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Application of water-assisted ultraviolet light processing on the inactivation of murine norovirus on blueberries

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ABSTRACT

In this study, a novel set-up using water-assisted UV processing was developed and evaluated for its decontamination efficacy against murine norovirus (MNV-1) inoculated on fresh blueberries for both small and large-scale experimental setups. Blueberries were skin-inoculated with MNV-1 and treated for 1–5 min with UV directly (dry UV) or immersed in agitated water during UV treatment (water-assisted UV). The effect of the presence of 2% (v/v) blueberry juice or 5% crushed blueberries (w/w) in wash water was also evaluated. Results showed that water-assisted UV treatment generally showed higher efficacies than dry UV treatment. With 12,000 J/m^2 UV treatment in small-scale setup, MNV reductions of >4.32- and 2.48-log were achieved by water-assisted UV and dry UV treatments, respectively. Water-assisted UV showed similar inactivating efficacy as 10-ppm chlorine wash. No virus was detected in wash water after UV treatment or chlorine wash. MNV-1 was more easily killed on skin-inoculated blueberries compared with calyx-inoculated berries. When clear water was used as wash water in the large-scale setup, water-assisted UV treatment (UV dose of 12,000 J/m^2) resulted in >3.20 log and 1.81 log MNV-1 reductions for skin- and calyx-inoculated berries, respectively. The presence of 2% blueberry juice in wash water decreased the decontamination efficacy of water-assisted UV and chlorine washing treatments. To improve the inactivation efficacy, the effect of combining water-assisted UV treatment with chlorine washing was also evaluated. The combined treatment had better or similar inactivation efficacy compared to water-assisted UV treatment and chlorine washing alone. Findings of this study suggest that water-assisted UV treatment could be used as an alternative to chlorine washing for blueberries and potentially for other fresh produce.

1. Introduction

Blueberry is a high-value fruit that has many benefits to human health, such as antioxidant and anti-inflammatory activities (Roy et al., 2009). However, blueberries have been linked to several recent outbreaks. In 2003, contaminated raw blueberries led to an outbreak of hepatitis A (Calder et al., 2003). In 2009, raw blueberries contaminated with *Salmonella* Muenchen resulted in a multistate outbreak, which caused 14 cases of illnesses (Centers for Diseases Control and Prevention [CDC], 2012a). Between 2007 and 2011, one norovirus outbreak associated with strawberries and 27 associated with raspberries were reported in the EU (European Food Safety Authority, 2014). In a massive human norovirus (HuNoV) gastroenteritis outbreak in 2012 that affected about 11,000 people, frozen strawberries were identified as the source of outbreak (Mäde et al., 2013). Indeed, norovirus is the leading cause of acute gastroenteritis in the United States, which causes 19–21 million illnesses and contributes to 56,000–71,000 hospitalizations and 570–800 deaths each year (CDC, 2013). Fresh blueberries are harvested manually or mechanically (Harris et al., 2003). Berries destined for the fresh market are not washed. They are field-packed into retail containers following harvesting. Ready-to-eat refrigerated berries and frozen berries are usually washed with chlorinated water before packaging or freezing. Since the berries are consumed raw or minimally processed, they could lead to food safety problems.

Chlorine has been widely used for fresh produce decontamination in the U.S. It is low in cost, has minimal impact on the quality of the food product and has been shown to be effective in killing pathogens in suspensions (Gonzalez et al., 2004; Gil et al., 2009). It is critical that a relatively constant level of free chlorine be maintained in washing solutions to ensure its efficacy against microbial contamination. Chlorine can react rapidly with organic matter in the washing solution and form by-products like trihalomethanes, haloketones and chloropicrin (Gil et al., 2009). To maintain a constant free chlorine level, it is necessary to replenish the chlorine during washing process, thus leading to the accumulation of toxic chlorine by-products and generation of harmful chlorine off-gas (Suslow, 2001). In several European countries, however, antimicrobial agents such as chlorine are prohibited in fresh-

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cut produce washing, enabling cross-contamination to the final fresh-cut product (Holvoet et al., 2014). Therefore, there has been a sustained effort to search for antimicrobial alternatives to treatment fresh produce.

Shortwave ultraviolet light (UVC, simplified as UV in this study) has been shown to be able to inactivate a wide range of microorganisms (Hijnen et al., 2006). UV light has been approved by the FDA as a treatment for controlling surface microorganisms and juice products (US Food and Drug Administration [FDA], 2013). Various studies have shown UV light treatment to be effective on bacterial and viral reduction on food surfaces such as blueberries, strawberries, lettuce and onions (Allende et al., 2006; Fino and Kniel, 2008; Kim and Hung, 2012). According to Fino and Kniel (2008), significant virus reductions could be seen on lettuce, green onions and strawberries with a UV dose of 2400 J/m^2. Commercially UV is used to treat wastewater, drinking water, and apple ciders (City of Boulder Colorado, 2012; CDC, 2012b; Geveke, 2005).

UV has far less detrimental effect on nutrients and can retain the fresh-like characteristics and flavors of foods compared with thermal processing (Falguera et al., 2011; Pala and Toklucu, 2013). In addition, the equipment setup is simple and relatively low in cost. However, the application of UV as a surface decontamination treatment for food is very limited due to this major UV shortcoming, very shallow penetration depth on an opaque food surface. In fact, the UV penetration depth is so shallow that microorganisms attached to the rough food surfaces or crevices are very difficult to be killed. In addition, microorganisms on a food surface must directly face a UV lamp to be inactivated (Shama, 1999). To overcome this UV limitation, a water-assisted UV system was developed in this study where blueberry samples were immersed in agitated water during UV treatment. The blueberry samples could randomly move and rotate in the agitated water, thus allowing all blueberry surfaces to be exposed to UV light and receive more uniform UV exposure. In the meantime, the vigorously agitated water would wash off microorganisms on blueberry surfaces into water (Pangloli and Hung, 2013), which could be easily killed by UV light since UV can penetrate well in clear liquid.

Since HuNoV cannot be propagated in cell cultures (Duizer et al., 2004), surrogate viruses that share similar molecular and/or pathological features with HuNoV are commonly used in research. Murine norovirus (MNV-1) is commonly used as a surrogate for HuNoV. Studies have shown that MNV-1 is environmentally stable (Bae and Schwab, 2007) and persistent over a wide range of pH values (Hirneisen and Kniel, 2013). MNV was also found to be more resistant to UV irradiation than Feline calicivirus (Park et al., 2011), which makes it a more suitable surrogate than feline calicivirus.

The overall goal of this study was to evaluate the decontamination efficacy of the water-assisted UV system against MNV-1 inoculated on blueberries. First, the effect of water-assisted UV irradiation on the inactivation of MNV-1 in comparison to UV irradiation alone (dry UV) and chlorine wash was evaluated. Second, the decontamination efficacy of this water-assisted UV system was assessed in the presence of organic load (2% blueberry juice or 5% crushed berries) in the wash water. Spent wash water was also tested for the presence of MNV-1 for all treatments to evaluate the possibilities of cross-contamination.

2. Materials and methods

2.1. Virus and cell lines

MNV-1 and murine macrophage cell line RAW 264.7 were kindly provided by Dr. Jianrong Li at the Ohio State University. RAW 264.7 cells were cultured in Dulbecco's modified Eagle medium (DMEM) (Gibco, Life Technologies Corporation, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco) and kept at 37 °C under 5% CO_2 atmosphere. To prepare MNV-1 stock, confluent RAW 264.7 cells were infected with MNV-1 at a multiplicity of infection (MOI) of 1. After 1 h of incubation at 37 °C under 5% CO_2 atmosphere, 25 ml of DMEM (supplemented with 2% FBS) was added. MNV-1 was harvested 2 days after inoculation by three freezing–thawing cycles and subsequent centrifugation. The virus was stored at −80 °C until use.

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. The blueberries were UV-treated (UV intensity of 0.2 mW/cm^2) for 10 min in a biosafety hood (NuAire Lab Equipment, Plymouth, MN, USA) at room temperature (22 °C) to reduce background microflora. MNV-1 of 50 μL was deposited on either the skin or the calyx tissue of blueberries in small droplets to simulate two contamination conditions. The inoculated blueberries were allowed to dry in the biological safety hood for 2 h at room temperature (22 °C).

2.3. MNV-1 recovery rate by the vegetable buffer homogenization method

To determine the recovery rate of MNV-1 from blueberries, blueberries were inoculated with 10-fold serial dilutions of the virus and dried as described above. MNV-1 was extracted from blueberries using the method described by Kingsley et al. (2002) with modifications. Individual blueberry samples were transferred into sterile stomacher filter bags (Whirl-Pak, Nasco, USA). Two volumes of vegetable buffer (100 mM Tris [Thermo Fisher Scientific Inc.], 50 mM glycine [Promega Corporation], 3% [m/v] beef extract [Becton Dickson Company], 50 mM $MgCl_2$ [Thermo Fisher Scientific Inc.], pH 9.5) were added to the bags and the samples were homogenized with a stomacher (Seward 400, Seward, London, U.K.) at 260 rpm for 1 min. The filtrate was taken and centrifuged at 2500 ×g for 10 min at 4 °C (Sorvall, Thermo Scientific, USA). The supernatants were used for subsequent plaque assays as described in Sections 2.8.

2.4. Efficacy of small-scale water-assisted UV treatment on MNV-1 inactivation on blueberries

The UV treatments were conducted using a Reyco UVC Emitter Table Top Test System (Meridian, ID, USA). The test system contained four parallel mercury lamps that emitted UV light at 254 nm (Fig. 1). UV intensity was measured before each treatment by placing the sensor of a UV radiometer (UVP, Upland, CA, USA) right above the surface of blueberry samples.

Skin-inoculated blueberries were either treated with UV directly (dry UV treatment) or immersed in agitated water during the UV

Fig. 1. Large-scale water-assisted UV treatment setup. UV lamps are on top of the chamber.