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The impact of pulsed light on decontamination, quality, and bacterial attachment of fresh raspberries

Wenqing Xu [a, b], Changqing Wu [b, *]

[a] School of Nutrition and Food Sciences, Louisiana State University AgCenter, Baton Rouge, LA 70808, USA
[b] Department of Animal and Food Sciences, University of Delaware, Newark, DE 19716, USA

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ABSTRACT

Raspberries have served as vehicles for transmission of foodborne pathogens through fecal-oral route and have resulted in 11 outbreaks in the United States from 1983 through 2013. However, because of its dedicated structures and perishability, water based sanitizer washing cannot be used for raspberry decontamination. As a non-thermal technique, pulsed light (PL) may have the potential to maintain both safety and quality of fresh raspberries. The first objective of our study was to investigate *Salmonella* and *Escherichia coli* O157:H7 inactivation efficacy of pulsed light (PL) on fresh raspberries during 10 days storage at 4 °C. The qualities of raspberries after PL treatment, including color, texture, total phenolic content (TPC), total anthocyanin content (TAC), total bacteria count (TBC) as well as total yeast and mold count (TYMC), have also been evaluated during the 10 days storage. Compared with the untreated control, all the PL treatments (5 s, 15 s and 30 s) maintained lower pathogen survival population during 10 days refrigerated storage. At day 10, all PL treated raspberries maintained significantly lower TBC and TYMC than the control. Although PL treatment for 30 s (with fluence of 28.2 J/cm^2) reduced most *Salmonella* and *E. coli* O157:H7 right after treatment, by 4.5 and 3.9 log 10 CFU/g respectively, it failed to maintain its advantage during storage. In addition, color and texture of these raspberries changed negatively after 10 days storage. PL 30 s provided the lowest TBC and TYMC at day 0, but failed to maintain its advantage during storage. To consider both safety and quality of fresh raspberries as well as the treatment feasibility, 5 s PL treatment with fluence of 5.0 J/cm^2 was recommended for decontamination. The second objective was to study attachment of bacteria as well as decontamination effect of PL on raspberries. Under the scanning electron microscopy (SEM), PL showed severe damage to the cell membrane on smooth surface. Surface structure of raspberries affected the attachment of bacterial cells and the surface roughness provided protection for pathogenic bacteria. Our research demonstrated for the first time that successful PL processing of raspberries should be evaluated for its impacts on both produce safety and quality during the storage. PL with fluence of 5.0 J/cm^2 maintained both safety and quality of fresh raspberries during the refrigerated storage.

1. Introduction

Raspberries are the third most popular berry in the United States for fresh use, after strawberries and blueberries. As the world's third-largest producer, most of raspberries growers in the U.S. are located in Washington, California and Oregon. However, only 15% of the domestic demand for raspberry fruit is met by production in the U.S. Most imports are arriving from Canada in July and August, and Mexico and Chile from November through May. Berries have been associated with outbreaks in both North America and Europe, and have caused numerous serious illnesses. Imported berries were often implicated, indicating that this problem extends beyond those countries where there have been outbreaks (FAO, 2008).

From 1983 to 2013, there were 11 outbreaks related with raspberries with total 4, 637 cases. In earlier time, raspberries outbreaks have been associated with *Cyclospora cayetanensis* (CDC, 1996, 1997, 1998; Ho et al., 2002), Calicivirus (Pönkä et al., 1999) and Hepatitis A (Reid and Robinson, 1987). Most recently, there were four raspberry outbreaks related with Norovirus (Cotterelle et al., 2005; Falkenhorst et al., 2005; Hjertqvist et al., 2006; Maunula et al.,

* Corresponding author.
E-mail address: changwu@udel.edu (C. Wu).

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2009). Fresh as well as frozen raspberries have both been reportedly associated with outbreaks. Outbreaks of foodborne illness in the United States associated with imported raspberries affects not only consumers and the growers of the contaminated product, but also frequently other suppliers to the U.S. market, including U.S. producers (Calvin, 2004, Calvin et al., 2003; Mishen, 1996).

C. cayetanensis, hepatitis A as well as norovirus contaminate raspberries through fecal-oral route. It indicates that other foodborne pathogens such as *Salmonella* and *Escherichia coli* O157:H7 which share the same transmission route all have the potential risk. Contamination of fresh raspberries may come from animal entrance into fields and packing houses, irrigation water, picking berries close to the soil line as well as unhygienic workers. The challenge for raspberry decontamination exists because, (1) raspberries are made up of many individual fruits (drupelets) held together by hairs (trichomes) and waxes (Mackenzie, 1979) which provides harbor for foodborne pathogens, and (2) unlike tree fruit, fresh market raspberries are not washed. Thus, there is no way to use liquid sanitizers to limit the potential microbial risk.

Pulsed light (PL) is a non-thermal method for food preservation that involves the use of intense, short duration pulses of a broad spectrum to ensure microbial decontamination on the surface of either foods or packaging materials (Elmnasser et al., 2007). PL treatment of foods has been approved by the FDA (1996) under the code 21CFR179.41 with the total cumulative treatment not exceeding 12.0 J/cm^2. The lamp can generate light from UV to near-infrared (100–1100 nm) and UV region was reported to be critical to efficiency of PL treatment (Takeshita et al., 2002). To our best knowledge, research about PL inactivation of *Salmonella* or *E. coli* O157:H7 on raspberries is still limited. For example, although Bialka (2007) reported that PL could reduce *E. coli* O157:H7 and *Salmonella* on raspberries but they did not study the pathogen survivial profile during the storage. Furthermore, none of previous research reported the impacts of the PL treatment on quality and associated bioactive compounds of raspberries during their refrigerated storage.

Raspberries are delicate fruits and are highly perishable for many reasons. Aesthetic quality of raspberries is mainly determined by color, firmness and mold content. Color change from pink to dark red leads to the loss of attractiveness to consumers. Raspberries grown for fresh market have to be able to retain firmness during harvest, handling and storage and have a shelf-life of 10 days or more (Toivenon et al., 1999). From prospective of health benefit, berries are a good source of polyphenols, especially anthocyanins, micronutrients, and fiber. In epidemiological and clinical studies, these constituents have been associated with improved cardiovascular risk profiles (Basu et al., 2010). Ripe raspberries are also highly susceptible to gray mold (*Botrytis cinerea*), which causes serious losses to some greenhouse growers of raspberries. Molds can also develop in harvested fruit if it is held too long before processing (FDA, 2013). Thus, in this study we not only investigated the decontamination efficacy of *Salmonella* and *E. coli* O157:H7 on fresh raspberries by using PL treatment (right after the treatment and during storage), but also evaluated potential quality change caused by PL during 10 days storage, including color, texture, total phenolic content (TPC), total anthocyanin content (TAC), total bacteria count (TBC) as well as total yeast and mold count (TYMC). For better understanding of mechanism of PL on pathogen inactivation, scanning electron microscopy (SEM) was applied to observe bacterial attachment on raspberries and the decontamination effect of PL.

2. Materials and methods

2.1. Bacterial strain and inoculum preparation

Single wild-type strains including *Salmonella* (*S.* Newport H1275, sprout outbreak isolate) and *E. coli* O157:H7 (250, sprout outbreak isolate) were used in our study. Wild strains were obtained from culture collection in Department of Animal and Food Sciences at University of Delaware. The *Salmonella* strain was adapted to grow in the presence of nalidixic acid (50 μg/mL, Fisher Scientific, Hampton, NH, USA) alone to create a single antibiotic resistance strain, while the *E. coli* O157:H7 strain was adapted to grow in the presence of nalidixic acid (100 μg/mL) plus streptomycin (100 μg/mL, Sigma, St. Louis, MO, USA) to create a double antibiotics resistance strain. Both resistance strains were grown on tryptic soy agar (TSA, Difco Laboratories, Sparks, MD, USA) plus 0.6% yeast extract (YE, Difco) supplemented with nalidixic acid only (TSAYE-N for *Salmonella*) or nalidixic acid and streptomycin (TSAYE-NS for *E. coli* O157:H7) for 2–3 days at 35 °C. Single colonies were picked and transferred to 10 mL of tryptic soy broth (TSB, Difco) plus 0.6% yeast extract (Fisher) supplemented with same single or double antibiotics (TSBYE-N for *Salmonella* or TSBYE-NS for *E. coli* O157:H7). The culture was incubated at 35 °C overnight and second-transferred to 10 mL of fresh TSBYE-N or TSBYE-NS to yield an approximate population of 10^9 CFU/mL after 24 h incubation at 35 °C. The culture was diluted to 10^8 CFU/mL using sterile 0.1% peptone water (Difco) and used as inoculum.

2.2. Raspberries preparation and inoculation

Fresh raspberries were purchased from a local supermarket and stored at 4 ± 2 °C for a maximum of 4 h before use. Medium size (~5 g) raspberries with pink color and ideal firmness were selected. The selected raspberries were intact and had no noticeable physical injury. To spot inoculate raspberries, 50 μL of 10^8 CFU/mL inoculum was deposited on the outside surface of raspberries as five droplets. Spot inoculation was applied to simulate the contamination caused by animal feces drops, irrigation water splashing or unhygienic touch. Inoculated raspberries were dried in the biosafety hood for 2 h before future use.

2.3. Inactivation of Salmonella and E. coli O157:H7 using PL treatment and pathogen survivor population during storage

PL was generated by a bench-top pulse light system (SteriPulse-XL, Model RS-3000C, Xenon Corp., Wilmington, Mass., U.S.). The system includes a controller module, a treatment chamber and an air cooling module. The 16 inch linear clear fused quartz PL lamp (LH840) generated PL in the wavelength of 200–1100 nm, with 40% of the energy being in the UV region. Pulses were delivered 505 J/pulse (1.27 J/cm^2) energy with 3 pulses/sec pulse rate, based on manufacturer's specification. The broadband energy of each pulse, expressed in J/cm^2, was quantified using a Vega laser power meter (Ophir Optronics Inc., Wilmington, MA) equipped with a pyroelectric energy sensor (PE-50C, Ophir Optronics). For PL treatment, raspberries were placed in a PL chamber in sterile petri dishes without covers. The distance between the lamp and the quartz window was 5.8 cm and the distance between the top of raspberries and the quartz window was about 13 cm. PL treatments were performed for a total time ranging from 5 s to 30 s, which included duration of pulses and the interval between each pulse. Since serious quality loss of raspberries was observed when PL was applied for more than 30s, we chose 5, 15 and 30 s as our treatments times. The quality loss included shrunk tissue, darker color and cooked smell. Fluence for our system was 5.0, 14.3 or 28.2 J/cm^2