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Evaluation of pulsed light treatments on inactivation of *Salmonella* on blueberries and its impact on shelf-life and quality attributes

Xinang Cao, Runze Huang, Haiqiang Chen*

Department of Animal and Food Sciences, University of Delaware, Newark, DE 19716-2150, USA

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ABSTRACT

Blueberry have a short shelf life when fully ripe and susceptible to contamination of various pathogens. Our study investigated the effect of pulsed light (PL) on inactivation of *Salmonella* on blueberries and its impact on shelf-life, quality attributes and health-benefit compounds of blueberries. Dry PL (6 J/cm^2) and water-assisted PL (samples were agitated in water during PL treatment; 9 J/cm^2) along with two controls, dry control (untreated) and water-assisted control (water washing without PL), were applied to blueberries with subsequent storages at room temperature (3 days) or 5 °C (7 days). For *Salmonella* inactivation, dry PL treatment achieved 0.9 and 0.6 log reduction of *Salmonella* for spot and dip inoculation, respectively; while the water-assisted PL treatment reduced *Salmonella* by 4.4 log and 0.8 log for spot and dip inoculation, respectively. The water-assisted PL treatment resulted in *Salmonella* populations significantly lower than the dry control after storage regardless of the storage temperature and inoculation method. Neither dry nor water-assisted PL treatments improved the shelf life of blueberries even though direct inactivation of natural yeasts and molds were achieved. Surface lightness was instantly reduced after both dry and water-assisted PL treatments. Compared with the dry control, the two PL treatments did not reduce the firmness of blueberries. Weight loss was increased for the dry PL treated samples, but not for the water-assisted PL treatment for both storage conditions. Delayed anthocyanins accumulation and reduced total antioxidant activity were induced by both PL treatments at the end of storage at room temperature, while slight enhancement in total phenolics content was achieved by water-assisted PL treatment. In conclusion, the water-assisted PL treatment could effectively decontaminate *Salmonella* on blueberries while showed minimal or no impact on the shelf-life, quality attributes and health-benefit compounds of blueberries. PL processing parameters need to be further evaluated and optimized before possible application in the blueberry industry.

1. Introduction

Due to the prevalence of chronic diseases, more and more people have become concerned about diet and nutrition value of food. Blueberry, a widely-known good source of antioxidants, has become increasingly popular and showed fast-growing markets in recent decades (Perez and Ferreira 2016). In 2014, USA was the largest blueberry producer around the world with total production almost 1.5 times as much as its nearest competitor, Canada (Food and Agricultre Organization of the United Nations 2017). Unfortunately, blueberries are susceptible to contamination by various foodborne pathogens, such as *Salmonella*, *Escherichia coli* O157:H7, human norovirus and hepatitis A virus, and have been implicated in several outbreaks (FAO/WHO 2008). The diversity of blueberry production chain and highly human relied harvesting process present multiple sources of contamination. In 2010, an outbreak of *Salmonella* Newport in Minnesota caused 6 infections and was traced back to fresh blueberries (Miller et al. 2013). In 2009, blueberries contaminated with *Salmonella* Muenchen led to an outbreak that caused 6 illness (Centers for Disease Control and Prevention 2009). Raw blueberries were also identified as the source of a hepatitis A virus outbreak in 2003 (Calder et al. 2003). Therefore, there is a need to develop effective decontamination technologies for blueberries.

Pulsed light (PL) is a novel nonthermal decontamination technology and has been proved to be effective against various bacteria, fungi and viruses on agar plates or in cell-culture medium by many researchers (Anderson et al. 2000; Elmnasser et al. 2007; Roberts and Hope 2003; Rowan et al. 1999). PL decontamination effect was also tested on minimally processed fresh vegetables and showed to be able to reduce the initial microbial load on vegetables (Gómez-López et al. 2005). However, the decontamination effect of PL was shown to be diminished due to the “shadow effect” which is caused by poor PL penetration

* Corresponding author.
E-mail address: haiqiang@udel.edu (H. Chen).

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ability (Lagunas-Solar et al. 2006; Wallen et al. 2001). Compared with short-wave ultraviolet (UV-C), PL uses a higher energy-density. Therefore, the cost of energy usage would be higher for PL processing. In addition, this higher energy used would result in surface heating and potentially damage food quality if dry PL is used (Bialka and Demirci 2007; Gómez et al. 2012; Huang and Chen 2014). However, the benefit of using PL processing is that a shorter processing time can be used to deliver a necessary light dosage for microbial inactivation. In the previous work in our lavatory, a water-assisted PL system where food samples were agitated in water while being exposed to PL was developed. The system was shown to be able to achieve higher decontamination effect as well as to avoid possible surface heating caused by PL radiation (Huang et al. 2015; Huang and Chen 2014). In this study, we intended to evaluate the potential of this water-assisted PL technology for blueberry decontamination and its effect on shelf life and nutritional quality.

For a processing technology to be commercial viable for food decontamination, it should have minimum impact on the shelf-life, sensory, physical, chemical and nutritional qualities of food. Blueberries have a short shelf-life due to their soft texture especially when they are fully ripe. In addition shelf-life of blueberries can be greatly reduced if not properly handled and stored, which could cause high post-harvest loss (Nunes et al. 2004; Sanford et al. 1991; Woodruff and Dewey 1959). During wet seasons, blueberries can be susceptible to fungal infection and are likely to show ripe rot, *alternaria* rot as well as green molds induced by *Colletotrichum acutatum*, *Alternaria* spp. and *Botrytis cinerea*, respectively (Retamales and Hancock 2012). Fresh blueberries on the market are usually not washed for the fear of possible fungal development, and in general, no additional processing technique is adopted besides sufficient pre-cooling and cold storage. Current lab-scale studies to extend blueberry shelf life have mainly focused on controlled atmospheric storage (Beaudry 1993; Schotsmans et al. 2007), vapor treatments (Chiabrando and Giacalone 2011) as well as edible coating (Duan et al. 2011). The effect of UV-C treatment on the reduction of fungal decay and influence of antioxidant contents for blueberries has also been studied (Perkins-Veazie et al. 2008).

The objectives of this study were to evaluate the effect of PL on inactivation of *Salmonella* on blueberries and determine the impact of PL processing on shelf-life, quality attributes and health-benefit compounds of blueberries.

2. Materials and methods

2.1. PL equipment and blueberries

PL treatments were conducted with a reconstructed PL system, which consisted of a commercial PL lamp with controlling and cooling modules (Xenon Steripulse-XL RS-3000, Xenon Corp., Wilmington, MA) and a self-designed, enclosed 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). 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 previous researches, 40% of its energy generated was within the UV spectrum (Hsu and Moraru 2011). Blueberries were purchased from local wholesale stores and stored at 5 °C in a refrigerator until use. Blueberries without any sign of rot or damage were selected.

2.2. Effect of PL treatments on inactivation of Salmonella spot- or dip-inoculated on blueberries

2.2.1. Salmonella inoculum preparation and blueberry sample inoculation

Four *Salmonella enterica* serotypes of Newport, Montevideo, St. Paul and Stanley previously adapted to be nalidixic-acid resistant were used in this study (Huang et al. 2013). The four strains were maintained on tryptic soy agar (Difco Laboratories, Sparks, MD) supplemented with 0.6% yeast extract (Difco Laboratories, Sparks, MD) and 50 μg/mL nalidixic acid (Fisher Scientific, Hampton, NH) at 2–4 °C (TSAYE-N). Individual strains were grown in 10 mL of tryptic soy broth (Difco Laboratories, Sparks, MD) supplemented with 0.6% yeast extract and 50 μg/mL of nalidixic acid (TSBYE-N) overnight at 35 °C. For spot inoculation, each *Salmonella* culture was transferred into 2 new tubes each containing 10 mL of TSBYE-N. The tubes were incubated at 35 °C for 24 h. The cultures were mixed to form a 4-strain cocktail of *Salmonella*. Bacterial cells were harvested by centrifugation at 4000 × *g* for 10 min (Sorvall ST16 R, Thermo Scientific) at 20 °C. The pellet was re-suspended in 6.5 mL of sterile 0.1% peptone water. Five hundred milliliters of the *Salmonella* cocktail was inoculated on the surfaces of 100 g blueberries in an 18-oz. PET clamshell. The spot-inoculated berries were dried in a biological safety hood for 2 h at room temperature with clamshell lid open. Blueberries were placed into 4.4 oz. clamshells and then stored at 4 °C overnight with clamshell lid closed to facilitate bacterial attachment. For dip inoculation, each *Salmonella* culture was transferred to 2 flasks containing 200 mL of TSBYE-N. The flasks were incubated, mixed and centrifuged as described above. The pellet was re-suspended in 1600 mL of 0.1% peptone water. Blueberries (1500 g) were then dipped in the *Salmonella* cocktail and gently stirred for 2 min. The blueberries were drained with a strainer and spread on trays lined with paper towel to be air dried for 2 h. The inoculated samples were then placed into 12 4.4-oz. clamshells (110 g blueberries/clamshell) which were stored at 4 °C overnight with clamshell lid closed.

2.2.2. PL treatments and storage conditions for blueberries

Two forms of PL treatments, dry PL (samples were exposed to PL directly) and water-assisted PL (samples were washed in agitated water while being exposed to PL), were used to treat inoculated blueberries. Two kinds untreated control groups were included. The dry control group consisted of blueberries that were not subjected to any treatments. The water-assisted control group consisted of blueberries washed in water without exposure to PL. A PL intensity of 0.066 J/cm^2 per pulse was used for both dry and water-assisted PL treatments. A PL dose of 6 J/cm^2 (30 s) was used for dry PL treatments and a PL dose of 9 J/cm^2 (45 s) was used for water-assisted PL treatments. The intensity and doses of PL treatments were selected based on results from preliminary trials.

For dry PL treatments, blueberries were removed from the clamshell and shaken on a round plate wrapped with aluminum foil at 180 rpm using an orbital shaker (Orbi-Shaker JR., Benchmark Scientific, Edison, NJ) while being exposed to PL for 30 s. The shaker allowed all surfaces of blueberries to be exposed to PL and aluminum foil could effectively reflect PL. After each treatment, the shaker was turned off and the ozone destruct unit was turned on for 150 s to remove ozone from the PL chamber. The treated blueberries were then placed into a new clamshell. Dry control samples were kept in their original clamshells with lid open at room temperature for 180 s. For water-assisted PL treatment, blueberries were removed from the clamshell and placed in 500 mL water in an 8-inch square baking dish (Pyrex, Corning Inc., Corning, NY) with a stirrer bar. The inside of the dish was wrapped with aluminum-foil to reflect PL. A magnetic stirrer (Fisher Scientific, Hampton, NH) underneath the dish was used to agitate the water in the dish to create turbulent flow so that random rotation and movement of food samples could be achieved during PL treatments. The blueberries were washed using the water-assisted PL system for 45 s followed by turning on the ozone destruct unit for 135 s. The water-assisted control samples were washed using the same washing system for 180 s without being exposed to PL. After treatments, the water-assisted PL-treated and un-treated control samples were poured on a stainless-steel strainer to remove water and then put into 18-oz. PET clamshells without lids. The bottoms of the clamshells were lined with paper towels to remove residual moisture. The clamshells were placed in the refrigerator at 5 °C. A five-inch portable fan (O2Cool, Chicago, IL) at high speed mode was