Salmonella and Escherichia coli O157:H7 Inactivation, Color, and Bioactive Compounds Enhancement on Raspberries during Frozen Storage after Decontamination Using New Formula Sanitizer Washing or Pulsed Light

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ABSTRACT

Berries are normally washed before they are frozen. Washing with sanitizer and treatment with pulsed light (PL) were studied for their effectiveness to inactivate foodborne pathogens on raspberries during frozen storage, while maintaining or enhancing major quality parameters. Raspberries were inoculated with Salmonella or Escherichia coli O157:H7 and then underwent a washing treatment with citric acid plus sodium dodecyl sulfate (CA+SDS) or citric acid plus thymol (CA+THY) or treatment with PL (dry PL, water-assisted [wet] PL, and PL-SDS). Pathogen survival was determined immediately after treatments and during frozen storage at –20°C for 3 months. Washing with CA+SDS or CA+THY significantly reduced Salmonella (by 3.6 and 3.2 log CFU/g, respectively) and E. coli O157:H7 (by 4.1 and 3.7 log CFU/g, respectively). At the end of storage, washing with CA+SDS reduced Salmonella to 0.6 log CFU/g and E. coli O157:H7 to 0.5 log CFU/g; washing with CA+THY reduced Salmonella to 0.9 log CFU/g and E. coli O157:H7 to 0.5 log CFU/g. PL-SDS showed decontamination efficacy on raspberries, with 0.7 log CFU/g Salmonella and 0.9 log CFU/g E. coli O157:H7 surviving at the end of storage; in comparison, in the control, 1.6 log CFU/g Salmonella and 1.5 log CFU/g E. coli O157:H7 survived. Pathogen survival in raspberries that had been washed or treated with PL-SDS was significantly lower than in untreated raspberries. Major quality parameters, including color, total phenolic content, total anthocyanin content, total bacterial count, and total yeast and mold counts, were evaluated on raspberries immediately after treatments and during frozen storage. Redness increased in PL-treated raspberries. At the end of storage, PL-treated raspberries had significantly higher total phenolic content and total anthocyanin content compared with control samples. Washing with sanitizers and treatment with PL decreased the total bacterial count and total yeast and mold counts on raspberries and maintained the low counts. Our findings suggest that washing with a sanitizer or treatment with PL could be used to process frozen raspberries for enhanced food safety and quality.

Key words: Decontamination; Frozen raspberries; Pulsed light; Quality; Sanitizer washing

Fresh and frozen common berries (i.e., blackberries, blueberries, raspberries, and strawberries) are popular and healthy foods. When berries are picked for fresh consumption, they are either placed directly in retail containers in the field or are packed in a packinghouse without washing because they are highly perishable. However, berries are usually washed before freezing, and they are not usually blanched or heat treated unless they are used in preserves or other processed products (15). Concerns regarding the microbial safety of berries and berry products have been increasing in recent years due to the implication of these small fruits in multiple foodborne outbreaks (2, 3, 9–11, 13, 21, 23, 31). A foodborne outbreak of Escherichia coli O157:H7 in Oregon in July and August 2011, which was traced to contaminated fresh strawberries, led to 15 illnesses, six hospitalizations, and four cases of hemolytic uremic syndrome; two of those with hemolytic uremic syndrome died (16).

During 1983 to 2013, there were 11 outbreaks linked to raspberries; of a total 4,637 cases, 7 were associated with frozen raspberries (24). In the 1990s, one outbreak was caused by calicivirus (25) and two by hepatitis A (27, 28). Most recently, there were four outbreaks caused by norovirus (4, 7, 12, 22). For the two hepatitis A outbreaks, pickers were believed to be the source of the contaminations, whereas for the norovirus outbreaks, the source of contamination was unknown. Foodborne pathogens, including bacteria such as Salmonella and E. coli O157:H7 and viruses such as hepatitis A and norovirus, all share the fecal-oral contamination route, which indicates that Salmonella and E. coli O157:H7 may possibly cause an outbreak on frozen raspberries. In a previous study, Knudsen et al. (15) spot inoculated sliced strawberries with E. coli O157:H7
(log 7.0 CFU per sample). After 30 days of frozen storage, the population of *E. coli* O157:H7 on frozen sliced strawberries had declined only by 2.2 log when 20% sucrose was not added, and by 0.7 log when 20% sucrose was added.

Unlike fresh raspberries, which are not washed in postharvest processing, frozen berries are washed before they are frozen. The washing process adds a glaze to the berries during freezing and could enhance product safety. Multiple studies have evaluated non–chlorine-based chemicals, including organic acids (17), surfactants (26), essential oils (18), and hydrogen peroxide (19, 38), for their antimicrobial efficacy in the decontamination of berries and tomatoes. Based on our previous study (37), washing with new sanitizer formulas, such as 2 mg/ml citric acid plus 4% sodium dodecyl sulfate (CA+SDS) and 2 mg/ml CA plus 0.2 mg/ml thymol (CA+THY), achieved a higher log reduction (4.5 to 5.3 log CFU/g) of *Salmonella* than washing with 200 ppm of chlorine (3.2 log CFU/g) on spot-inoculated green onions. In another study of ours (35), a novel application of pulsed light and SDS (PL-SDS) could potentially be used for decontamination of *E. coli* O157:H7 on green onions. The combination of PL and 100 ppm of SDS reduced the effective treatments were evaluated in this study for decontamination of raspberries used for frozen processing, immediately after treatments and during storage at −20°C for 3 months. Also important was to investigate whether these decontamination treatments affected the quality of the frozen berries. It remains critical that frozen berries are safe while maintaining their nutritional value and functional phytochemical properties. Major quality parameters of raspberries, including color, total phenolic content (TPC), total anthocyanin content (TAC), total bacterial count (TBC), and total yeast and mold counts (TYMC), were evaluated immediately after treatments and during storage.

**MATERIALS AND METHODS**

**Bacterial strain and inoculum preparation.** Single wild-type strains, including *Salmonella* Newport H1275 and *E. coli* O157:H7 strain 250 (both sprout outbreak isolates), were used in our study. Wild strains were obtained from the culture collection in the Department of Animal and Food Sciences at the University of Delaware (MD). The *Salmonella* strain was adapted to grow in the presence of nalidixic acid alone (100 µg/ml; Fisher Scientific, Hampton, NH) to create a single antibiotic-resistant strain, whereas 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) to create a double antibiotic-resistant strain. Both resistant strains were grown on tryptic soy agar (TSA; Difco, BD, Sparks, MD) plus 0.6% yeast extract (YE; Difco, BD) supplemented only with nalidixic acid ([TSAYE-N] for *Salmonella*) or with nalidixic acid and streptomycin ([TSAYE-NS] for *E. coli* O157:H7) for 2 to 3 days at 35°C. Single colonies were picked and transferred to 10 ml of tryptic soy broth (TSB; Difco, BD) plus 0.6% YE (Fisher) supplemented with the 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 a second transfer was made to 10 ml of fresh TSBYE-N or TSBYE-NS to yield an approximate population of 10⁹ CFU/ml after a 24-h incubation at 35°C. The culture was diluted to 10⁶ CFU/ml using sterile 0.1% peptone water (Difco) and was used as inoculum.

**Preparation and inoculation of raspberries.** Fresh raspberries were purchased from a local supermarket and were stored at 4 ± 2°C for a maximum of 4 h before use. Medium-size (~5 g) raspberries with pink color and firm texture were selected. The selected raspberries were intact and had no noticeable physical injury. To spot inoculate raspberries, five droplets (50 µl) of 10⁸ CFU/ml of inoculum was deposited on the outside surface of raspberries. Spot inoculation was applied to provide a controlled pathogen level. Inoculated raspberries were dried in the biosafety hood at room temperature for 2 h before use.

**Inactivation of *Salmonella* and *E. coli* O157:H7 using sanitizer washing.** Based on our previous study (37), raspberries that had been spot inoculated with *Salmonella* and *E. coli* O157:H7 were treated with the sanitizer combinations CA+SDS and CA+THY. Distilled water was also used for comparison. One raspberry (5 g) was submerged in 100 ml of washing solution in a beaker with a stirring bar (0.37 by 1 in. [0.9 by 2.5 cm]); the beaker was placed over an ultrathin magnetic stirrer (~700 rpm; Lab Disc, Fisher Scientific) with continuous agitation for 1 min at room temperature.

**Inactivation of *Salmonella* and *E. coli* O157:H7 using PL treatment.** Based on our previous studies (35, 37), three PL treatments were used: dry PL (15 s), water-assisted (wet) PL (60 s), and PL-SDS (100 ppm) combinations. PL was generated by a bench-top pulse light system (SteriPulse-XL, model RS-3000C, Xenon Corp., Wilmington, MA). The 16-in. (40.6-cm) linear clear fused quartz PL lamp (LH840) delivered 505 J per pulse (1.27 J/cm²) energy with three pulses per s. For dry PL treatment, raspberries were placed in a PL chamber in sterile Petri dishes without covers, with the inoculated side facing the lamp. Two berries were treated at a time. The treatment time (15 s) was chosen according to our previous study (35). Fluence for the system was 14.3 J/cm².

For wet PL and PL-SDS, two raspberries were placed in a beaker with 200 ml of distilled water or 100 ppm of SDS and a stir bar. An ultrathin magnetic stirrer (0.37 by 1 in. [0.9 by 2.5 cm], ~700 rpm; Lab Disc, Fisher Scientific) was placed under the PL chamber to create turbulent flow inside the beaker so that raspberries could rotate freely. PL treatment was conducted in the same manner as described above.

**Freezing of the raspberries.** Treated and untreated raspberries (control) were then placed separately on a tray and frozen at −20 ± 1°C. After freezing for 3 h, raspberries were bagged in zip-lock bags and stored in the freezer until they were tested. Samples were analyzed at months 0 (immediately after freezing), 1, 2, and 3.

**Microbial analyses.** Raspberries were microbiologically analyzed at months 0, 1, 2, and 3. They were transferred into sterile stomacher bags with Dey-Engley neutralized broth (Difco, BD) (1.9, wt/vol) where they were homogenized (400 Circulator, Seward Co., West Sussex, UK) at 260 rpm for 1 min to help release and evenly distribute pathogens. The homogenate was serially diluted using 0.1% peptone water and was plated on TSAYE-N (*Salmonella*) or TSAYE-NS (*E. coli* O157:H7) plates using a sterile spreader. Colonies were enumerated after incubation for 48 hours at 35°C.
Pathogen survivor population was reported as log CFU per gram.

Effect of sanitizer washing and PL on color of raspberries. Raspberries without artificial inoculation were either washed with a sanitizer, were treated with PL as described above, or were used as a control and then stored in a freezer for 3 months. The raspberries’ color was analyzed before thawing at months 0, 1, 2, and 3. Color was tested by using Chroma Meter (Minolta CR-400, Minolta, Osaka, Japan). Color parameters were quantified in the Hunter L* (lightness-darkness), a* (redness-greenness), and b* (yellowness-blueness).

Effect of sanitizer washing and PL on TPC and TAC of raspberries. TPC of uninoculated raspberries with or without PL treatment was determined as described by Xu et al. (36), with slight modification. Raspberries were homogenized in stomacher bags with 25 ml of ethanol (1:5, wt/vol). The bags were heat sealed and allowed to sit for 30 min, with gentle massaging to extract the polyphenol compounds. Extractions were centrifuged at 4,000 rpm for 10 min (Microfuge, Beckman Coulter, Inc., Indianapolis, IN), and supernatants were collected. Thirty microliters of supernatants for 10 min (Microfuge, Beckman Coulter, Inc., Indianapolis, IN), and was incubated for 30 min at 35 °C, where they were homogenized (260 rpm for 1 min). The homogenate was plated on either TSA for TBC enumeration or on potato dextrose agar plates were incubated at 25 °C, whereas potato dextrose agar plates were incubated at 25°C for 3 to 5 days (28).

Statistical analyses. All experiments were replicated three times, and results were reported as mean ± SD. JMP software (SAS Institute, Cary, NC) was used for statistical analyses. Salmonella and E. coli O157:H7 survival was reported as log of the survival population (log CFU/g). For survival population of pathogens and quality of raspberries (including color, TBC, TMC, TPC, and TAC) during storage, Dunnett’s test was used to determine differences between treated and untreated (control) samples in months 0, 1, 2, and 3. Student’s t test was used to determine the difference between each of the treated samples and the same treatment on different days. Significant difference was reported when P < 0.05.

RESULTS AND DISCUSSION

Inactivation of Salmonella and E. coli O157:H7 using sanitizer washing during frozen storage. Figure 1 shows the survival populations of Salmonella and E. coli O157:H7 during 3 months of frozen storage after raspberries.
FIGURE 2. Inactivation of Salmonella and E. coli O157:H7 using PL and pathogen survivor population during storage. Fresh raspberries were spot inoculated with Salmonella or E. coli O157:H7 and then were treated using dry PL for 15 s, wet PL for 60 s, or PL-SDS combination for 60 s. Untreated raspberries served as control. Pathogen survival populations were evaluated at months 0, 1, 2, and 3 during storage at −20°C. *, values were significantly different (P < 0.05) from their respective controls at that same time point.

were washed using sanitizer combinations. The initial populations of Salmonella and E. coli O157:H7 on the raspberries were 4.9 and 5.1 log CFU/g, respectively. Washing the raspberries with sanitizer combinations CA+SDS or CA+THY reduced the Salmonella populations to 1.3 and 1.7 log 10 CFU/g, respectively, and reduced the E. coli O157:H7 populations to 1.0 and 1.4 log CFU/g, respectively. All reductions of the two pathogens were significant compared with the untreated control, whereas no significant differences were detected between the two sanitizer combinations.

During 3 months of frozen storage, the Salmonella and E. coli O157:H7 populations (4.9 and 5.1 log CFU/g, respectively) in the untreated raspberries decreased to 1.6 and 1.5 log CFU/g, respectively. There were significant decreases during the first 2 months, whereas in the last month, no significant change as observed. After 3 months, raspberries treated with CA+SDS had survival populations of 0.6 (Salmonella) or 0.5 log CFU/g (E. coli O157:H7). Raspberries treated with CA+THY had survival populations of 0.9 (Salmonella) or 0.5 log CFU/g (E. coli O157:H7). The control groups (untreated raspberries) had survival populations of 1.6 (Salmonella) or 1.5 log CFU/g (E. coli O157:H7). Salmonella and E. coli O157:H7 were shown to survive under frozen conditions (15).

At the end of 3 months, the populations of Salmonella and E. coli O157:H7 in washed raspberries were significantly lower than in the untreated raspberries (P < 0.05), which indicated that sanitizer washing prior to freezing enhanced the pathogenic microbial inactivation in the frozen berries. Although the exact mechanism for the enhancement remained unclear, it is possible that sanitizers caused sublethal damage, followed by inactivation during the freezing process.

Inactivation of Salmonella and E. coli O157:H7 using PL during frozen storage. Unlike fresh raspberries, which are usually unwashed, frozen raspberries require washing before freezing. For this washing, all three PL treatments, dry PL, wet PL, and PL-surfactant combinations, have potential applications in the frozen raspberry industry. As shown in Figure 2, after 15 s of dry PL treatment, the Salmonella and E. coli O157:H7 populations decreased to 1.5 and 1.8 log CFU/g, respectively. Wet PL (60 s) had lower inactivation efficacy compared to dry PL treatment and only decreased the populations of Salmonella and E. coli O157:H7 to 3.0 and 3.3 log CFU/g, respectively. However, PL combined with surfactant (SDS) solution, lowered pathogen survival populations to 1.7 and 2.0 log CFU/g, respectively. Comparing the inactivation efficacy of the three PL treatments, dry PL (15 s) > PL-SDS > wet PL (60 s); and significant differences were found among treatments for both Salmonella and E. coli O157:H7. Significantly higher populations were seen in the control: Salmonella (4.9 log CFU/g) and E. coli O157:H7 (5.1 log CFU/g). This is in good agreement with our previous studies on green onions (35, 37).

During storage at −20°C for 3 months, further decreases in the survival populations of Salmonella and E. coli O157:H7 were observed on all treated raspberries; decreases were also seen on the control, with 1.6 and 1.5 log CFU/g for Salmonella and E. coli O157:H7, respectively. Salmonella and E. coli O157:H7 populations on raspberries treated with dry PL decreased to 1.2 and 1.5 log CFU/g, respectively; this was not a significant drop compared with the pathogen population immediately after the dry PL treatment. It is possible that treatment with dry PL was more severe than treatment with wet PL; dry PL may have caused direct death of the pathogens so that no further decrease of the population was observed during frozen storage. Raspberries treated by wet PL and PL-SDS showed significant decreases of both Salmonella and E. coli O157:H7 after 3 months, although there were no significant differences between months. At the end of the frozen storage, the Salmonella population decreased to 1.8 and 0.7 log CFU/g for wet PL and the PL-SDS combination, respectively. The survival population of E. coli O157:H7 decreased to 1.8 and 0.9 log CFU/g for wet PL and PL-SDS, respectively. Compared with the control, raspberries treated with PL-SDS had significantly lower pathogen populations at the end of 3 months of frozen storage (P < 0.05); these populations were also significantly lower than those
TABLE 1. Effect of sanitizer washing and PL treatment on the color (L* and a*), TPC, and TAC of raspberries during 3 months of storage at −20°C

<table>
<thead>
<tr>
<th>Month</th>
<th>L*</th>
<th>a*</th>
<th>TPC (mg of GAE/100 g of FW)</th>
<th>TAC (mg of cyanidin-3-glucoside equivalents/100 g of FW)</th>
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<tr>
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<tr>
<td>Month 0</td>
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<tr>
<td>Control</td>
<td>32.3 ± 1.2</td>
<td>22.8 ± 2.1</td>
<td>38.7 ± 5.0</td>
<td>31.1 ± 0.2</td>
</tr>
<tr>
<td>Water</td>
<td>32.2 ± 0.3</td>
<td>20.9 ± 1.2</td>
<td>36.4 ± 3.8</td>
<td>32.4 ± 1.0</td>
</tr>
<tr>
<td>CA+THY</td>
<td>31.6 ± 0.3</td>
<td>22.1 ± 0.8</td>
<td>37.3 ± 1.8</td>
<td>33.5 ± 2.2</td>
</tr>
<tr>
<td>CA+SDS</td>
<td>32.2 ± 0.9</td>
<td>21.6 ± 0.2</td>
<td>35.4 ± 2.0</td>
<td>33.0 ± 5.5</td>
</tr>
<tr>
<td>Dry PL (15 s)</td>
<td>31.8 ± 0.8</td>
<td>22.3 ± 1.1</td>
<td>42.6 ± 5.1</td>
<td>34.0 ± 0.1</td>
</tr>
<tr>
<td>Wet PL (60 s)</td>
<td>32.0 ± 1.2</td>
<td>21.9 ± 0.4</td>
<td>41.5 ± 5.0</td>
<td>33.6 ± 1.0</td>
</tr>
<tr>
<td>PL+SDS</td>
<td>30.9 ± 1.3</td>
<td>19.8 ± 0.6</td>
<td>39.4 ± 4.0</td>
<td>35.6 ± 1.9</td>
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<tr>
<td>Month 1</td>
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</tr>
<tr>
<td>Control</td>
<td>32.8 ± 1.6</td>
<td>22.8 ± 1.3</td>
<td>37.8 ± 2.5</td>
<td>27.8 ± 2.8</td>
</tr>
<tr>
<td>Water</td>
<td>31.3 ± 0.8</td>
<td>22.9 ± 0.8</td>
<td>39.7 ± 3.4</td>
<td>30.1 ± 6.7</td>
</tr>
<tr>
<td>CA+THY</td>
<td>31.3 ± 1.1</td>
<td>24.3 ± 1.2</td>
<td>38.4 ± 1.0</td>
<td>29.4 ± 2.4</td>
</tr>
<tr>
<td>CA+SDS</td>
<td>30.8 ± 0.9</td>
<td>22.8 ± 0.9</td>
<td>36.3 ± 4.2</td>
<td>30.9 ± 3.7</td>
</tr>
<tr>
<td>Dry PL (15 s)</td>
<td>31.8 ± 0.6</td>
<td>25.6 ± 0.7**</td>
<td>37.1 ± 3.5</td>
<td>33.0 ± 1.5**</td>
</tr>
<tr>
<td>Wet PL (60 s)</td>
<td>32.2 ± 0.7</td>
<td>22.9 ± 0.6</td>
<td>38.8 ± 5.7</td>
<td>35.0 ± 2.6**</td>
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<td>PL+SDS</td>
<td>30.5 ± 0.5</td>
<td>23.3 ± 0.0</td>
<td>40.5 ± 0.9</td>
<td>34.7 ± 3.8**</td>
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<td>Month 2</td>
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<tr>
<td>Control</td>
<td>33.0 ± 1.4</td>
<td>23.5 ± 1.6</td>
<td>34.1 ± 1.3</td>
<td>25.0 ± 3.5</td>
</tr>
<tr>
<td>Water</td>
<td>30.9 ± 0.9</td>
<td>24.0 ± 0.0</td>
<td>34.4 ± 0.7</td>
<td>27.0 ± 1.8</td>
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<tr>
<td>CA+THY</td>
<td>31.7 ± 0.6</td>
<td>26.6 ± 0.3***</td>
<td>34.9 ± 2.2</td>
<td>29.2 ± 1.3</td>
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<tr>
<td>CA+SDS</td>
<td>30.8 ± 0.6</td>
<td>24.2 ± 0.5</td>
<td>35.9 ± 1.5</td>
<td>28.5 ± 3.4</td>
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<td>26.3 ± 0.5</td>
<td>38.6 ± 1.0</td>
<td>33.5 ± 1.6**</td>
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<td>Wet PL (60 s)</td>
<td>31.4 ± 0.0</td>
<td>25.0 ± 0.4</td>
<td>37.3 ± 4.4</td>
<td>31.8 ± 2.1**</td>
</tr>
<tr>
<td>PL+SDS</td>
<td>31.2 ± 0.8</td>
<td>27.9 ± 1.1**</td>
<td>36.5 ± 1.3</td>
<td>35.2 ± 4.2**</td>
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<td>Month 3</td>
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</tr>
<tr>
<td>Control</td>
<td>33.5 ± 1.6</td>
<td>24.9 ± 1.1</td>
<td>33.8 ± 0.8</td>
<td>21.4 ± 1.6</td>
</tr>
<tr>
<td>Water</td>
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<td>34.7 ± 1.6</td>
<td>31.4 ± 4.3**</td>
</tr>
</tbody>
</table>

* Data represent mean values ± standard deviations.

** Values were significantly different (P < 0.05) from their respective controls on that same day.

recovered in raspberries treated by dry PL or wet PL. PL-SDS might cause more injury in the bacterial cells, leading to fewer survivors during frozen storage. In the PL-SDS system, raspberries were submerged in agitated SDS aqueous solution during PL treatment, which could provide two major benefits. (i) The presence of water substantially reduced heating of the sample, thus preserving its sensory quality, as demonstrated in the next section. A lower temperature could also better preserve nutrients in food because some bioactive compounds such as anthocyanin and vitamins may be degraded at high temperature. (ii) The agitated SDS solution allowed produce to move and rotate freely, so that all surfaces of the berries had uniform PL exposure; also, the SDS reduced water surface tension, allowing more bacterial cells to be released into water, where they were injured or killed by PL. The injured cells were subsequently inactivated by frozen processing.

Effect of sanitizer washing and PL on color, TPC, and TAC of raspberries during frozen storage. Three color parameters were quantified: L* (brightness-darkness), a* (redness-greenness), and b* (yellowness-blueness). However, only L* and a* values were reported in this study because the unattractive color changes of the raspberries were mainly from light pink to dark red. As shown in Table 1, there was no significant color change immediately after the treatments. L* values ranged from 30.9 to 32.3 and a* values from 19.8 to 22.8.

During storage, there was no significant change in the brightness (L*) of untreated and treated raspberries. The redness of the untreated raspberries increased slightly from 22.8 to 24.9 after 3 months, which, however, was not a significant change. Raspberries washed by sanitizer combinations also increased slightly in redness, whereas raspberries treated by PL had a significantly higher a* value (redness). At month 3, a* values for dry PL, wet PL, and PL-
SDS combinations were 27.9, 28.2, and 28.8, respectively. The results indicated that neither sanitizer washing nor PL treatments had a significantly negative impact on the color of raspberries. PL treatment increased the redness of raspberries without causing darkening; this was not considered an unattractive outcome in this study. PL has been applied to improve the color of figs; Rodov et al. (29) exposed poorly colored figs to PL for just 10 to 90 s and enhanced their red coloration in a dose-dependent manner.

Ripe raspberries have a high antioxidant capacity and TPC compared with most other berries (8). In the present study, the TPC of untreated raspberries (Table 1) did not change significantly during 3 months of storage (38.7 to 33.8 mg of GAE/100 g of FW). Similar results have been reported by de Ancos et al. (5), who investigated the ellagic acid, vitamin C, TPC, and radical scavenging capacity in frozen raspberry fruits. Their results showed no significant change of TPC at the end of long-term frozen storage (12 months). In our study, for the raspberries treated with sanitizer combinations or PL treatments, there was no significant difference between control and treated berries at month 0, ranging from 35.4 to 42.6 mg of GAE/100 g of FW. Similar results have been reported by Luksiene et al. (20), who evaluated the impact of high-power PL on microbial control and nutritional properties of strawberries. Their findings showed no significantly different TPC between raspberries before and immediately after PL treatment.

Our studies, however, showed that, after 3 months, raspberries treated by dry PL (15 s) (40.5 mg of GAE/100 g of FW) and wet PL (60 s) (38.0 mg of GAE/100 g of FW) showed significantly higher TPC than the control (33.8 mg of GAE/100 g of FW). The dynamic changes of TPC in PL-treated raspberries indicate that PL might have potential benefits to increase bioactive phytochemicals in the small fruits.

Anthocyanins belong to flavonoid groups and are responsible for the attractive colors, ranging from red to blue, of flowers and fruits. As shown in Table 1, there was no significant change of the TAC right after decontamination treatments. TAC of the untreated berries was 38.7 mg of cyanidin-3-glucoside equivalents per 100 g of FW, whereas TAC of the treated raspberries ranged from 35.4 to 42.6 mg of cyanidin-3-glucoside equivalents per 100 g of FW. Neither sanitizer washing nor PL treatments affected the TAC of raspberries significantly. During 3 months of frozen storage, however, the TAC decreased in all samples, including the control. TAC of the control decreased from 31.1 to 21.4 mg of cyanidin-3-glucoside equivalents per 100 g of FW. TAC of raspberries washed with sanitizer also decreased from 33.5 (CA+THY) or 33.0 (CA+SDS) to 23.6 (CA+THY) or 24.8 (CA+SDS), and there was no significant difference among the control raspberries and those washed with sanitizer at the end of 3 months.

However, at the end of storage, TAC of dry PL (15 s) (30.4 mg of cyanidin-3-glucoside equivalents per 100 g of FW), wet PL (60 s) (29.7 mg of cyanidin-3-glucoside equivalents per 100 g of FW), and PL-SDS (31.4 mg of cyanidin-3-glucoside equivalents per 100 g of FW) were significantly higher than that of the control (21.4 mg of cyanidin-3-glucoside equivalents per 100 g of FW). This suggested that PL was able to enhance TAC content during storage. The increased TAC in PL-treated raspberries might contribute to their increased redness at month 3. In addition to the effect of decontamination processing, anthocyanin stability during freezing and frozen storage also depends on the season of harvest. Also, the anthocyanin profile has an effect because compounds show different degradation rates. Cyanidin-3-glucoside was found to be the most easily affected by the degenerative reactions that take place during processing and frozen storage (6). Detailed research on TAC profile is ongoing in our lab to study the impacts of PL on TAC content during storage.

The influence of decontamination methods on the quality of produce varies depending on the type of treatment, contact time, types of produce, etc. For example, strawberries and raspberries showed great sensitivity to anthocyanin bleaching at bactericidal peroxide levels (30). In addition, quality defects may not occur immediately after treatment, but they could develop during the product’s shelf life (1). Our current findings demonstrate that, immediately after treatments and at the end of storage, washing with our sanitizer combinations or treatment with PL were able to inactivate foodborne pathogens in raspberries while maintaining or enhancing major quality parameters during frozen storage.
Interestingly, enhanced TPC or TAC were observed in PL-treated raspberries in our study. In previous studies, some nutrients or phytochemicals were increased in PL-treated produce, such as vitamin D in mushroom (14, 33), TAC in figs (29), and total lycopene and carotene in tomatoes (1). More research is needed to understand the interaction of PL with phytochemical changes during frozen storage.

Effect of sanitizer washing and PL on TBC and TYMC of raspberries during frozen storage. The initial TBC and TYMC on raspberries were about 1.8 and 2.4 log CFU/g, respectively (Fig. 3). Raspberries washed with sanitizer or treated with PL had significantly lower TBC and TYMC compared with untreated raspberries. Immediately after decontamination treatments, TBC was reduced by 0.6 to 1.1 log CFU/g, and TYMC was reduced by 1.0 to 1.4 log CFU/g. Further decreases in TBC and TYMC were seen in all raspberries (including control) after frozen storage for 1 month; however, all treated raspberries had significantly lower TBC and TYMC values. At the end of frozen storage, the TBC and TYMC of untreated raspberries decreased to 0.6 and 0.8 log CFU/g, respectively, values similar to those in treated raspberries. The results indicate that frozen storage itself had the ability to lower the population of natural microflora (TBC and TYMC). Because lethal effects are difficult to quantify for filamentous molds, the effects of freezing on vegetative fungi have been investigated mostly with yeasts. As with bacteria, the susceptibility of vegetative cells to freezing damage varies widely with the growth phase of the cells, the conditions under which they are cultivated, and exposure to other stresses before freezing (32). In our study, washing with sanitizer and PL treatment had the ability to lower the natural microorganism population and maintain the low population during frozen storage, perhaps because of the stress generated by the decontamination treatment.

As a potential commercial application of PL, PL lamps could be installed on top of a tunnel structure, and berries could be soaked in shallow water or sanitizer solutions as they move through the tunnel. The exposure time would be the time it takes for berries to move through the whole tunnel. Future research is still needed to scale up the current PL system and to understand whether lower concentrations of CA or SDS are more effective on raspberries. Lower water-to-product ratios should be studied to simulate some commercial practices.

Sanitizer combinations (CA+THY and CA+SDS) as well as PL could potentially be used for decontamination of Salmonella and E. coli O157:H7 on raspberries before frozen storage. Both sanitizer washes resulted in more than 3-log reductions on Salmonella and approximately 4-log reductions on E. coli O157:H7. Dry PL, wet PL, and PL-SDS all showed promising decontamination efficacy on raspberries. All treated raspberries maintained the low pathogen populations during 3 months of storage at −20°C. Although the process of freezing itself reduced foodborne pathogens, populations of Salmonella and E. coli O157:H7 were significantly lower in raspberries that had been washed or treated with PL-SDS than in untreated raspberries at the end of 3 months of frozen storage (P < 0.05). This indicated that washing with sanitizer or treatment with PL-SDS prior to freezing enhanced pathogenic microbial inactivation in frozen berries. The brightness of raspberries was not affected by decontamination treatments. Increased redness, however, has been observed in PL-treated raspberries, but this was not considered unattractive. No negative impact was observed on TPC and TAC after the treatments or during the 3 months of storage. At the end of storage, PL-treated raspberries had higher TPC and TAC compared with control samples. Washing with sanitizer and treatment with PL decreased the TBC and TYMC on raspberries and maintained the low natural microorganism population during storage. Our findings suggest that sanitizer combinations and PL could be used to process raspberries before frozen storage to enhance their safety and quality. PL could be very attractive for commercial use because of its short processing time (<1 min) and its potential enhancement of nutrition and microbial safety.

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