157:H7 under controlled and uncontrolled water temperatures was tested. Temperature of water samples placed on an ice bath did not increase above 22 °C during 60 min of HPU treatment. In contrast, the temperature of the HPU-treated samples without control of temperature reached 72 °C after 20 min (Fig. 3a). The inactivation of *E. coli* O157:H7 in water by HPU (0.28 kW/L) with and without control of temperature was compared (Fig. 3b). During the first 8 min, similar microbial reductions were observed in HPU-treated water with and without control of temperature although temperature greatly differed (18 and 49 °C, respectively). After 10 min, the temperature of the samples without control of temperature increased more than 50 °C, resulting in a drastic reduction of bacteria survival. In fact, after 20 min of sonication, no microbial survivors were detected. However, a slower disinfection was observed in the water samples with control of temperature, and HPU treatment of 60 min was needed to reduce 5 log units of the initial *E. coli* O157:H7 inoculum. Madge and Jensen (2002) reported that of the total kill produced by ultrasound, approximately 52 % was attributed to heat, 36 % to mechanical stresses associated with ultrasonically induced cavitation, and 12 % to uncharacterized synergistic effects. As expected, due to the heat, we found high disinfection capacity in the samples without temperature control (6 log reductions *E. coli* O157:H7 after 60 and 20 min with and without temperature

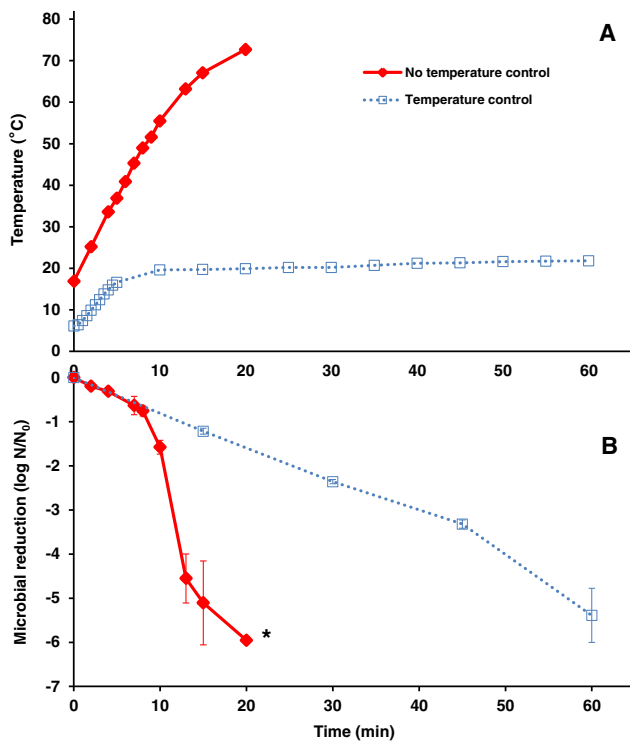


Fig. 3 Effect of controlled (a) and uncontrolled (b) temperatures on *Escherichia coli* O157:H7 inactivation by high-power ultrasound (HPU) at 0.28 kW/L. Bars represent standard deviation. Asterisks, counts below the detection limit of 1 log CFU/mL

control, respectively). This is also in agreement with Salleh-Mack and Roberts (2007) who observed that after 3 min, 6.29 log unit reductions were achieved with no temperature control, while a reduction of only 1.79 log was achieved when the temperature was controlled. In that study, the average temperatures of the samples after 3 and 4 min with no temperature control were 60 and 68 °C, respectively. Therefore, the reported reduction was not only reached by sonication but also by thermal inactivation, and this temperature effect could be advantageous under some circumstances. However, water recirculation back into the processing line at high temperature can affect the food texture and enhance deterioration of sensory quality, particularly in fresh fruits and vegetables (Seymour et al. 2002; Madigan and Martinko 2006). In fact, the fresh-cut industry uses cold water (4 °C) and short times (1–2 min) for washing. Therefore, HPU can be a suitable technology to recycle/disinfect process water but requires a water cooling system before water is reused in the processing line.

Effect of Water Alkalinity on *E. coli* O157:H7 Inactivation by HPU

The efficacy of HPU (0.28 kW/L) against *E. coli* O157:H7 inoculated in TW (very hard water) and in DW (soft water) was investigated (Table 1). More than 3 log reductions of

E. coli O157:H7 were achieved after 45 min of HPU treatment in TW and DW containing very different levels of alkalinity (253 and 0 mg HCO₃⁻/L, respectively) (Fig. 4). Differences observed were lower than 0.5 log units. After 60 min, counts were lower than the detection limit in both types of water (4 log reductions). No reports on the effect of alkalinity and the interferences of scavenged hydroxyl radicals on the disinfection capacity using ultrasonic technology have been found in the literature. However, treatments such as H₂O₂/UV and ozone, which generate a high content of hydroxyl radicals, were very sensitive to scavenging (Liao et al. 2001; Hofmann and Andrews 2006). When bicarbonate or carbonate scavenges the hydroxyl radicals, fewer hydroxyl radicals were present in the wash water and this could reduce the disinfection capacity of the treatment. However, we observed that high water alkalinity did not reduce HPU efficacy against *E. coli* O157:H7. These results indicate an advantage of HPU treatment over electrochemical and ozone disinfection treatments and also over chlorine, the most widely used sanitizer in the fresh-cut industry since Pangloli and Hung (2013) have recently demonstrated that the disinfection efficacy of chlorine also decreases with water hardness.

Effect of Organic Matter Content on *E. coli* O157:H7 Inactivation by HPU

Process water obtained from the processing of lettuce showing three different CODs (244, 466, and 3,525 mg O₂/L for PW1, PW2, and PW3, respectively) and TP (COD 18 mg O₂/L) was compared to examine the differences in microbial inactivation of *E. coli* O157:H7 by HPU. Although alkalinity, pH, and turbidity were also different in the water types, the greatest differences among these samples were the COD levels (Table 1). Selma et al. (2008) found that COD levels of process water obtained in a fresh-cut industry after

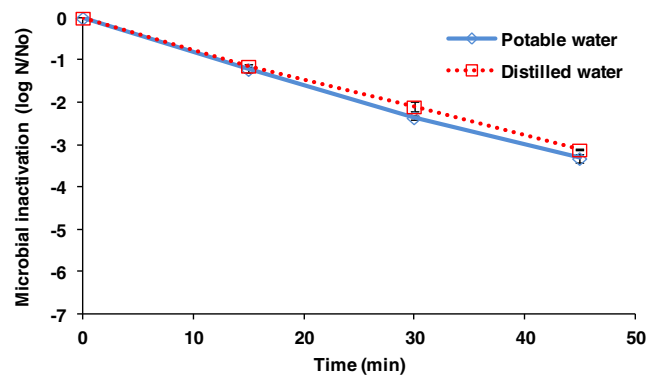


Fig. 4 Effect of alkalinity on *Escherichia coli* O157:H7 inactivation by high-power ultrasound (HPU) at 0.28 kW/L. Distilled water (DW) and tap water (TW) alkalinity were 0 and 253 mg HCO₃⁻/L. Bars represent standard deviation

washing for 2 h six different types of vegetables ranged from 18 to 750 mg O₂/L. Therefore, the COD range of the different types of water used in the present study represented the process water characteristics that can be found in the fresh-cut industry, although in some cases, it can be even worse (3,525 mg O₂/L).

Process water was inoculated with *E. coli* O157:H7 at two inoculum levels (1×10^3 and 6×10^5 CFU/mL). High-power ultrasound (HPU) treatments of 30 and 60 min were needed for *E. coli* O157:H7 inactivation in water samples inoculated with 1×10^3 and 6×10^5 CFU/mL, respectively (Fig. 5a, b). Linear inactivation constants in the different types of water (TW, PW1, PW2, and PW3) inoculated with high inoculum levels were 0.168–0.181/min with $R^2 > 0.99$ and mean sum of square roots of 0.0016–0.0084. Mean *D* value obtained for the different water types was 13.2 ± 0.4 min. Thus, the quality of the water did not significantly affect the inactivation of *E. coli* O157:H7 in PW using HPU. This is a very encouraging result because the COD of PW in the fresh-cut industry is very dynamic during the working cycle, starting with clean water and increasing continuously with the entrance of produce. This is an important advantage compared with chlorine which has a high variability on the disinfection capacity depending on the COD (Van Haute et al. 2013). Madge and Jensen

(2002) also found no effect on the physical and chemical parameters on ultrasound disinfection of municipal water.

There is little information about the effect of organic matter in the PW on microbial disinfection. In literature, some papers discuss the effect of the physical and chemical parameters on microbial disinfection by ultrasound in different liquids. Most of them are for disinfection of drinking water, wastewater, and some other liquid foods such as milk, orange juice, and liquid whole eggs. The effect of pH on the ultrasonic treatment (700 kHz) on *E. coli* was discussed by Utsunomiya and Kosaka (1979) who observed that *E. coli* suspension in saline solution (pH around 7) resulted in 0.2 % survival after 30 min. However, the survival of *E. coli* in milk containing 10 % orange juice at pH 2.6 increased to 0.3 %. In our study, the pH differences were lower than those mentioned in that study, so it was expected that pH differences did not cause differences in the disinfection efficacies. These authors also described the potential effect of organic matter on the disinfection efficacy as orange juice contains higher organic matter level than a saline solution (Utsunomiya and Kosaka 1979). Lee et al. (1989) also reported that ultrasonic treatment (10 min) of *Salmonella* reduced up to 4 log CFU/mL in peptone water and only 0.78 log units in milk chocolate even after treatment for 30 min. Our results showed that the organic matter did not affect the disinfection efficacy of HPU treatment as samples with huge differences in COD levels were compared and the disinfection efficacy did not show significant differences. Therefore, other physical and chemical parameters such as viscosity could influence ultrasound disinfection efficacy. Viscosity affects HPU efficacy because the cohesive forces present in a liquid oppose the occurrence of cavitation (Gogate et al. 2003). When *Salmonella* Typhimurium was treated in BHI broth, cell numbers decreased by more than 3 log units compared with 1 log

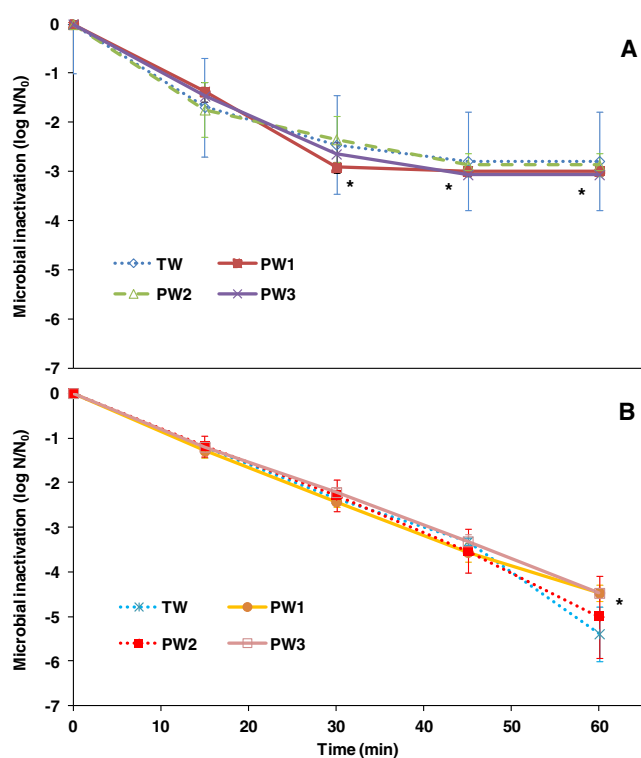


Fig. 5 Effect of chemical oxygen demand (COD) on *Escherichia coli* O157:H7 inactivation by high-power ultrasound (HPU) at 0.28 kW/L. a *E. coli* O157:H7 initial inoculum (2×10^3 CFU/mL). b *E. coli* O157:H7 initial inoculum (6×10^5 CFU/mL). Tap water (TW) and different types of process water (PW1, PW2, and PW3). Bars represent standard deviation

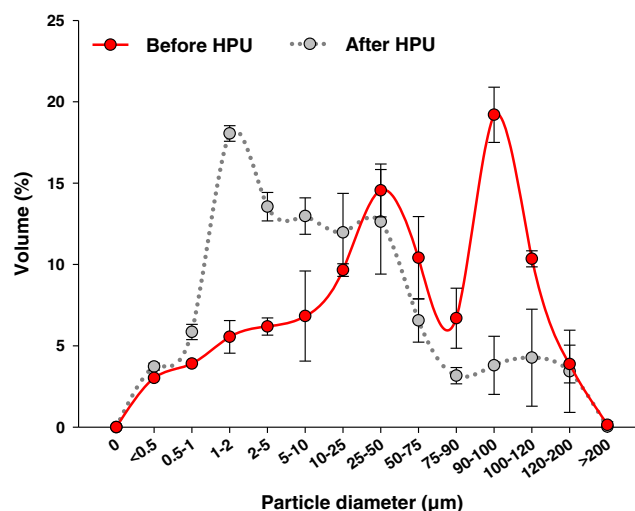


Fig. 6 Particle size distribution before and after high-power ultrasound (HPU) application in process water (PW3) at 0.28 kW/L for 45 min

reduction when cells were in liquid whole egg (Wrigley and Llorca 1992).

Effect of HPU on the Physical and Chemical Quality Characteristics of Process Water

The effect of HPU (0.28 kW/L) on the physical and chemical quality characteristics of lettuce process water (PW3) was evaluated. No reductions in alkalinity, COD, and pH were observed after 60 min of HPU treatment. A higher HPU power density (0.56 kW/L) was also ineffective in reducing alkalinity, COD, and pH. A previous study also showed a low COD reduction ($\leq 5\%$) of landfill leachate after 60 min of ultrasound treatment when the initial COD was 4,470 mg O₂/L (Wang et al. 2008). However, these authors showed that the initial COD concentration affects COD reduction efficiency. Therefore, COD reduction efficiency could be improved by diluting the PW before HPU treatment.

The effect of operating ultrasound on the particle size distribution was also evaluated (Fig. 6). Before sonication, agglomerates bigger than 90 μm in diameter represented 34.4 % of the total particle composition. After 45 min of sonication, this proportion of particles larger than 90 μm was reduced to only 11.5 %. In contrast, the proportion of particles between 1 and 25 μm diameters increased from 38.1 to 66.1 % after the 45-min HPU treatment. Therefore, HPU operating at low frequency (20 kHz) was able to bring down the size of agglomerates of particles by declumping and reduce particles higher than 90 μm in diameter. This phenomenon could facilitate ultrasonic disinfection because of declumping of bacterial aggregates (Joyce et al. 2011) that would be then more susceptible to radicals generated by sonication. Previous studies also mentioned the reduction capacity of ultrasonic waves on the particle size of wastewater (Blume and Neis 2003; Gogate 2007). For this reason, ultrasound has been proposed as a more suitable water pretreatment for UV-C disinfection than sand filters which are expensive in construction and cleaning maintenance (Blume and Neis 2003).

Conclusions

The efficacy of HPU on the inactivation of *E. coli* O157:H7 increased with power density and temperature but was unaffected by lettuce process water hardness and COD. High-power ultrasound was also able to bring down the size of agglomerates of particles in the vegetable process water. The efficiency of ultrasound technology to disinfect process water together with the broad efficacy for very different water quality characteristics typical in the processing lines of fresh-cut produce makes it suitable for recycling process water

generated in the fresh-cut industry, which would have positive consequences for the industry and the environment in terms of lower consumption of water and lower generation of wastewater and disinfection by-products.

Acknowledgments The research leading to these results has received funding from the European Community's Seventh Framework Program (FP7) under grant agreement no. 244994 (project VEG-i-TRADE, www.veg-i-trade.org) and the MICINN (AGL2009-08603 and IPT-2012-0169-060000). The support provided by the COST ACTION FA1202 BacFoodNet is also appreciated.

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