Effects of Pulsed Electric Field Processing on Microbial Survival, Quality Change and Nutritional Characteristics of Blueberries

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Abstract

Whole fresh blueberries were treated using a parallel pulsed electric field (PEF) treatment chamber and a sanitizer solution (60 ppm peracetic acid [PAA]) as PEF treatment medium with square wave bipolar pulses at 2 kV/cm electric field strength, 1 μ s pulse width, and 100 pulses per second for 2, 4, and 6 min. The effects of PEF on native microbiota and artificially-inoculated *Escherichia coli* K12 and *Listeria innocua* populations on blueberries were determined. Color, texture, anthocyanins and total phenolic compound concentrations were also evaluated. The combination of PEF and PAA was able to achieve up to 3 log reduction of *E. coli* and *Listeria* as well as 2 log/g reduction of native microbiota. PEF treatments did not cause any changes in color and appearance of the blueberries. The treatments did, however, cause the blueberries to soften in texture. Anthocyanins and phenolic compounds in blueberries increased by 10 and 25%, respectively, after PEF treatments. The results demonstrate the potential of PEF applications to enhance the safety and improve the quality and nutritional value of fruits and their derived products.

Key words: pulsed electric fields, blueberries, microbial, quality, nutrition, sanitizer

Chemical compounds studied in this article

Peroxyacetic acid (PubChem CID: 195769); Hydrogen peroxide (PubChem CID: 784); Sodium chloride (PubChem CID: 5234)

1 Introduction

Small fruits such as raspberries and blueberries are high value commodities for their well-reported health benefits. In terms of U.S. fruit consumption, blueberries rank only second to strawberries in popularity of berries. Blueberries are not only popular, but also repeatedly ranked in the U.S. diet as having one of the highest antioxidant capacities among all fruits, vegetables, spices and seasonings. Antioxidants are essential to optimizing health by helping to combat the free radicals that can damage cellular structures as well as DNA (Whfoods, 2016).

Berry fruits are usually grown in open fields and are constantly exposed to sources of potential pre-harvest contamination such as soils, insects, birds, and irrigation water as well as human contact during harvesting. Therefore, they are susceptible to contamination caused by pathogenic bacteria. In recent years, an increasing number of foodborne illness outbreaks has been associated with small fruits such as raspberries and blueberries (Calder et al., 2003; Luna & Griffin, 2010; Miller et al., 2013; Sarvikivi et al., 2012). Two outbreaks and 52 illnesses were related to the consumption of blueberries in 2014 (CDC, 2015). In addition, the warm temperature during the harvest season prompts fruit spoilage. Wu and Kim (2007) observed that visible signs of decay can develop on warm, wet blueberries in less than 12 h. Therefore, proper intervention controls are needed and should be applied as soon as the fruits are harvested. The intervention processes currently being used may not be sufficient for controlling the biological hazards in these foods, creating urgency for the development of new intervention technologies.

Fruit washing with sanitizers has been used by farms, processors, and distributors to reduce initial microbial loads, but the effectiveness has been limited. Chlorinated water sprays (100 ppm) have demonstrated reductions of less than 1 log CFU/g against APC on the surface of low bush blueberries, when compared to unwashed berries (Crowe et al., 2005). Alexandre et al.

(2012) reduced total aerobic mesophilic bacteria on strawberries by 1.5 and 2.2 log CFU with 1% and 5% hydrogen peroxide, respectively. Hung et al. (2010) reduced *E. coli* O157:H7 populations artificially inoculated onto strawberries by 1.17 log CFU/g when treated with electrolyzed oxidizing water at 4°C for 1 minute, and by 1.58 log CFU/g when the treatment time was increased to 5 minutes. When ultrasonication was added, *E. coli* O157:H7 populations on strawberries were further reduced by 1.49 and 1.89 log CFU/g, respectively. The efficacy of electrostatic sprays of electrolyzed oxidizing water, UV light, ozone, and a combination of ozone and UV light in inactivating *E. coli* O157:H7 artificially inoculated onto blueberries was studied and a synergistic effect for the combination was observed (Kim & Hung, 2012). Tsunami® 100 is an Environmental Protection Agency (EPA) registered antimicrobial water additive for pathogen reduction in fruit and vegetable processing water, composed of acetic acid, peracetic acid and hydrogen peroxide. Tsunami 100 is recommended for use in the process waters of post harvest, fresh cut, and processed fruits and vegetables in both batch and continuous operations. ~~The Environmental Protection Agency~~ (EPA (1998) authorizes the use of peroxyacetic acid-based additives for fruits and vegetables and washed water up to 80 ppm. Alvaro et al. (2009) used the peracetic acid sanitizer (Tsunami®100, Ecolab) for sweet pepper, tomato and cucumber (500 mg/L for 2 min) and stated that the perorganic acid compound is better for washing fruit and improving postharvest shelf life as it is environmentally friendly, breaking down into acetic acid and oxygen after use, does not have any adverse effects on human health, and does not affect the taste characteristics of fruits and vegetables.

PEF treatments have been studied for killing foodborne pathogenic microorganisms, such as *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* as well as spoilage bacteria, molds and yeasts (Guo et al., 2014; Gurtler et al., 2011, 2010; Jin et al., 2015, 2014, 2009a, 2009b, Min

et al., 2003; Reina et al., 1998). PEF treatments have exhibited the potential of maintaining the physico-chemical quality of liquid food products, without substantially impacting the sensory properties and health-related compound makeup (Guo et al., 2014; Jin & Zhang, 1999). Most of these studies have been focused on liquid foods, such as juices and beverages. There is an increasing interest in PEF pretreatment of fresh produce products, such as potato and apple (Angersbach et al., 2000; Bazhal et al., 2001; Lebovka et al., 2004). However, there is no information available for inactivation of microbial contaminants on fresh whole fruits, particularly combined with the use of a sanitizing solution as a PEF treatment medium, and their impact on microbial reduction, product quality and nutritional value of whole berry fruits. Fresh berries are, traditionally, a seasonal food, and their consumption is highest during the summer months. Frozen berries, berry jams, juices/nectars, and dehydrated berries are all typically available at other times of the year.

The central idea behind this current study was to evaluate PEF processing for its potential use as a pretreatment that would extend the shelf-life, enhance the safety and improve nutritional and other characteristics of whole fresh fruits and their derived constituent products (i.e., juice, puree, jam, pastes, raisin and other dehydrated products). The specific objectives of this first study were to: 1) investigate the antimicrobial efficacy of PEF treatment in combination with sanitizing solution for blueberries; 2) evaluate the effects of these treatments on the quality and nutritional attributes of fresh fruits, and 3) assess the potential of these treatments to manufacture derived products.

2 Materials and Methods

2.1. Blueberry preparation

Fresh blueberries were obtained from local supermarkets, stored in a refrigerator at 4°C until the time of the experiment and used within *ca.* 1 week of purchasing. Blueberries with a diameter of about 12-14 mm and average weight of 2 g/piece were selected for the experiments.

2.2. Inoculation of blueberries

E. coli K12 and *Listeria innocua* were used as surrogates of pathogenic *E. coli* O157:H7 and *Listeria monocytogenes*, respectively. *E. coli* K12 (ATCC 23716) and *Listeria innocua* (ATCC 33090) were obtained from the culture collection of the U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center (Wyndmoor, PA, USA). Frozen stock cultures of each strain were cultured independently in 9 mL Tryptic Soy Broth (TSB, BBL/Difco Laboratories, Sparks, MD, USA) in sterile 150 mm glass tubes at 37°C for 18h.

For studies involving the evaluation of quality and nutritional properties or natural microbiota, blueberries were only washed with tap water. For studies involving the artificial inoculation of *Listeria* or *E. coli*, blueberries were further sprayed with 70% ethanol to remove background microorganisms and dried for 2 h in biohood prior to inoculation. Blueberries were immersed into 100 mL of the inoculum for 1 min and then placed on a sterile tray in a laminar flow hood and dried under continuous circulating laminar flow for 2 h at $22 \pm 2^\circ\text{C}$ to facilitate microbial attachment on the fruit surface.

2.3 Sanitizing solution and PEF treatment

Tsunami 100 (EcoLab, St. Paul, MN) (15.2% peroxyacetic acid, 11.2% hydrogen peroxide) was used in this study. One liter of sterile distilled water was conditioned at 22°C

overnight, and 250 μ l or 500 μ l Tsunami 100 (T100) was added to the sterile distilled water just before each experiment. The concentration of peroxyacetic acid in each sanitizing solution was determined using a test kit according to the manufacturer's instruction. The concentration of peroxyacetic acid corresponding to 0.25% and 0.5% solutions were 30 and 60 ppm, respectively.

A bench-scale PEF processing system (OSU-4H Model) located at the Eastern Regional Research Center, Agricultural Research Service, USDA (Wyndmoor, PA, USA) was used for this study (Figure 1). The system provided bipolar square waveform pulses with a maximum peak voltage of ± 11 kV. The high voltage pulse generator operated at a maximum repetition rate of 2000 pulses per second and a pulse width of 1~10 μ s. Pulses were monitored with a high voltage probe (VD-60; Northstar, Albuquerque, NM, USA), current monitors (Model 110; Pearson, Palo Alto, CA, USA) and oscilloscopes (TDS-210; Tektronix, Beaverton, OR, USA). A specially designed parallel-electrode (stainless steel) PEF treatment chamber with 2.5 cm gap distance and an area of 56 cm² (7 $\times$ 8 cm) was used to treat blueberry samples (Figure 1).

Before PEF treatment, fresh blueberries (30 pieces, ca. 60 g) were placed in the treatment chamber, and then the chamber was filled with the salt solution (0.025% w/v; 500 μ S/cm electrical conductivity) with or without sanitizer. The salt solution was used to improve electrical contact between electrodes and have a more homogeneous electric field distribution. The PEF treatment conditions in this study were 2 kV/cm field strength, 1 μ s pulse width, and 100 pulses per second. The treatment times were 2, 4, and 6 min and treatment temperatures were 22°C. Approximately 4, 6, and 8°C temperature increase was observed for 2, 4, and 6 min treatment time, respectively. These PEF parameters were selected based on our previous trials considering the PEF process system power limit, operation stability, and microbial inactivation.

After PEF treatment, the blueberry samples were taken out from the chamber, blotted with paper towels to remove excess water, weighed, and subject to microbial and quality analyses.

2.4 Microbiological analysis

Untreated and treated blueberry samples were transferred into individual sterile stomacher bags and then stomached in 10 mL of 0.1% peptone water for 1 min. Serial dilutions of the resultant suspensions were made in 0.1% peptone water and surface-plated (100 µl/plate) onto PALCAM agar plates (BBL/Difco, Sparks, MD) with PALCAM selective supplement (Oxoid, England) for *Listeria*, tryptic soy agar (TSA: BBL/Difco) for *E. coli*, Plate Count Agar (PCA, BBL/Difco) plates for aerobic mesophilic bacteria and dichloran rose bengal chloranphenicol agar (DRBC, Merck, Germany) plates for yeasts and molds (Y&M). PALCAM, TSA, and PCA plates were incubated at 37°C up to 48 h, while DRBC plates were incubated at 25°C for 4 to 5 days. Colony forming units (CFU) were then enumerated.

2.5 Color and Texture analysis

Color analysis was conducted at $25 \pm 2^\circ\text{C}$ using a portable General Colorimeter (JZ-300, Kingwell Instrument Col. Ltd., Guangdong, China) in the reflectance mode. Color was expressed in L^* , a^* and b^* values. Color analysis was conducted using ten blueberries for each pretreatment and the results were averaged.

Compression tests were performed to evaluate any changes in the hardness of blueberry samples. The hardness value (g) is the peak force that occurs during the first compression. The force required to compress each blueberry fruit was measured individually using a TA-XT2i texture analyzer (Texture Technologies, Scarsdale, NY, USA) with a 3mm diameter stainless

cylinder probe TA-212. The probe height was calibrated to 20 mm above the TA-90 base platform so that the blueberry could be aligned directly under the probe. The test settings were as followed: pretest speed of 2 mm/s and test speeds of 2 mm/s with an automatic trigger set to 5 grams of force; and test distance of 3mm into the blueberries. Each time a set of fruits was measured, the results were the average of 10 berries.

2.6 Determination of total phenolics and anthocyanins

Blueberry samples (20 g) were homogenized and mixed with 30 mL of ethanol. After the mixture was centrifuged at 4000 x g for 5 min, blueberry residue was extracted with 85% (v/v) ethanol two times, and supernatant (total 100 mL) was used to determine total phenolics content and total anthocyanin content.

Total phenolics contents in the supernatant were determined via the Folin–Ciocalteu method as described by Yu et al. (2014). Quantification was based on the calibration curve generated with gallic acid standard solutions, and contents were expressed as Gallic Acid equivalent (GAE)/g dry matter.

Anthocyanins contents in the supernatant were determined using the pH differential method as described by Lee et al. (2005). The absorbance of each sample diluted with pH 1.0 and pH 4.5 buffers at both 520 and 700 nm was read versus a blank cell filled with deionized water using a spectrophotometer (Genesys 10 UV, Thermo Electron Corporation, Pennsylvania, USA). The results were expressed as cyanidin-3-glucoside (C3G) equivalents/g dry matter.

To determine the compounds in peels or juice separately, blueberry samples were homogenized and filtered using double-layer cheesecloth. The solid and liquid portions of the berry homogenate were analyzed following the previously-described procedures.

2.7. Statistical analysis

Ten blueberries were sampled in each experimental trial and experiments were conducted in triplicate for each treatment. The microbial counts for each replicate were transformed to log CFU/g before data analysis. Analysis of variance (ANOVA) was performed with SAS version 9.1 (SAS Institute, Inc., Cary, NC). Mean comparisons were made between the control and each treatment replicate using LSD to determine significance. Significant differences were defined at $P < 0.05$.

3 Results

3.1 Inactivation of Microorganisms

The first trial was conducted to investigate the efficacy of the PEF treatment conditions in microbial inactivation for blueberries in salt water without addition of sanitizer (T100). Figure 2 shows that approximately 1 log CFU/g of artificially inoculated *E. coli* on blueberries was reduced due to “washing” effect by soaking samples in salt solution (CK1). The PEF treatment achieved additional 0.5 log reduction after 4 and 6 min. There were no significant ($p > 0.05$) difference in microbial inactivation between 4 and 6 min treatments. Therefore, the treatment time of 6 min was excluded for further studies with sanitizing solutions.

Figure 3 shows the effect of sanitizer concentrations on the survival of artificially inoculated *E. coli* on blueberries after PEF treatments. The initial *E. coli* cell populations were approximately 4.3 log CFU/g (CK0). *E. coli* populations were reduced by 1 and 1.5 log CFU/g on non-PEF (CK1) and PEF treated (PEF) samples in the solution without sanitizer (0%T100), which was similar to the result in Figure 2. Addition of 0.25% sanitizer (0.25%T100)

significantly increased the inactivation rate and further reduced *E. coli* populations by 1.8 and 2.9 log CFU/g respectively. However, an increase in the concentration to 0.5% (0.5% T100) did not show further reduction of *E. coli* populations. Similar result was observed for *Listeria* on blueberry samples (Figure 4). Therefore, the treatment solution with 0.25% T100 was used for further studies.

Figure 5 shows the effect of PEF treatment time on the inactivation of *Listeria* on blueberry samples. PEF treatment at 2 and 4 min reduced *Listeria* populations by 2.3 and 2.6 log CFU/g, respectively. *Listeria* populations in blueberry samples (CK1) which were soaked in sanitizing solution and not subject to PEF treatment were reduced by less than 1 log CFU/g.

Same PEF treatments were applied to natural microflora on blueberries. Figure 6 shows the effects of PEF treatment on the inactivation of native bacteria (A) and yeasts and molds (B) on blueberry samples. PEF treatment reduced total bacterial counts from 2.6 log CFU/g to undetectable levels (< 0.7 log CFU/g) after 2 min, achieving more than 2.5 log reduction (Figure 6A). Blueberries had higher initial Y&M counts than native bacteria (viz., ca. 4.8 log CFU/g). After PEF treatments, populations were reduced to 3.5, and 3.1 log CFU/g for 2 and 4 min, respectively (Figure 6B).

Unlike *E. coli* and *Listeria*, native microorganisms were less sensitive to sanitizer solutions. When blueberry samples were placed in the sanitizing solution for 4 min without PEF treatment (CK1), only 0.2 log CFU/g reduction of bacteria and 0.7 log CFU/g reduction of Y&M were obtained (Figure 6).

3.2 Changes in Quality and Nutrient Concentration

The effect of PEF on the visual appearance, surface color, compression firmness, and total anthocyanins and phenolic compounds in blueberries was determined immediately after treatments. Based on visual inspection, blueberries did not display any signs of gross physical damage, compared to the control (Figure 7). Surface color measurements (L^* , a^* , b^* values) were not significantly impacted by PEF treatments (Table 1). However, a trend towards reduction in compression firmness was detected when blueberries were treated with sanitizer solution and PEF (Table 1).

Anthocyanin concentration in whole fruit increased from 101 units to 110 units after the PEF treatment, and total phenolics were increased from 111 to 152 units, which corresponded to a 10% and 23% increase in anthocyanins and total phenolics, respectively, compared to the controls (non-PEF treated) (Figure 8).

In addition, PEF treatments also affected the distribution of anthocyanins and total phenolics in blueberries. Higher anthocyanin concentration and total phenolics were observed in the juice portions, while lower concentrations were present in the peel/skin portions following PEF treatment (Figure 9). Ninety percent of anthocyanins in peel and 10% in juice changed to 85% in peels and 15% in juice after PEF treatments. Similarly, for total phenolics, 86% in peel and 14% in juice were changed to 82% in peel and 18% in juice.

4 Discussion

PEF is a promising alternative to current juice and beverage processing technologies. Nevertheless, PEF was not effective in reducing the numbers of microorganisms on whole fruits without the aid of sanitizing solutions, potentially, because the applied field strength we used (2 kV/cm) is limited in efficacy compared to the higher field strengths (viz., 25 - 45 kV/cm) used

with liquid products. To compensate for low field strength, a longer treatment time (2-6 min) was used in this study compared to 250 μ s for juice pasteurization (Guo et al., 2014). In addition, a sanitizing solution was used in combination with PEF treatments. It is a general rule that a liquid medium with a specific conductivity must be used for PEF processing to provide uniform PEF treatment. Liquid food itself, or a salt water carrier for solid food, is usually used. Two advantages were observed when the sanitizer solution was used in combination with PEF. One advantage is that the combination increased the inactivation rates of *E. coli*, *Listeria*, and native microorganisms on blueberries. When salt water was used alone, the PEF treatment only achieved less than 0.5 log CFU/g reduction of *E. coli* populations (Figure 2). The combination was able to achieve up to a 2.9 and 3 log CFU/g reduction of *E. coli* (Figure 3) and *Listeria* (Figure 4 and 5), as well as 2 log CFU/g reduction of native microorganisms (Figure 6). Use of the sanitizing solution alone caused a very limited reduction in bacterial populations. Davidson et al. (2013) reduced *E. coli* O157:H7 populations, which were artificially inoculated onto iceberg lettuce by 0.93 log CFU/g when washed for 90s with 50 ppm of peroxyacetic acid (Tsunami 100), which was not significantly ($P < 0.05$) more effective than water alone. It has been reported that PEF treatments with different treatment conditions cause death or injury of microbial cells and the sub-lethally injured cells could recover depending on growth environment (Jin et al., 2014; Gurtler et al., 2010). Sanitizing solution or other antimicrobials increases destruction of the microbial cytoplasmic membrane as well as preventing cell repair of survivors from PEF treatments (Nguyen & Mittal, 2007). Therefore, the combination in the present study can enhance their killing effect on microorganisms, particularly on those injured during PEF processing. The other advantage is that the combination treatment inactivated microorganisms in the solution and reduced the risk of cross-contamination, if the solution is re-circulated. Our test

demonstrates that there were no detectable cells in the carrier solution used for all treatments (data not shown).

The softening of PEF treated blueberries may be due to cell membrane breakdown by PEF treatment, known as electroporation. A number of publications have reported changes in plant tissue integrity after PEF processing that resulted in texture softening (Asavasanti et al., 2010; De Vito et al., 2008; Gonzalez et al., 2010a, 2010b; Lebovka et al., 2004). From a sensory standpoint, maximum force represents the hardness of the food sample (Bourne, 2002). Decreases in hardness may negatively affect sensory quality of whole fresh fruit that requires a firm texture (or may impute a positive effect for those fruits in which soft texture is preferred). Therefore, the results from the present study suggest that PEF processing is more suitable for fruits that require softening before consumption.

Anthocyanins reside in vacuoles, mostly found on the skin of blueberries (Bjoroy et al., 2009). Total anthocyanins demonstrated a significant increase in C3G equivalents after PEF treatment (Figure 8). This result indicates that in this particular range of processing conditions, the PEF treatment may modify the mechanism of anthocyanins degradation by affecting the molecules involved in the kinetics of the reaction, such as enzymes. Barba et al. (2013) and Ferrari et al. (2010) observed similar results when they studied blueberry and pomegranate juice properties after high pressure treatments. Similarly, phenols were significantly increased (23%), after PEF treatment. This increase in total phenolic content may be related to increased extractability of some of the antioxidant components following PEF processing. Other studies have reported an increase in total phenolic content of blueberry juice (Barba et al., 2013) and grape by-products (Corrales et al., 2008), following high pressure processing treatment. In

contrast, some other non-thermal interventions, such as cold plasma (Lacombe et al., 2015) and ozone (Tiwari et al., 2009), significantly reduced anthocyanins in fruits or juices.

PEF treatment increased the concentrations of anthocyanins and total phenolics and changed their distribution throughout the blueberries. Higher concentrations were obtained in juices than in peels after PEF treatment (Figure 9). It can be explained that PEF treatment increases the permeabilization of cell membranes due to the electroporation phenomenon (Kotnik et al., 2012). Studies on extraction of intracellular compounds from plant tissues treated with PEF have shown that a higher yield of active compounds was obtained after PEF treatment (Jemai & Vorobiev, 2006; Praporscic et al., 2007; Schilling et al., 2007; Turk et al., 2012). PEF treatment has recently shown good results for the enhancement of polyphenols and anthocyanins extraction from grape wastes (Boussetta et al., 2012, 2009; Brianceau et al., 2015; Corrales et al., 2008). Increases of those nutrients in the juice portion after PEF treatment would benefit juice processing by allowing for the production of juice with better nutritional properties.

Blueberries can be consumed as fresh fruit, and also can be used to make juice, jam, or dehydrated products. PEF treatment significantly reduced initial microbial loads and improved the nutritional values of fresh whole fruits, which is an important step in producing high quality and safe products derived from berries. The FDA requires that juice pasteurization reduce target pathogens by 5 log CFU. This mandatory requirement is challenging for most nonthermal processes without scarifying the quality and nutritional properties of juice products. However, multiple (i.e., hurdle) treatments are permitted to meet this requirement, as long as juices are processed in the same facility (FDA, 2004), hence, two-stage processing is possible. In the first stage, PEF pretreatment could be applied to whole fruits to achieve a ca. 2 log reduction, allowing for the production of juice with high nutritional value. In the second stage, the juice

would be subject to further treatment, such as PEF, at high intensity and short time or by other nonthermal processing treatments, to achieve an additional 3 log reduction. In this way, less severe processing conditions could be applied for each treatment, not only allowing for the preservation or improvement of quality and nutritional properties, but also meeting the FDA 5-log reduction requirement for juice pasteurization. Similarly, PEF has the potential for flexible applications in other derived fruit products.

To our best knowledge, this is the first study that investigates the use of a sanitizing solution as a PEF treatment medium and PEF-induced inactivation of potential pathogenic and spoilage microorganisms on blueberries, while considering its effect on their quality and nutrition. Future studies will address the feasibility for scale-up of this technology to pilot plant as well as its application in derived products. Optimal PEF parameters (field strength, pulse width, frequency, total treatment time), and other parameters (treatment temperature, type of treatment liquid media, etc.) will be further evaluated.

5 Conclusion

The capability of PEF to be utilized as a postharvest sanitization process was demonstrated in this study by the logarithmic reduction of *E. coli*, *Listeria* and native microorganisms. This combination of PEF plus a sanitizer solution will potentially allow for efficient decontamination of blueberries and other small fruits. The significance of PEF pretreatment in inactivation of microorganisms on whole fruits is to make the products safer at the beginning of processing; thus, producers have more flexibility to make pathogen-free products, such as fresh-like whole fruits, or their derived products such as purees, pastes, or juices with reduced populations of pathogenic or spoilage contaminants. In addition, PEF

processing preserved the berries visual appearance and improved their nutritional properties, while softening their texture, suggesting the usefulness of further research for various fruits and their derived products.

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Figure Legends

Figure 1. Bench-scale PEF processing system (OSU-4H Model). A: Pulse generator with controllers and oscilloscopes; B: Parallel-electrode (stainless steel) PEF treatment chamber.

Figure 2. Effects of treatment time on survival of *E. coli* on blueberries in salt solution without sanitizer. CK0: samples without PEF treatment and not in salt solution; CK1: samples in salt solution without PEF treatment; PEF: samples in salt solution and PEF treated. Error bars represent the standard deviation of the means.

Figure 3. Effects of sanitizer concentration on survival of *E. coli* on blueberries after PEF treatments. CK0: samples in sanitizing solution without PEF treatment; CK1: samples in sanitizing solution without PEF treatment for 4 min; PEF: samples in sanitizing solution and PEF treated for 4 min. Error bars represent the standard deviation of the means.

Figure 4. Effects of sanitizer concentration on survival of *L. innocua* on blueberries after PEF treatments. CK0: samples in sanitizing solution without PEF treatment; CK1: samples in sanitizing solution without PEF treatment for 4 min; PEF: samples in sanitizing solution and PEF treated for 4 min. Error bars represent the standard deviation of the means.

Figure 5. Effect of treatment time on survival of *L. innocua* on blueberries after PEF treatments. CK1: samples in sanitizing solution without PEF treatment; PEF: samples in sanitizing solution and PEF treated. Error bars represent the standard deviation of the means.

Figure 6. Effects of treatment time on survival of total aerobic bacteria (A) and Y&M (B) on blueberries after PEF treatments. CK1: samples in sanitizing solution without PEF treatment; PEF: samples in sanitizing solution and PEF treated. Error bars represent the standard deviation of the means.

Figure 7. Appearance of blueberries after PEF treatment for 0 min (A), 2 min (B), and 4 min (C).

Figure 8. Changes in anthocyanins and total phenolics concentrations in blueberries after PEF treatment. CK1: samples in sanitizing solution without PEF treatment; PEF: samples in sanitizing solution and PEF treated for 4 min. Unit of anthocyanins: cyanidin-3-glucoside (C3G) equivalents mg/100g wet weight; unit of total phenolics: gallic acid equivalent (GAE) mg/100g wet weight. Error bars represent the standard deviation of the means.

Figure 9. Changes in the distribution of anthocyanins and total phenolics between blueberry peel and juice after PEF treatment for 4 min.

A

B

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Table 1

Color and Firmness of Blueberries after PEF treatment

Treatment	L*	a*	b*	Hardness (g)
CK0	30.93±1.39 a	0.35±0.55 a	-3.45±0.79 a	499.8±134.6 a
CK1(4M)	29.76±2.28 a	0.30±0.55 a	-3.06±0.95 a	328.9±90.8 b
PEF2M	30.89±2.37 a	0.22±0.93 a	-3.55±1.06 a	143.6±44.2 c
PEF4M	30.12±1.58 a	0.29±0.65 a	-3.64±0.75 a	119.1±39.1 c

Data are means $\pm$ standard deviation. Means in the same column with different letters are significantly different ($p < 0.05$). CK0: samples without PEF or sanitizing solution treatment; CK1(4M): samples in sanitizing solution for 4 min without PEF treatment; PEF 2M and PEF4M: samples in sanitizing solution and PEF treated for 2 or 4 min.