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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