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A novel water-assisted pulsed light processing for decontamination of blueberries

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ABSTRACT

Sample heating and shadowing effect have limited the application of pulsed light (PL) technology for decontamination of fresh produce. In this study, a novel setup using water-assisted PL processing was developed to overcome these limitations. Blueberries inoculated with *Escherichia coli* O157:H7 or *Salmonella* were either treated with PL directly (dry PL treatment) or immersed in agitated water during the PL treatment (wet PL treatment) for 5–60 s. Although both pathogens were effectively inactivated by the dry PL treatments, the appearance of the blueberries was adversely affected and a maximum temperature of 64.8 °C on the blueberry surface was recorded. On the other hand, the visual appearance of blueberries remained unchanged after wet PL treatments and sample heating was significantly reduced. The wet PL treatments were more effective than chlorine washing on inactivating both pathogens. After a 60-s wet PL treatment, the populations of *E. coli* O157:H7 inoculated on calyx and skin of blueberries were reduced by 3.0 and >5.8 log CFU/g, respectively. *Salmonella* on blueberry calyx and skin was reduced by 3.6 and >5.9 log CFU/g, respectively. No viable bacterial cells were recovered from the water used in the wet PL treatments, demonstrating that this setup could prevent the risk of cross-contamination during fresh produce washing. Our results suggest that this new water-assisted PL treatment could be a potential non-chemical alternative (residue free) to chlorine washing since it is both more effective and environmentally friendly than chlorine washing.

1. Introduction

Fresh berries are highly valued for their high antioxidant and vitamin content. Many bioactive compounds in berries have been shown to provide significant health benefits (Hannum, 2004; Szajdek and Borowska, 2008). As a result, the consumption of berries has been increasing rapidly in recent years in the United States. From 2000 to 2010, a five-fold increase was observed for the consumption of fresh blueberries (ERS, 2012) and the U.S. has become the largest producer of blueberries in the world (FAO, 2012).

Unfortunately, these small fruits are susceptible to contamination by various pathogenic microorganisms since they are constantly exposed to soils and irrigation water in the fields and human contacts during harvesting. Moreover, berries destined for fresh market are usually picked by hands and not washed before sale for the sake of fruit quality and shelf-life. Recently, the implication of blueberries and other berries in several foodborne outbreaks has raised concerns about their microbial safety. In 2003, contaminated blueberries were reported to be the source of an outbreak of hepatitis A virus (Calder et al., 2003). A multistate outbreak of *Salmonella* Muenchen reported in 2009 was traced back to consumption of blueberries, which caused 14 cases of illnesses (CDC, 2013). In 1999, the U.S. Food and Drug Administration (FDA) initiated a survey focused on high-volume imported fresh produce; *Salmonella* was isolated from 1 of 143 samples of strawberries imported into the U.S. (FDA, 2001). In 2011, fresh strawberries from a farm in Oregon were linked with an *Escherichia coli* O157:H7 outbreak, which caused at least 15 illnesses including one death. Deer droppings in the strawberry field were later confirmed as the source of contamination (Oregon Health Authority, 2011). Therefore, effective intervention methods with minimal effect on the quality and shelf-life of blueberries are urgently needed.

Pulsed light (PL) is an emerging nonthermal technology that utilizes short, intense pulses of broad spectrum light (wavelength = 180–1100 nm) to inactivate microorganisms (Gomez-Lopez et al., 2007). This technology was adopted by the U.S. FDA for food processing in 1996 (FDA, 1996). The efficacy of PL for inactivating bacteria, fungi and viruses *in vitro* is well documented (Anderson et al., 2000; Roberts and Hope, 2003; Rowan

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et al., 1999). Use of PL to process food products such as apple juice, milk, minimally processed vegetables, berries, alfalfa seeds, hot dogs and salmon fillets have been studied with the intention of extending shelf-life and/or inactivating pathogens (Oms-Oliu et al., 2010). The primary mechanism of microbial inactivation is the photochemical dimerization of nucleic acids in microorganisms, caused mainly by UV-C part of the PL spectrum (Gomez-Lopez et al., 2007). This dimerization inhibits transcription and replication, thus resulting in cellular inactivation. In addition, it has also been shown that both the visible and infrared regions of PL in combination with its high peak power also contribute to the killing effect on microorganisms (Elmnasser et al., 2007).

The application of PL to enhance the safety and/or shelf-life of fresh produce, including berries, has been studied previously (Bialka and Demirci, 2007; Gomez et al., 2012; Ramos-Villarroel et al., 2011, 2012). Currently there are two main challenges that limit the PL application in the fresh produce industry. One issue is that PL treatment causes substantial heating of the samples, which might damage the quality of fresh produce. Another issue is that microorganisms on an opaque food surface must directly face the PL-strobe to be inactivated due to the shallow penetration depth of PL. In addition, samples positioned in different parts of the PL chamber might be exposed to different doses of PL. To overcome these two limitations, we developed a water-assisted PL system in which blueberry samples were immersed in agitated water during PL treatment. With this new system, the temperature increases of water and the samples were minimized due to the large specific heat of water. Moreover, the blueberry samples could randomly move and rotate in the agitated water, thus allowing more uniform PL exposure of all the blueberry surfaces.

The objectives of this study were to evaluate the efficacy of PL on the inactivation of *E. coli* O157:H7 and *Salmonella* on blueberries and to use this novel setup to minimize the adverse effects of PL on the physical and sensorial properties of blueberries.

2. Materials and methods

2.1. Bacterial strains and inoculum preparation

Five *E. coli* O157:H7 strains and four *Salmonella enterica* strains of different serotypes were used in this study. The *E. coli* O157:H7 strains were kindly provided by Dr. Joerger and Dr. Kniel, University of Delaware; and the *Salmonella* strains by Dr. Gurtler, U.S. Department of Agriculture. Detailed strain information is shown in Table 1.

The individual wild-type strains were adapted to become nalidixic-acid-resistant as described by Huang et al. (2013). The cultures were maintained on tryptic soy agar (Difco Laboratories, Sparks, MD) supplemented with 0.6% yeast extract (Difco) and 50 μg/mL of nalidixic acid (Fisher Scientific, Hampton, NH) (TSAYE-N) at 10 °C. Individual cultures were grown in tryptic soy broth (Difco) supplemented with 0.6% yeast extract and 50 μg/mL of nalidixic acid (TSBYE-N) overnight at 35 °C and transferred into 10 mL of fresh TSBYE-N for another 24-h incubation at 35 °C. One mL of each culture was mixed to form a 5-strain cocktail of *E. coli* O157:H7 or a 4-strain cocktail of *Salmonella*. Bacterial cells were harvested by centrifugation at 2450× *g* for 10 min (Centra CL2, Centrifuge, Thermo Scientific). The supernatant was discarded and the pellet was resuspended in 1 mL of sterile 0.1% peptone water (Fisher Scientific) to yield a final concentration of ~10^9 CFU/mL.

Table 1
Bacterial strain information.

Species	Serotype	Strain	Origin
E. coli	O157:H7	H1730	Lettuce outbreak
E. coli	O157:H7	cider	Cider outbreak
E. coli	O157:H7	250	Sprout outbreak
E. coli	O157:H7	251	Lettuce outbreak
E. coli	O157:H7	J58	Lettuce isolate
S. enterica	St. Paul	02-517-1	Cantaloupe outbreak
S. enterica	Newport	H1275	Sprout outbreak
S. enterica	Montevideo	G4639	Tomato outbreak
S. enterica	Stanley	HO588	Sprout outbreak

2.2. Inoculation of blueberries

Fresh blueberries were purchased from local grocery stores the day before each experiment and stored at 4 °C until use. All the berries were UV-treated (254 nm) in a biosafety hood for 10 min to reduce the impact of background microflora before each experiment. The blueberries were then spot-inoculated with 25 μl of the 5-strain cocktail of *E. coli* O157:H7 or the 4-strain cocktail of *Salmonella*. Inoculum was deposited on either the skin or calyx tissue of blueberries in small droplets to simulate two contamination conditions. After inoculation, the samples were air-dried in a biosafety hood at 22 ± 2 °C for 2 h to facilitate bacterial attachment. The final inoculation levels of *E. coli* O157:H7 and *Salmonella* on berries were 6.8–7.0 log CFU/g.

2.3. PL treatment

The PL treatments were performed with a laboratory scale PL system (Steripulse-XL RS-3000, Xenon Corp., Wilmington, MA), which consisted of a controller module, a treatment chamber and an air cooling module. The lamp was capable of generating PL in the wavelength of 180–1100 nm, with 40% of the energy being in the UV region (Hsu and Moraru, 2011). The pulses were generated at a rate of 3 pulses/s with pulse width of 360 μs. According to the manufacturer's specifications, each pulse delivers 1.27 J/cm^2 for an input of 3800 V at 19.3 mm from the quartz window of the lamp.

In this study, PL treatments were performed in two modes, dry and wet PL treatments. For dry PL treatments, three blueberries were placed on a sterile petri-dish with the inoculation site facing the PL lamp. The samples was placed at the center of the PL chamber and directly illuminated by PL for 5, 15, 30 and 60 s. The distance between the lamp and the quartz window was 5.8 cm and the distance between the top of blueberries and the quartz window was ~15 cm. For wet PL treatments, three blueberries were immersed in 150-mL agitated tap water during the PL treatment in a 1-L glass beaker containing a 2.5-cm stirring bar. An ultra-thin magnetic stirrer (Lab Disc, Fisher Scientific) was placed under the PL chamber to agitate the water in the beaker so that random rotation and movement of blueberries could be achieved. The treatment durations were 5, 15, 30 and 60 s. The distance between the top of blueberries and the quartz window was also ~15 cm and the distance from the top of blueberries to the water surface was about 1 cm. For comparison, blueberries were also washed with 150-mL agitated tap water or 10 ppm chlorinated water for 60 s in the beaker with the stirring bar. The 10 ppm chlorinated water was chosen since it was generally used by the food industry to wash blueberries intended for further processing such as frozen storage. Chlorinated water (pH 6.5) was prepared by adding commercial bleach (Clorox, Oakland, CA, USA) into DI water to obtain 10 ppm of free chlorine. Chlorine concentration was determined by free chlorine micro check test strips (HF Scientific, Ft. Myer, FL).