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Evaluation of inactivating *Salmonella* on iceberg lettuce shreds with washing process in combination with pulsed light, ultrasound and chlorine

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ABSTRACT

This study was conducted to investigate the *Salmonella* inactivation effects of washing in combination with pulsed light (PL), ultrasound, and chlorine on lettuce shreds. First, the effect of washing combined with PL and chlorine on the inactivation of *Salmonella* on lettuce and in wash water was evaluated in a small-scale study with clear tap water and turbid tap water containing lettuce extract and silicon dioxide. In general, water wash combined with PL (PL wash) and chlorine wash combined with PL (PL-Cl) were significantly more effective on killing *Salmonella* on lettuce than the chlorine wash and water wash regardless the wash water quality and inoculation method. We then tested washing combined with PL, ultrasound and chlorine using a large-scale UV setup with turbid wash water. Increasing the sample size decreased the decontamination efficacy of all the treatments. All the treatments resulted in < 2 log reductions of *Salmonella* on lettuce shreds. For both small- and large-scale studies, treatments involving chlorine could keep the *Salmonella* population in wash water under the detection limit of 2 CFU/mL for almost all the replicates. Taking everything into consideration, we concluded that the combined PL-Cl treatment could be a better alternative to the chlorine wash for lettuce decontamination since it was in general more effective on inactivating *Salmonella* on lettuce than chlorine wash and could maintain the *Salmonella* level in wash water under the detection limit of 2 CFU/mL regardless the inoculation method, water quality and sample size, preventing the potential cross contamination through wash water.

1. Introduction

As the consumption of fresh produce is increasing over the decades and food chain became more complicated, there is a need to develop an effective method for fresh produce decontamination. A variety of pathogens are frequently associated with fresh produce, such as human norovirus, *Salmonella* and *Escherichia coli* O157:H7, which raised public concerns (Wadamori et al., 2017; Yeni et al., 2016). According to the statistics of Centers for Disease Control and Prevention (CDC), the number of foodborne illness outbreaks in the USA associated with fresh produce fluctuated around 57 per year (Wadamori et al., 2017). Lettuce, one of the top consumed leafy vegetables in the USA, also served as a transmission vehicle for many pathogens (Berger et al., 2010). In 2018, romaine lettuce contaminated with *E. coli* O157:H7 caused 172 cases, 75 hospitalizations and 1 death in the U.S. (CDC, 2018). In 2016, an outbreak of *Salmonella* Anatum linked to pre-packaged lettuce caused 10 cases in Australia (Whitworth, 2016). In 2014, lettuce was identified as one of the transmission vehicles of two *E. coli* O96:H19 outbreaks in the UK, leading to 27 cases (Newitt et al., 2016). In 2011, a multistate outbreak of *E. coli* O157:H7 in USA was traced back to romaine lettuce, which caused 58 cases and 34 hospitalizations (Slayton et al., 2013).

Washing process has been widely used in the fresh produce industry to remove soil, debris and dust from fresh produce as well as improve microbiological safety of fresh produce (Gil et al., 2009). However, the washing process alone is not able to inactivate internalized pathogens in fresh produce (Erickson et al., 2010; Park et al., 2012). Another problem is that wash water itself could also serve as a contamination source for fresh produce (Olaimat and Holley, 2012). Thus, sanitizers, such as hypochlorite, chlorine dioxide and peroxyacetic acid, are used in the washing process to help inactivate pathogens on fresh produce and prevent cross-contamination through wash water (Olaimat and Holley, 2012).

Various processing technologies have been tested for the decontamination efficacy on lettuce, such as shortwave ultraviolet (UV), pulsed light (PL), ultrasound and cold plasma (Gómez-López et al., 2005; Guo et al., 2017; Millan-Sango et al., 2015; Schnabel et al., 2015). Pulsed light is a nonthermal processing technology which involves the use of intense pulses of a short time and a broad spectrum (200–1100 nm) (Gómez-López et al., 2005). PL is approved by the U.S. Food and Drug

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Administration to be used during the production, processing, and handling of food with a total dose of < 12 J/cm^2 (21 C.F.R. § 179.41). Many studies have shown that PL is effective against bacteria, fungi and viruses (Anderson et al., 2000; Huang et al., 2017). The microbiological inactivation mechanism of PL is believed to be mainly due to the UV part of PL spectrum and photothermal effect also contributes to the microbiological inactivation (Oms-Oliu et al., 2010; Rowan et al., 1999; Wekhof et al., 2001). The energy cost of PL is limited and it does not leave chemical residuals on fresh produce (Oms-Oliu et al., 2010; Rowan et al., 1999). Drawbacks of PL inactivation of pathogens on fresh produce includes sample heating (Gómez-López et al., 2005) and shadow effect (Oms-Oliu et al., 2010). Ultrasound has been used in many aspects of food processing, such as filtration, emulsification, drying and freezing (Majid et al., 2015). Some studies also showed that high energy ultrasound (> 1 W/cm^2; frequencies between 20 and 500 kHz) could be used for decontamination of fresh produce, which was attributed to the mechanical effect generated by cavitation bubbles (Awad et al., 2012; Seymour et al., 2002). It has been shown that the efficiency of ultrasound inactivation of pathogens is affected by the frequency and wave amplitude of ultrasound (Awad et al., 2012).

To achieve better microbial inactivation and/or better preservation of quality and nutrition of fresh produce, combination of different technologies (hurdle technology) is studied by many researchers. By combining different technologies, the intensities of individual technologies could be reduced to minimize negative impact on food quality and achieve better microbiological inactivation effect (Rico et al., 2007). Guo et al. (2017) reported that a chlorine wash (10 ppm free chlorine) combined with UV (2 min; ~29 mW/cm2) showed ~ 1 log higher reduction of *Salmonella* on lettuce shreds than the chlorine wash alone. Ge et al. (2013) also showed that a 10-min combined treatment of UV (1.5 mW/cm^2) and chlorine wash (200 ppm) could reduce internalized *Salmonella* in lettuce by ~ 2.4 log while a 10-min chlorine wash (200 ppm) alone could only reduce by 1.0 log. A previous study showed that combined treatments of ultrasound and sanitizers (chlorine, acidified sodium chlorite, peroxyacetic acid and acidic electrolyzed water) significantly enhanced the reduction of *E. coli* O157:H7 on spinach leaves by 0.7 to 1.1 logs than wash with sanitizers alone (Zhou et al., 2009). Sagong et al. (2013) also demonstrated that a 5-min ultrasound treatment (40 kHz, 30 W/L) combined with 0.1% Tween 20 could achieve a 1-log higher reduction of *Bacillus cereus* spores on lettuce than ultrasound alone. Huang et al. (2006) found that a 10-min treatment of 40 ppm aqueous chlorine dioxide could reduce 2.4 log of *Salmonella* on lettuce, and the reduction increased to 3.0 log when the chlorine dioxide treatment was combined with a 170 kHz ultrasonication treatment. Chen and Zhu (2011) reported that a combined treatment of 40 ppm aqueous chlorine dioxide and 40 kHz ultrasound achieved synergistic germicide effect on plums, and the combined treatment could prolong the shelf life of plums for 25 days.

The aims of this study were to 1) determine the *Salmonella* inactivation effect of washing process in combination with PL, ultrasound and chlorine on iceberg lettuce shreds and 2) determine the effect of some selected treatments on *Salmonella* inactivation using a larger sample size.

2. Materials and methods

2.1. Bacterial strains and inoculum preparation

Four nalidixic acid-resistant mutant strains of *Salmonella enterica* (*S.* Montevideo G4639, *S.* Newport H1275, *S.* Saintpaul 02-517-1 and *S.* Stanley HO588) were used in this study and details of origins were described by Huang and Chen (2015). The nalidixic acid-resistant mutants strains were selected as described by Huang et al. (2013). The working cultures were 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) and 50 μg/mL nalidixic acid (Fisher Scientific, Pittsburgh, PA; TSAYE-N). 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 resuspended in sterile 0.1% peptone water (Becton, Dickinson and Company, Franklin Lakes, NJ) to yield final concentrations of ~10^9 CFU/mL (dip-inoculation) or ~10^{10} CFU/mL (spot-inoculation).

2.2. Inoculation on iceberg lettuce shreds

Fresh iceberg lettuce was bought from local markets the day before inoculation and stored at 4 °C until use. Damaged outer layers of lettuce were removed, and lettuce was cut into small pieces (~10 cm^2/piece) with a sterile knife. Two different inoculation methods were used in this study, spot-inoculation and dip-inoculation. For the small-scale study (15 g lettuce shreds), 800 μL of the *Salmonella* cocktail (~10^{10} CFU/mL) was deposited randomly onto the samples in small droplets (10 μL/droplet; spot-inoculation) or samples were dipped in 360 mL of the *Salmonella* cocktail (~10^9 CFU/mL) with stirring for 2 min (dip-inoculation). The initial inoculation levels of *Salmonella* on iceberg lettuce shreds in the small-scale study were 7.61 ± 0.24 and 8.18 ± 0.14 log CFU/g for spot- and dip-inoculation, respectively. For the large-scale study with medium sample size (60 g lettuce shreds), 800 μL of the *Salmonella* cocktail (~10^{10} CFU/mL) was deposited randomly onto the samples in small droplets (10 μL/droplet; spot-inoculation) or samples were dipped in 1000 mL of the *Salmonella* cocktail (~10^9 CFU/mL) with stirring for 2 min (dip-inoculation). For the large-scale study with large sample size (300 g lettuce shreds) and spot-inoculation, 4 mL of the *Salmonella* cocktail (~10^{10} CFU/mL) was deposited randomly onto the samples in small droplets (10 μL/droplet; spot-inoculation). For the large-scale study with large sample size (300 g lettuce shreds) and dip-inoculation, a batch (100 g per batch) of lettuce was dipped in 2 L of *Salmonella* cocktail (~10^9 CFU/mL) with stirring for 2 min and three batches were combined to achieve the 300 g sample size. The initial inoculation levels of *Salmonella* on iceberg lettuce shreds in the large-scale study were 7.66 ± 0.13 and 7.97 ± 0.32 log CFU/g for spot- and dip-inoculation, respectively. 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. System setups and wash water quality

The PL unit consisted of a commercial PL lamp with controlling and cooling modules (Xenon Steripulse-XL RS-3000, Xenon Corp., Wilmington, MA) and a homebuilt stainless-steel chamber (inner size 60 cm (L) × 45 cm (W) × 70 cm (H)) connected with a high flow ozone destruct unit (Ozone Solutions Inc., Hull, IA) (Fig. 1A). The PL lamp was enclosed in a lamp housing mounted at the top of the chamber. Pulses at wavelength of 180–1100 nm were generated at 3 pulses/s with a pulse width of 360 μs. According to a previous study, 40% of its energy generated was within the UV spectrum (Hsu and Moraru, 2011). The intensity of PL was measured with a Vega laser power meter (Ophir Optronics, Wilmington, MA) coupled with a pyroelectric energy sensor (PE-50C, Ophir Optronics, Wilmington, MA). The wavelength setting was 300 nm with pulse width of 500 mm. The intensity of PL, measured in triplicates at the height of wash water surface, was ~0.14 J/cm^2 per pulse based on the method described by Huang et al. (2015).

For the small-scale study, the washing unit consisted of a glass container (950 mL) and a stir bar (6 cm) sitting on a magnetic stirrer (Fisher Scientific, Pittsburgh, PA; Fig. 1B). During treatments, the stir bar could agitate the water in the glass container to create turbulent flow so that random rotation and movement of food samples would be