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Strategies to enhance fresh produce decontamination using combined treatments of ultraviolet, washing and disinfectants

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ABSTRACT

This study investigated the effect of a water-assisted ultraviolet system (WUV; samples were treated by UV while being immersed in agitated water) on the inactivation of *Salmonella* on baby spinach, iceberg lettuce, blueberry, grape tomato, and baby-cut carrot. The *Salmonella* inactivation effect of the WUV system was tested in two scales, and three disinfectants, chlorine, peroxyacetic acid (PAA) and hydrogen peroxide (H_2O_2), were tested in combination with the system to see whether the *Salmonella* inactivation effect could be enhanced. The fresh produce samples were dip-inoculated with a *Salmonella* cocktail to final concentrations of 4.6–7.6 log CFU/g. To simulate the washing process in the industry, fresh produce extracts and/or silicon dioxide were added in the wash water to adjust chemical oxygen demand to ~2000 mg/L and turbidity to > 60 NTU. In general, the decontamination efficacy of WUV treatments followed this order: Tomato > Carrot > Lettuce ≈ Blueberry > Spinach. In the small-scale study, WUV alone was able to achieve 0.9, 2.6, > 3.6, 1.7, and 2.0 log CFU/g reductions of *Salmonella* on fresh produce for spinach, lettuce, tomato, blueberry, and carrot, respectively. For all fresh produce items, WUV combined with PAA could achieve significantly ($P < 0.05$) higher *Salmonella* reduction on fresh produce than chlorine wash and PAA wash. The WUV treatments combined with chlorine or PAA were able to keep residual *Salmonella* in wash water below the detection limit (2 CFU/mL) for almost all the replicates. Similar *Salmonella* reductions on fresh produce and in wash water were found in the large-scale study. Considering the decontamination efficacy on fresh produce, the ability to disinfect the wash water, and the cost, we recommend chlorine wash for baby spinach, WUV alone for grape tomato and WUV combined with PAA for iceberg lettuce, blueberry and baby-cut carrot.

1. Introduction

Fresh produce is a major contributor of essential vitamins and minerals to the diet of world population (Goodburn and Wallace, 2013). According to Food and Agriculture Organization (FAO), the production of fruits and vegetables increased from 1.26 billion tons in 2000 to 1.94 billion tons in 2016 (FAO, 2017). Between 1996 and 2006, fresh fruits and vegetables were associated with 98 foodborne illness outbreaks in the USA (FAO and WHO, 2008). From 2002 to 2012, the number of foodborne illness outbreaks in the USA related to fresh produce fluctuated around 57 per year (Wadamori et al., 2017). Important pathogens associated with fresh produce include *Salmonella* spp., pathogenic *Escherichia coli*, *Shigella* spp., *Yersinia* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium* spp., human norovirus, and hepatitis A virus (Wadamori et al., 2017; Yeni et al., 2016). Among foodborne illness outbreaks associated with fresh produce in the USA from 1996 to 2012, human norovirus was the leading cause, followed by *Salmonella* and pathogenic *E. coli* (Wadamori et al., 2017). From 2010 to 2014, 63 foodborne illness outbreaks in the USA were related to *Salmonella* (Crowe et al., 2015). In 2011, 23 cases in UK were confirmed for an outbreak of *Salmonella braenderup*, which was linked to the consumption of iceberg lettuce (Gajraj et al., 2012). In 2011, an outbreak of *Salmonella* Strathcona associated with datterino tomatoes caused 43 cases in Denmark and 28 additional cases in Germany, Italy, Austria, and Belgium (Müller et al., 2016). In 2007, baby spinach contaminated with *Salmonella* Java caused 228 cases in Denmark, UK, Norway, etc. (Denny et al., 2007).

Fresh produce could be microbiologically contaminated via various routes, including agricultural practices (organic fertilizer, irrigation water, soil, and spray of pesticide and insecticide) and post-harvest practices (handling, collection, washing, processing, transportation, and packaging) (Rajwar et al., 2015). Many methods have been studied for fresh produce decontamination, including washing or spraying with chemicals, ultrasound and shortwave ultraviolet (UV-C, simplified as UV in this study), but there is no guarantee for the safety of minimally processed fresh produce (Gil et al., 2009; Goodburn and Wallace,

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2013). Washing with disinfectants such as chlorine, a common method adopted by industry of fresh produce, could only reduce potential contamination but does not eliminate it (Goodburn and Wallace, 2013; Parish et al., 2003). Moreover, previous studies showed that wash water could be a transmission vehicle of pathogens, especially when wash water is reused (Gil et al., 2009; Holvoet et al., 2014; Selma et al., 2008a). Therefore, disinfectants serve an important role of preventing the transfer of microbial pathogens via wash water.

UV is an effective surface decontamination technology for fresh produce at wavelength of 190–280 nm (Artés et al., 2009). UV light could induce the formation of pyrimidine dimers, distort DNA helix and interfere cell replication, which leads to microorganism inactivation (Lado and Yousef, 2002). UV has been proved to be effective in inactivating various microorganisms, including *Salmonella*, *E. coli*, and *Listeria*. Although UV processing is easy to setup and produce no chemical residuals (Artés et al., 2009; Fan et al., 2017), application of UV in fresh produce decontamination is limited due to its shallow penetration ability, sample heating, and shadowing effect (Liu et al., 2015b). To overcome these limitations, we developed a water-assisted UV decontamination system for fresh produce, which used agitated water to wash fresh produce during UV treatments (Guo et al., 2017; Liu et al., 2015b). This system enabled fresh produce to move and rotate randomly during UV treatments to achieve full exposure of all surfaces of fresh produce to UV. Water could also help remove pathogens from food surfaces, which could be more easily killed in wash water. Another benefit of this system was that it prevented the heating and drying of fresh produce by direct UV exposure without the presence of water (referred to as dry UV treatment in this study).

Chlorine has been widely used for prevention of cross-contamination via wash water and for surface decontamination of fresh produce in the food industry due to its relatively low cost and ease of use (Goodburn and Wallace, 2013). However, even high concentrations of chlorine (50–200 ppm) could not completely eliminate bacteria on produce, especially for internal contamination (Ramos et al., 2013). Hydrogen peroxide (H_2O_2) has bacteriostatic and bactericidal activity related to its oxidizing property. Using H_2O_2 as a disinfectant would not leave any residual as it can be easily degraded into water and oxygen (Juven and Pierson, 1996; Ölmez et al., 2009). However, its efficacy of inactivating pathogens on fresh produce is low when used at low concentrations, 1–2% (Park and Beuchat, 1999). Peroxyacetic acid (PAA) is approved to be used in washing for vegetables and fruits (FDA, 21 C.F.R. §173.315, 1999). Comparing to chlorine, PAA does not generate harmful disinfection by-products and is stable in the presence of organic matters (Van Haute et al., 2015). PAA has also been demonstrated to be effective in inactivating various pathogens via the production of reactive oxygen species, which leads to damage of DNA and lipids, membrane disruption and blockage of enzymatic and transport systems (Banach et al., 2015).

The aim of this study was to evaluate the effect of a water-assisted UV system for inactivating *Salmonella* on fresh produce and to investigate whether chlorine, H_2O_2 or PAA could be used to enhance the UV decontamination effect.

2. Materials and methods

2.1. Bacteria strains and inoculum preparation

Four *Salmonella enterica* strains of different serotypes (*S*. Heidelberg 45955, *S*. Montevideo 51, *S*. Newport H1073 and *S*. Typhimurium 14028) were used in this study. The *Salmonella* strains were kindly provided by Dr. Vivian Wu at the USDA Agricultural Research Service. The individual wild-type strains were adapted to grow in the presence of 100 μg/mL of nalidixic acid (Fisher Scientific, Pittsburgh, PA) and 100 μg/mL of streptomycin (Fisher Scientific, Pittsburgh, PA) and maintained at 4 °C on tryptic soy agar (Becton, Dickinson and Company, Franklin Lakes, NJ) supplemented with 0.6% yeast extract (Becton, Dickinson and Company, Franklin Lakes, NJ), 100 μg/mL of nalidixic acid and 100 μg/mL of streptomycin (TSAYE-NS) as described previously (Huang and Chen, 2011). Individual strains were grown in tryptic soy broth (Becton, Dickinson and Company, Franklin Lakes, NJ) supplemented with 0.6% yeast extract and 50 μg/mL of nalidixic acid (TSBYE-N) for 24 h at 35 °C and transferred into a new tube or flask of TSBYE-N for another 24-h incubation at 35 °C. Each culture was mixed to form a 4-strain cocktail of *Salmonella*. Bacterial cells were harvested by centrifugation at 4000 × *g* for 10 min at 20 °C. The pellet was re-suspended in sterile 0.1% peptone water (Becton, Dickinson and Company, Franklin Lakes, NJ) to yield final concentrations of ~10^9 CFU/mL.

2.2. Inoculation on fresh produce

Baby spinach, iceberg lettuce, grape tomato, blueberry and baby-cut carrot were purchased from local markets the day before inoculation and stored at ~20 °C (tomatoes) and 4 °C (other items) until use. Fresh produce free of visible wounds and bruises were chosen for experiments and warmed up to room temperature (~20 °C) before bacterial inoculation. For iceberg lettuce, damaged outer leaves were removed, and lettuce was cut into small pieces with sizes of 8–10 cm^2. For small-scale treatments, the sample sizes were 15 g, 15 g, 150 g, 150 g and 100 g for baby spinach, iceberg lettuce, grape tomato, blueberry and baby-cut carrot, respectively. Samples were dipped in 800 mL of *Salmonella* cocktail (~10^9 CFU/mL) with stirring for 2 min. For large-scale treatments, the sample sizes were 200 g, 800 g, 3000 g, 3000 g and 3000 g for baby spinach, iceberg lettuce, grape tomato, blueberry and baby-cut carrot, respectively. Batches of samples were dipped in 2 L of *Salmonella* cocktail (~10^9 CFU/mL) with stirring for 2 min and several batches were combined to achieve desired sample sizes. Inoculated samples were then dried in a biological safety hood for 2 h at room temperature and stored at 4 °C for 24 h to facilitate bacterial attachment.

2.3. WUV setups

UV treatments were conducted using a home-built UV system, which consisted of a stainless-steel chamber (120 cm (L) × 40 cm (W) × 60 cm (H)) with a see-through plastic door and four 90-cm long amalgam UV lamps (265 W/lamp; Heraeus Noblelight, Buford, GA) situating on the top of the chamber. Prior to each experiment, the UV unit was run for 10 min to stabilize UV output. A UV radiometer (ILT77, International Light Technologies, Peabody, MA) was used to determine the UV intensity before each treatment. The distance from the UV lamp to the water surface is ~17 cm and the UV intensity (dosage) varied between 23 and 28 mW/cm^2 (27.6 and 33.6 kJ/m^2) during the treatment.

For small-scale treatments, samples at room temperature (~20 °C) were placed in 500 mL wash water (30 °C for grape tomato and 4 °C for other produce items) in a 900-mL glass container (Pyrex, Corning Inc., Corning, NY) with a stirrer bar. The wash water temperature of 30 °C for tomato was chosen based on the guideline provided by the US Food and Drug Administration (FDA), which states that the temperature of wash water should be at least 5.6 °C warmer than that of tomatoes to prevent infiltration (FDA, 2015). A magnetic stir plate (Fisher Scientific, Hampton, NH) underneath the container was used to agitate the water in the container to create turbulent flow so that random rotation and movement of samples could be achieved during UV treatments. For large-scale treatments, samples were placed in 10 L wash water in a stainless-steel pan (53 cm (L) × 33 cm (W) × 15 cm (H); Winco, Lodi, NJ). Two stirrer systems (a 150 RPM gear box motor attached to a beater blade) (Fig. 1) was used to agitate the wash water to create random rotation and movement of samples during UV treatments.