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2 **The Impact of Pulsed Light on Decontamination, Quality, and Bacterial**

3 **Attachment of Fresh Raspberries**

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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 *E. 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/cm²) 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/cm² 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/cm² maintained both safety and quality of fresh raspberries during the refrigerated storage.

Keywords

Raspberries; Pulse Light; *Salmonella*; *E. coli* O157:H7; quality; attachment

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., 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 and 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 *E. 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/cm². The lamp can generate light from UV to near-infrared (100 to 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 et al (2007) reported that PL could reduce *E. coli* O157:H7 and *Salmonella* on raspberries but they did not study the pathogen survival 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 10⁹ CFU/mL after 24 h incubation at 35 °C. The culture was diluted to 10⁸ 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 10⁸ 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 joules/pulse (1.27 joules/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 for 5, 15 or 30 s PL treatments, respectively. Treated and control (inoculated but not treated) raspberries were then tested immediately or placed into sterile bags with openings for breathing and then stored at $4 \pm 1^\circ \text{C}$ for 10 days. Three independent trials were conducted using pathogenic bacteria growing on three different days and raspberries have been processed on three different days.

2.4 Microbial analyses

Raspberries were microbiologically analyzed at day 0, day 5 and day 10. They were transferred into sterile stomacher bags with D/E neutralized broth (Difco) at 1:9 ratio (w:v) and homogenized by stomacher (400 Circulator, Seward Co., West Sussex, UK) at 260 rpm for 1min to help releasing and evenly distributing pathogens. The homogenate was serially diluted using 0.1% peptone water and plated on TSAYE-N (for *Salmonella*) or TSAYE-NS (for *E.coli* O157:H7) plates using sterile spreader. Colonies were enumerated after incubation for 48 h at 37°C. Pathogen survivor population was reported as log 10 CFU/g.

2.5 Effect of PL on color and texture of raspberries

Raspberries without artificial inoculation were either treated by PL as described above or served as control (without PL) and then stored in clean bags with openings at 4±1 °C for 10 days. The color and texture of raspberries were analyzed at day 0, day 5 and day 10. Color was tested by using chromameter (Minolta CR-400, Minolta, Osaka, Japan). Color parameters were quantified in the Hunter L* (lightness/darkness), a* (redness/greenness), and b* (yellowness/blueness) color space. Texture of raspberries was measured as shear strength using a texture analyzer (TA. XT2i, Texture Technologies, New York, USA). Samples were placed on the TA-91 platform and compression tests were carried out. The maximum force (Fmax) needed to compress the samples were reported.

2.6 Effect of PL on TPC and TAC of raspberries

TPC of raspberries with or without PL treatment was determined as described by Xu et al. (2010) with slight modification. Raspberries were homogenized in stomacher bags with 25mL of ethanol with the ratio of 1:5 (w/v). The bags were heat sealed and let sit for 30 min with gentle massaging to extract the polyphenol compounds. Homogenates were centrifuged at 4000 rpm for

10 min (Beckman Coulter, Inc. Microfuge[®], Indianapolis, IN, U.S.) and supernatants were collected. Thirty μ L of supernatants (dilute if necessary) was added into clear 96-well plate with a clear bottom followed by adding 60 μ L of the Folin–Ciocalteu reagent (1×10^{-4} M) and 120 μ L of sodium carbonate solution (20 %, w:v). The plate was covered with clear lid, shook horizontally to mix and incubated for 30 min at 35°C. A serial of known concentration of gallic acid was used as standard. Absorbance was measured at 765 nm using the Synergy^{TM 2} multi-mode microplate reader (BioTek Instruments, Inc. Winooski, VT, U.S.). The results were expressed as μ g of gallic acid equivalents (GAEs) per 100 g of fresh weight (FW).

TAC of raspberries was measured using pH differential method reported by Bunea et al. (2011) with slight modification. Raspberries with or without PL treatments were placed in 50 mL conical centrifuge tubes with 30 mL of methanol-water solution (80:20, v:v) containing 0.1 mL/L acetic acid. The mixture was homogenized by ultra-turrax (IKA Works, Inc., Wilmington, NC) for 2 min, incubated in the dark for 1 h and then ultrasonicated for 15 min to aid anthocyanin extraction. After centrifugation at 5000 rpm for 30 min at room temperature, the volume of the supernatants, which was raspberry anthocyanin extract, was recorded. The anthocyanin extract was mixed with a 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5) with a dilution factor at 6.0. The absorbance of each mixture was measured at 510 nm and 700 nm for the correct haze. The absorbance (A) of anthocyanin extract was calculated by the following formula:

$$A = (A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.5}}$$

where A was the absorbance of the anthocyanin extract. A₅₁₀ meant the absorbance measured at 510nm, and A₇₀₀ meant the absorbance measured at 700nm.

The monomeric anthocyanin pigment concentration in the original raspberry sample was expressed in equivalence of cyaniding-3-glucoside using the following formula:

$$\text{anthocyanin content} = (A \times \text{MW} \times \text{DF} \times 1000) / (\epsilon \times 1)$$

where MW (449.2 g/mol) was the molecular weight of cyanidin-3-glucoside; DF was the dilution factor (6 in our study); and ϵ was the molar absorptivity, which equal to 26, 900 for cyanidin-3-glucoside.

2.7 Effect of PL on TBC and TYMC of raspberries

Raspberries without artificial inoculation were used for TBC and TYMC evaluation. At day 0, day 5 and day 10, raspberries with or without PL treatment were placed in sterile stomacher bags with 45mL of 0.1% peptone water (1:9 ratio, w:v) after the PL treatment and homogenized by stomacher at 260 rpm for 1 min. The homogenate was either plated on TSA for TBC enumeration or on Potato Dextrose Agar (PDA, Difco) for TYMC enumeration. Before counting colonies, TSA plates were incubated at 35°C for 48 h while PDA plates were incubated at 25 °C for 3-5 days (Tournas et al., 2001).

2.8 Scanning Electron Microscopy (SEM) sample preparation

To observe the behavior of foodborne pathogens on the surface of raspberries, dip inoculation was applied to provide maximum attachment possibilities. *E. coli* O157:H7 was used as model pathogen for its attachment and decontamination in response to PL treatment. Double antibiotic resistant strain was prepared as in 2.1. Raspberries were fully submersed in 10⁸ CFU/mL inoculum of *E. coli* O157:H7 in a sterile stomach bag in the ratio of 1:5 (weight of raspberries: volume of the inoculum). Bags were heat-sealed immediately and slight shaking was applied for 1 min. Raspberries were taken out by sterile tweezers, placed in clean petri dishes and

air dried in the biosafety hood for 4 h before the decontamination treatments. Longer drying time was used because more volume of bacteria culture liquid involved in the dip inoculation than the spot inoculation. PL treatment (15 s) was applied as described in 2.3.

Treated and untreated raspberries were cut into small pieces then placed in sterile clear 6-wells cell culture plates and bacterial cells were fixed with 2% of glutaraldehyde for 1 h before critical points drying (CPD) to stabilize the tissue components and to reduce their extraction in the dehydration and transitional solvents. Fixation was conducted in a flammable fume hood.

To compare the different behavior of *E. coli* O157:H7 on smooth surface and raspberry, coverslips were inoculated with *E. coli* O157:H7 and treated by PL for 15s. Fisherbrand™ microscope coverslips (Circles No. 1; 0.13 to 0.17 mm thick; Size: 18 mm) were placed in clean petri dishes. 0.1% of poly-L-lysine was applied in the center of the coverslips and incubated at room temperature for 5 min. Liquid was pipetted out and the coverslips were dried in the biosafety hood at room temperature for 12h. *E. coli* O157:H7 inoculum (0.3 mL) was placed on center of coverslips coated with poly-L-lysine and incubated at 37°C for 30 min. Extra inoculum was removed and a single layer of bacteria cells was formed on the surface. Inoculated coverslips were placed in sterile petri dishes without covers in a PL chamber and treated for 15s as described 2.3. Inoculated coverslips without PL treatment served as control. Treated and untreated cover glasses were then placed in sterile clear 6-wells cell culture plates and bacterial cells were fixed by 2% of glutaraldehyde for 1h before critical points drying (CPD) to stabilize the tissue components and to reduce their extraction in the dehydration and transitional solvents. Fixation was conducted in a flammable fume hood.

2.9 Scanning Electron Microscopy (SEM)

After fixation, coverslips and raspberries were washed 3 times (30 min/time) with filtered buffer (1×PBS) and went through postfixation process by incubating with Osmium Tetroxide (OsO₄) for 2 h at room temperature. Then coverslips and raspberries were rinsed thrice with filtered ultrapure water (30 min/time) and then dehydrated in series of ethanol (25%, 50%, 75%, 95% and 100%) with 30 min for each concentration. The 100% ethanol was applied twice to ensure complete dehydration. Coverslips and raspberries were transferred to the metal carrier baskets that came with CPD apparatus. The dehydrated samples were dried immediately by autosamdri-815B Critical Point Dryer (Tousimis® Rockville, MD, USA) followed by mounting onto SEM stubs and sputter-coating (Denton Vacuum Bench Top Turbo III, Moorestown, NJ, USA) with a thin layer of gold-palladium. The coated samples were observed under SEM (Hitachi S4700 FESEM; Hitachi Ltd, Tokyo, Japan) at an accelerating voltage of 3kV. Pictures were acquired at different magnifications, ranging from 2.5 K to 20.0 K.

2.10 Statistical analyses

All experiments were replicated 3 times in each independent trial, all the experiments have been conducted in three independent trials, and results were reported as Mean±SD. JMP 10 (SAS Cary, NC, USA) software was used for statistical analyses. For survival population of pathogens and quality of raspberries (including color, texture, TBC, TYMC, TPC and TAC) during storage, Dunnett's test was used to determine differences between treated and untreated (control) samples on day 0, day 5 and day 10 and Student's t test was used to determine the difference between each treated samples and same treatment on different days. Significant difference was reported when $P < 0.05$.

3. Results and discussion

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

As shown in Fig. 1, PL inactivated *Salmonella* in a time-dependent manner (from 5 s to 30 s) which was also a fluence-dependent manner (from 5.0 to 28.2 J/cm²). The 5s PL treatment significantly lowered the *Salmonella* population from 5.5 (control) to 2.1 log CFU/g. *Salmonella* population was lowered to 1.5 or 1.0 log CFU/g after 15 or 30s PL treatment, respectively. The population decrease was significant at the alpha level of 0.05. The highest log reduction (4.5 log CFU/g reduction) of *Salmonella* was provided by 30 s PL treatment.

During 10 days storage, there was no significant difference for the control ranging from 5.5 to 5.0 log CFU/g. PL 5s treated raspberries showed a slight population increase in the first 5 days and a slight decrease in the last 5 days. Raspberries treated by 15s PL, however, had a significantly increased *Salmonella* population overall. During the first 5 days of storage, the population increased significantly from 1.5 to 2.2 log CFU/g and there was no significant increase after day 5 through the end of the storage. The 30s PL showed basically the same trend as PL 15s. There was a significant increase in *Salmonella* population during first half of the storage, from 1.0 to 2.3 log CFU/g, and no significant change has been observed from day 5 to day 10 even though the population did slightly decrease to 2.0 log CFU/g. At day 10, end of storage, *Salmonella* survivor population for PL 15s (2.3 log CFU/g) and PL 30s (2.0 log CFU/g) treatments were still significantly lower than the control (5.0 log CFU/g) but there was no more significant difference compared with PL 5s (1.8 log CFU/g), which indicated that a longer exposure time of PL provides high inactivation efficacy on *Salmonella* right after the treatment, however the advantage is lost after 10 days storage.

Inactivation of *E. coli* O157:H7 using PL is shown in the same figure (Fig.1). Population of *E. coli* O157:H7 decreased significantly from 5.7 (control) to 2.1 log CFU/g after 5 s of PL treatment, which showed that PL has reduced *E. coli* O157:H7 by 3.6 log CFU/g. Different from *Salmonella*, with increase of PL treatment time from 5 s to 30 s, there was no significant further improvement of pathogen inactivation. *E. coli* O157:H7 population on 5 s PL treated raspberries stayed low during the first 5 days and lowered significantly from day 5 (2.1 log CFU/g) to day 10 (1.2 log CFU/g). The 15s PL led to a significantly lower pathogen survivor population on day 10 (1.5 log CFU/g) compared with day 0 (2.3 log CFU/g). For 30s PL treatment, *E. coli* O157:H7 survivor population did not change significantly during storage. At the end of 10 days, there was no significant difference among three PL treatments, which was also observed for *Salmonella* population.

Our results showed that PL was an efficient technique to inactivate *Salmonella* and *E. coli* O157:H7 on fresh raspberries and maintained the lower pathogen survivor population during the 10 days storage. PL has been reported to inactivate microorganisms naturally present (Gómez-López et al., 2005; Hoornstra et al., 2002) as well as inoculated (Fine and Gervais, 2004) on food surfaces. Our previous study showed that PL provided 4.9 and 5.2 log reduction on *E. coli* O157:H7 spot inoculated on green onion stems and leaves, respectively (Xu et al., 2013). Sharma and Demirci (2003) inoculated alfalfa seeds with *E. coli* O157:H7 and treated with PL at different distance (8 and 13 cm) for 75s. They achieved 4.89 log reduction at distance of 8 cm, but only 1.42 log reduction at 13 cm, which indicated the PL decontamination efficacy is dependent on the distance. It also seems that fresh produce with smoother surface and simpler structure was easier to decontaminate by using PL. Reported in Bialka's study (2007), PL was applied to different berries to inactivate artificially inoculated *E. coli* O157:H7 and *Salmonella*.

Maximum reductions of 4.3 and 2.9 log₁₀ CFU/g were achieved on blueberries after a UV dose of 22.6 J/cm² for *E. coli* O157:H7 and *Salmonella*, respectively. On raspberries, however, the maximum reductions of *E. coli* O157:H7 and *Salmonella* were 3.9 and 3.4 log₁₀ CFU/g at much higher fluence (72 and 59.2 J/cm², respectively).

3.2 Effect of PL on color and texture of raspberries

Attractive color is one of the main sensory characteristics of berry fruits and products (Rein and Heinonen, 2004). Color changing from pink to red to dark red may not be attractive in some markets. In this study we monitored the L*, a* and b* values, but only reported L* and a* which indicated the change of brightness/darkness and redness/greenness, respectively. As shown in Table 1, there was no significant change for the brightness (L*) after the PL treatment at day 0 ranging from 31.6 to 32.3. During 10 days storage, brightness of raspberries without PL treatment decreased significantly from 32.3 to 27.1 while the redness did not change significantly. The 5s PL treated raspberries showed the same results with decrease of brightness and no significantly change of redness. After being treated for 15 s or 30 s, raspberries became darker red including significantly decrease of L* value and increase of a* value after 10 days storage. Although not included in Table 1, b* value of raspberries ranging from 11.7 to 12.6 did not show any significant change after the PL treatment or during storage.

Firmness is another quality indicator for raspberries not only for fresh market but also for processing. Texture of the raspberries showed the similar trend as color, as shown in Table 1. When texture analysis was conducted immediately after PL treatment, those have been treated for 5s showed the significantly softer texture which may because of the individual variation. During 10 days storage, the control and all PL treated berries resulted in significantly softer

texture at day 10 comparing with day 0. PL 30s caused the significant softening during 10 days of storage.

Over the last decade, consumers have an increasing requirement for natural and fresh food quality. Quality and safety are both critical for the fresh produce industry. People want to develop techniques with satisfying antimicrobial capacity without compromising quality. In this study, 30s PL treatments did not affect the quality of raspberries right after treatment, however, the influence on quality became significantly greater during first 5 days of storage. In Bialka and Demirci's study (2007), PL was applied at three distances (3, 8 and 13 cm) for 60s with UV-dose of 32.4, 22.6, and 12.8 J/cm², respectively for the decontamination of blueberries. There was no significant color difference between treated and untreated blueberries. However, no quality during storage was monitored. Charles et al. (2013) has evaluated the impact of PL on firmness and color of fresh-cut mangoes. Mangoes were treated by PL with fluence of 8 J/cm² and placed in glass jar and stored at 6°C for 7 days. The results showed that PL preserved the tissue firmness and color of mangoes during storage. This difference may be because of higher fluence used in our study (28.2 J/cm² for 30s PL treatment) as well as tougher texture of mangoes compared with raspberries used in our study.

3.3 Effect of PL on TPC and TAC of raspberries

Although polyphenols are widely distributed in fruits and vegetables, not all foods are equally good sources, and soft fruits such as strawberries, raspberries and blueberries are now recognized as especially rich sources (Beattie et al., 2005). Berries contain polyphenols including phenolic acids, flavonoids, tannins and stilbenes. As shown in Table 1, TPC was not affected by PL right after the treatment. This is similar as findings from Luksiene et al. (2013) who treated strawberries with high-power pulsed light (3.6–10.8 J/cm²) and no TPC decrease was observed

after the treatment. Other researches, on the other hand, have reported conflicted results. Murugesan (2012) has studied the changes in total polyphenolics in elderberry (*Sambucus nigra*) following treatment with various doses of pulsed light. The results showed that PL treatments enhanced the antioxidant properties of elderberry fruits by increasing their TPC. They also found that the increase of energy from 4500J/m²/pulse to 6000J/m²/pulse brought a decrease in the TPC. Guner (2012) treated blueberries with PL and concluded that 60 s or 90 s of PL significantly increased the total phenolic contents of blueberries. These previous findings suggested that TPC varied among different energy and treatment duration used in PL.

In our study, during 10 days storage, TPC of the control decreased significantly from 39.1 to 29.6 mg Gallic acid equivalents per 100 g FW. Similar results have been reported by Verde et al. (2013). In their study, fresh raspberries were stored at 4°C for 14 days and the results of TPC indicated a decrease with the storage. In our study, TPC in PL treated raspberries also decreased during 10 days storage. However, significant drop of TPC in the first 5 days has only been observed in raspberries treated by 15 or 30s PL. At the end of storage, there was no significant difference on TPC among the untreated and PL treated raspberries, which indicated that although PL did not improve TPC of raspberries, neither did it affect the TPC compared with untreated raspberries.

TAC was recorded since anthocyanins contribute to the red color as well as antioxidant abilities of raspberries. Raspberries contain a distinct spectrum of 11 different anthocyanins, the most abundant being cyanidin-3-sophoroside, cyanidin-3-(2G-glucosylrutinoside), and cyanidin-3-glucoside (Mullen et al., 2002). As shown in Table 1, PL 5s (38.7 mg cyanidin-3-glucoside equivalents /100 g FW) and 15s (44.8 mg cyanidin-3-glucoside equivalents /100 g FW) did not affect the TAC of raspberries compared with untreated berries (42.6 mg cyanidin-3-glucoside

equivalents /100 g FW) right after treatment. PL 30s, however, significantly increased the TAC to 48.6 mg cyanidin-3-glucoside equivalents /100 g FW. It has been reported (Rodov et al., 2012) that brief postharvest exposure to pulsed light (10-90s) stimulates coloration and anthocyanin accumulation in fig fruit (*Ficus carica* L.).

In our study, during 10 days storage, TAC in the control and treated samples significantly decreased while all PL treated berries showed higher TAC at the end of storage. TAC has been reportedly affected by various factors, such as fruit maturity, storage temperature and pulsed light treatment. Wang et al. (2009) showed that cyanidin-based anthocyanins of raspberries increased during the postharvest period, and red raspberries harvested at different developmental stages continued their anthocyanins development during storage even under the dark conditions. Kalt et al. (1999) reported that temperature above 0 °C has been shown to increase the anthocyanin contents of red raspberries on storage. Rodov et al. (2012) who studied the influence of PL on fig fruit also reported that after 5 days of storage in the dark at 20 °C, the anthocyanin content of the skin of fruit exposed to 90–300 s PL was 20 times higher than that observed for the untreated control. In our study we didn't observe significant increase of TAC for the untreated raspberries, but we did agree with other researchers on the positive effect of PL on TAC during storage.

No evaluation of vitamin C was conducted in the current study due to limitation of research time and resource. Other researchers (Luksiene et al. 2013) reported that vitamin C in strawberries would not be affected by PL treatment and there was no significant change before and after PL treatment, however, no kinetic changes of vitamin C were evaluated during the storage of strawberries. In the future research, it is important to determine the vitamin C profile in raspberries right after PL treatment and during the shelf-life.

3.4 Effect of PL on TBC and TYMC of raspberries

Raspberries are usually rotten by softening and growth of mold and/or bacteria. Initial population of total bacteria of raspberries was 2.5 log 10 CFU/g. PL treatment decreased significantly populations of aerobic bacteria in raspberries compared with the control. As shown in Fig. 2, after PL treatment, TBC in raspberries were 1.8, 1.5 and 1.0 log 10 CFU/g for 5, 15 and 30s of PL treatment, respectively. After 10 days of storage at 4°C, the control had 4.2 log 10 CFU/g of TBC, while raspberries treated with 5, 15 and 30s of PL had the TBC of 2.5, 2.3 and 3.3 log 10 CFU/g, respectively which were significantly lower than that of control. PL 30s showed the highest (1.5 log 10 CFU/g reduction) of TBC at day 0. At the end of storage, although still significant lower than the control, TBC of PL 30s was significantly higher than PL treatment with shorter time (5 and 15s). This may be because the longer duration PL treatment affected the structure of raspberry fruits which benefits bacteria growth, which might be related with softer texture observed in PL 30 s treated raspberries.

Same inactivation efficacy of PL has been found on yeasts and molds (Fig. 3). The initial TYMC of raspberries was 3.4 log 10 CFU/g while after treatment, population of yeasts and molds for raspberries were 2.4, 2.0 and 1.8 log 10 CFU/g with 5, 15 and 30s of PL treatment, respectively. PL 30s provided 1.6 log 10 CFU/g reduction of TYMC in raspberries at day 0 which was comparable with Guner's (2012) result. In her research, blueberries were treated with 120s PL and 1.27 log 10 CFU/g reduction of total yeasts and molds has been observed. During our 10 days storage, TYMC of the control reached 4.2 log 10 CFU/g while treated raspberries with 5, 15 or 30s of PL had 2.5, 2.2 and 2.8 log 10 CFU/g, respectively. At day 10, PL treated raspberries maintained significantly lower TYMC than the control. There was no significant

difference between each PL treatment although raspberries treated with PL 30s had slightly but not significantly higher TYMC.

3.5 Scanning Electron Microscopy (SEM)

Fig. 4 A shows the *E. coli* O157:H7 cells on coverslips without PL treatment while Fig.4 B showed the *E. coli* cells treated by dry PL for 15s. Comparing A and B, there was a clear structural difference between untreated and treated *E. coli* O157:H7 cells on coverslips. The untreated *E. coli* O157:H7 cells were about 2.0 μm long and displayed a smooth and intact surface (Fig. 4A). Some treated cells lost their membrane integrity completely which led to flattening out of the cells from the edges. This may be because PL treatment suddenly increased the temperature of intracellular fluid which led to water vaporization in a relatively short time (Wekhof, 2000). This observation was comparable with Cheigh et al., (2012) who used transmission electron microscopy (TEM) analysis to observe the cell damage induced by intense pulsed light. Their results revealed the destruction of cellular structures such as the cell wall and cytoplasmic membrane. Various mechanisms have been proposed to explain the lethal effect of PL. It has been confirmed that the UV region is crucial to the efficiency of PL treatment (Takeshita et al. 2002). Photochemical mechanism has been widely accepted because the germicidal effect of UV light has been attributed primarily to a photochemical transformation of pyrimidine bases in the DNA of pathogens to form dimers. However, some other researchers proposed that the effect of PL may not wholly dependent on DNA damage and the lethal action of PL also can be due to photothermal effect (Wekhof, 2000). UV light absorption by bacteria can be attributed to overheating, intercellular water vaporization and then membrane disruption, which has been confirmed by our SEM findings. To contaminate fresh produce and transfer in the food producing chain, foodborne pathogens have to attach themselves on the plant surface to

survive or proliferate. Pathogens use wounds and natural openings like stomata, hydathodes, and lenticels as natural gateways for passage into internal plant tissues where they grow and begin to cause disease (Hirano and Upper, 2000; Melotto et al., 2006). The surface roughness may affect pathogen attachment (Gómez-López, 2012). The ability of *E. coli* O157:H7 attaching to raspberries has been proven under SEM (Fig. 4C and D). Fig. 4 C represented the untreated raspberries while Fig. 4D represented PL treated ones. During dip inoculation, bacteria showed specific attachment preference when bacterial cells had free access to the plants and can move freely. Raspberries are made up of many individual fruits (drupelets) held together by hairs (trichomes) and waxes (Mackenzie, 1979). As shown in Fig. 4 C, it seems that *E. coli* O157:H7 was preferred for the trichomes of the raspberries. Although to our knowledge, no research has been conducted to investigate how the surface structure of raspberries would affect *E. coli* O157:H7 attachment, Sirinutsomboon (2011) built the surface structures on silicon to mimic stomata, trichomes, and grooves between plant epidermal cells using photolithography as a microfabrication method to determine the effect of microstructure on bacterial attachment. The results showed that the trichome base had attachment levels 3-5 times as large as those of the more distant areas, which might help to explain the attachment preference for the raspberries trichomes in our study.

After PL treatment for 15s, *E. coli* O157:H7 cells on raspberries (Fig.4 D) showed different morphology with those on coverslips (Fig.4 B). *E. coli* O157:H7 cells on raspberries looked corrugated, and there were some dimples in these cells, but their average length remained unaltered. No complete membrane rupture has been observed as on the coverslips. The relatively minor damage to the *E. coli* O157:H7 cells may be because the complex microstructure of raspberry surface protected the bacteria from being inactivated by PL. Observed in a previous

study by Wang et al. (2009), an increase in fruit surface roughness would introduce protection to microbes entrapped on fruit surface resulting in reduced washing efficacy.

4. Conclusion

Our research demonstrated for the first time that successful PL processing of raspberries should be evaluated for its impacts on both safety and quality, because the most effective decontamination by PL treatment could lead to quality defects during the storage even these quality defects may not develop right after treatment. Right after treatment, *Salmonella* reduction by PL showed a fluence dependent manner with PL 30s providing highest log reduction of *Salmonella* (4.5 log 10 CFU/g). *E. coli* O157:H7 reduction, however, was not significantly affected by fluence. PL 30s provided slightly but not significantly higher *E. coli* reduction (3.9 log 10 CFU/g) on fresh raspberries. All the PL treatments maintained lower pathogen survival populations during 10 days storage at 4 °C compared with the control, and PL 30 s failed to maintain its inactivation advantage during the storage. Color and texture of raspberries treated by PL 30s also changed negatively in 10 days storage. PL 30s provided the lowest TBC and TYMC at day 0, but failed to maintain its advantage during storage. PL 30s did not have a negative impact on TPC and TAC. To consider both safety and quality of fresh raspberries as well as the treatment cost, 5 s PL treatment with fluence of 5.0 J/cm² was recommended. Under 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.

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Figure captions

Fig. 1 Inactivation of *Salmonella* and *E. coli* O157:H7 using PL treatment and pathogen survivor population during storage. Fresh raspberries were spot inoculated with *Salmonella* or *E. coli* O157:H7 and then treated using PL for 5, 15 or 30s. Fluence generated by the system was 5.0, 14.3 or 28.2 J/cm² for 5, 15 or 30 s PL treatments, respectively. Untreated raspberries served as control. Pathogen survival populations were evaluated at day 0, day 5 and day 10 during storage at 4 °C. Values marked by * were significantly different ($P < 0.05$) from their respective controls on that same day.

Fig. 2 Effects of PL on TBC of raspberries during storage. Raspberries without inoculation were used in total bacterial count (TBC) test. Fresh raspberries were treated with PL for 5, 15 or 30s. Fluence generated by the system was 5.0, 14.3 or 28.2 J/cm² for 5, 15 or 30 s PL treatments, respectively. TBC were evaluated were evaluated at day 0, day 5 and day 10 during storage at 4 °C.

Fig. 3 Effects of PL on TYMC of raspberries during storage. Raspberries without inoculation were used in total yeasts and molds count (TYMC) test. Fresh raspberries were treated with PL for 5, 15 or 30s. Fluence generated by the system was 5.0, 14.3 or 28.2 J/cm² for 5, 15 or 30 s PL treatments, respectively. TYMC were evaluated were evaluated at day 0, day 5 and day 10 during storage at 4 °C.

Fig. 4 SEM micrographs of *E. coli* O157:H7 on coverslips and raspberries. Coverslips represented the smooth surface. After inoculated with *E. coli* O157:H7, (A) showed the coverslips without PL treatment while (B) showed coverslips after 15s PL treatment. Fluence generated by the system was 14.3 J/cm² for 15 s PL treatment. Raspberries provided unique

produce surface structure. After inoculated with *E. coli* O157:H7, (C) showed the raspberries without PL treatment while (D) showed raspberries after 15s PL treatment.

Fig. 1 Inactivation of *Salmonella* and *E. coli* O157:H7 using PL treatment and pathogen survivor population during storage

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Fig. 2 Effect of PL on TBC of raspberries during storage.

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Fig. 3 Effect of PL on TYMC of raspberries during storage.

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Fig. 4 SEM micrographs of *E. coli* O157:H7 on coverslips and raspberries

Table 1 Effect of PL treatment on the color (L* and a*), texture, TPC and TAC of raspberries during 10 days storage at 4 °C.

	color (L*)	color (a*)	Texture (N)	TPC (mg GAE/100 g FW)	TAC (mg cyanidin-3-glucoside equivalents /100 g FW)
<i>Day 0</i>					
Control	32.3±1.2	22.8±2.1	2.6±0.8	39.1±6.0	42.6±2.8
PL 5s	32.2±0.3	20.9±1.2	1.9±0.5*	38.3±7.3	38.7±6.8
PL 15s	31.6±0.3	18.7±1.2*	2.4±0.6	43.0±3.9	44.8±3.0
PL 30s	32.2±1.2	21.6±1.1	2.5±0.8	32.1±5.9	48.6±2.7*
<i>Day 5</i>					
Control	31.5±0.6	20.6±2.0	2.5±0.5	33.1±6.9	35.3±1.5
PL 5s	30.9±0.2	20.1±1.3	2.0±0.5	30.5±5.3	33.7±4.9
PL 15s	31.2±2.1	21.4±2.1	2.1±0.2	27.3±3.7*	44.0±0.2*
PL 30s	30.6±1.3	22.0±2.3	1.5±0.4*	25.4±2.6*	42.4±4.4*
<i>Day 10</i>					
Control	27.1±0.4	21.3±0.6	1.9±0.1	29.6±8.4	24.6±4.0
PL 5s	26.6±1.5	20.6±0.9	1.8±0.1	31.3±1.1	27.0±2.4
PL 15s	30.2±0.6*	23.0±0.4*	2.2±0.3	27.5±2.1	30.0±2.4*
PL 30s	22.0±2.3*	25.4±0.8*	0.6±0.3*	25.5±3.7	25.6±4.2

Fluence generated by the system was 5.0, 14.3 or 28.2 J/cm² for 5, 15 or 30 s PL treatments, respectively. Data representing mean values ± standard deviation. For individual testing, Values marked by * were significantly different ($P < 0.05$) from their respective controls on the same day.