



Effect of Pulsed Ultraviolet Light on Natural Microbial Load and Antioxidant Properties of Fresh Blueberries

Senem Güner^{1*}, Zeynal Topalcengiz²

¹Department of Food Engineering, Faculty of Engineering, Afyon Kocatepe University, 03200 Afyonkarahisar, Turkey

²Department of Food Engineering, Faculty of Engineering and Architecture, Muş Alparslan University, 49250 Muş, Turkey

ARTICLE INFO

Research Article

Received 15 January 2018

Accepted 24 April 2018

Keywords:

Blueberries

PUV

Microbial load

Antioxidant enzyme

DPPH

*Corresponding Author:

E-mail: sguner@aku.edu.tr

ABSTRACT

Blueberries have been drawn more attention in recent years for their antioxidant capacities and proposed health benefits. Pulsed ultraviolet (PUV) light is known for its disinfection effects on the surface of fresh fruits. This study's aim was to examine the effects of PUV light on naturally present microbial load, antioxidant capacity, and antioxidant enzyme activity of fresh blueberries. Fresh blueberry samples were treated for 30, 60, 90, and 120 s. Samples were stabilized at 13 cm away from the PUV lamp before treatment. Total Aerobic Plate Count (TAPC) and yeast and mold count reduction were observed the highest in samples treated for 90 s, 1.97 and 1.27 log CFU/g, respectively. Antioxidant capacity levels were significantly different for treated samples for 60 and 90 s in FRAP analysis, whereas no differences were observed in results of DPPH analysis. Superoxide dismutase (SOD) activity as antioxidant enzyme had no difference, however, glutathione peroxidase (GPx) increased by 50% with PUV treatment for 120 s. PUV light treatment of fresh blueberries for 90 s was determined as ideal exposure time among tested time intervals of this study. PUV light illumination can generally enhance antioxidant capacity and antioxidant enzyme activity of fresh blueberries and decrease the population of natural microorganisms of fresh blueberries and similar small fruits.

DOI: <https://doi.org/10.24925/turjaf.v6i6.733-739.1797>

Introduction

Fresh fruits have an appropriate medium for growth of several types of fungi and bacteria. Fruits can be contaminated in the field, during harvest and/or transportation, on market, or by the consumer (Tournas and Katsoudas, 2005). Spoilage microorganisms are a part of natural flora of fruits and vegetables. They dramatically influence the shelf-life and consumer acceptance of fresh products. Fresh fruits and vegetables should be prepared with proper processing and sanitation procedures to have ready-to-eat status based on food regulations (Williams et al., 2012).

Pulsed ultraviolet light (PUV) is a novel technology for food processing with a broad spectrum of ultraviolet, infrared and visible light. The PUV equipment produces the wavelength between 100 and 1100 nm and consists of one or more lamps filled with inert gases (xenon or krypton), a power unit, and a high voltage connection, providing the transfer of a high electrical current into a pulse of PUV light (Oms-Oliu et al., 2010). Applications of PUV light in food processing include decontamination of food surfaces and packing materials, pasteurization of milk, and mitigation of food allergens (Chung et al., 2008; Demirci and Panico, 2008). The ultraviolet light has been

used for several decades in the food industry to inactivate microorganisms, especially pathogens. Studies showed that PUV applied to fresh small fruits has an inactivation effect on pathogens and spoilage microorganisms including *Salmonella* spp. and *Escherichia coli* O157:H7 (Bialka and Demirci, 2007; Bialka et al., 2008; Hsu and Moraru, 2011; Oms-Oliu et al., 2010). The PUV treatment can be an alternative disinfection application for inactivation of both spoilage and pathogenic microorganisms in fresh small fruits including blueberries. While product safety is an essential criterion for public health and safety, the food's nutritional quality and shelf life should not be compromised.

Blueberries are believed to be a rich source of natural antioxidant compounds, including anthocyanins. Antioxidants are the defense mechanism of plants against the harmful effects of oxidative stress caused by decay organisms. They can effectively scavenge free radicals and suppress the reactive oxygen species in plants (Wang et al., 2010). The major antioxidant activity levels can be measured by single electron transfer (ET) reaction based assays. In ET assays, a change in color is monitored as the reaction occurs (Tabart et al., 2009). Ferric Reducing

Antioxidant Power (FRAP) and DPPH (α -diphenyl- β -picrylhydrazyl) assays are ET assays used to measure the antioxidant activity levels, spectrophotometrically. The FRAP assay is one of the common assays used for the measurement of antioxidant capacities of fruits and vegetables. The reduction of the intense blue ferric 2,4,6-tripyridyl-s-triazine (TPTZ) complex to its ferrous form enables this colorimetric assay to measure the antioxidant activity level of the sample (Molan et al., 2010). In DPPH assay, the reduction of α -diphenyl- β -picrylhydrazyl free radical by an antioxidant or by a radical species results in a color change, meaning a change of absorbance in 515 nm (Fukumoto and Mazza, 2000).

The antioxidant enzyme system is a part of the defense mechanism of fruits and vegetables against oxidative stress. Plant cells have enzymatic and non-enzymatic defense mechanisms for prevention from oxygen toxicity. Free radicals are controlled by an array of enzymes, such as superoxide dismutase (SOD), catalase, ascorbate peroxidase, and glutathione reductase (Lushchak, 2014). Superoxide dismutases (SODs), metalloenzymes, are a part of the antioxidant defense. They can catalyze the dismutation of O_2^- into H_2O_2 while catalases and/or peroxidases remove H_2O_2 (Lacan and Baccou 1998; Ballester et al., 2006). Glutathione is oxidized to glutathione disulfide. Hydroperoxides, including hydrogen peroxide, are reduced by glutathione peroxidase (GPx) using reduced glutathione to catalyze the reaction. These mechanisms can delay the harmful effects of free radicals (Prior, 2015).

As the fruits and vegetables mature and senescence, the appropriate mediums (e.g. higher water activity, materials released from cells with cell rupture) allow more microbial growth. The UV light, used as a postharvest treatment, is proved to be effective on delaying senescence in fruits after harvest and controlling decay process in many fruits and vegetables (Lemoine et al., 2010; Duarte-Molina et al., 2016), which may be related to the antioxidant capacity (Erkan et al., 2008). Reduction of spoilage microorganisms and maintaining antioxidant capacity in blueberries is important to have nutritional benefits and to lower the costs by increasing the quality, shelf life, and consumer acceptance. The objective was to assess the PUV light disinfection effectiveness on the natural microbial load and antioxidant properties of fresh blueberries.

Materials and Methods

Blueberries

Fresh mature highbush blueberries within the same grove were hand-picked from a farm located in Gainesville, FL. Collected blueberries were rapidly frozen for further analysis in two hours after transfer to the laboratory. To conduct assays, frozen blueberries were kept at room temperature until complete thawing.

Pulsed UV Light Treatment

Blueberries were weighed 10 ± 1 g for microbiological analysis, 20 ± 2 g for antioxidant capacity and 3.0 ± 0.5 g for antioxidant enzyme analyses in 70 mL aluminum dishes (Fisher Scientific, Pittsburgh, PA). Samples, prepared for microbiological and antioxidant capacity

analysis, were placed on the tray of a Xenon PUV light system model RS-3000C (Xenon Corporation, Wilmington, MA). Samples were treated at 3 pulses per second and for different time periods (30, 60, 90 and 120 s). During the treatment, samples were stabilized 13 cm away from the PUV lamp.

Microbiological Analysis

Total aerobic mesophilic and yeast and mold count of fresh blueberries were counted using a modified method by Lamikanra et al. (2005). One gram of PUV light treated blueberries was placed in 99 mL 0.1% peptone water (Bacto™ Peptone, Sparks, MD) resulting in an initial 1:100 dilution and subsequently homogenized with a stomacher for 90 s. Serial dilutions of the samples were prepared in 0.1% peptone water. 1 ml of the homogenate was inoculated onto Total Aerobic Plate Count (TAPC) and Yeast and Mold Petrifilm plates (3M™ Microbiology, St. Paul, MN). The inoculum was evenly distributed over a circular area of the film with spreader designed for Petrifilm plates. TAPC and yeast and mold counts were enumerated after incubation at 35 °C for 48 h and 120 h, respectively.

Determination of Antioxidant Capacity

Blueberry samples (control or PUV treated), each 20 g, were homogenized with a hand-held homogenizer. For extraction, a mixture of 20 ml of an acetone/water/acetic acid (70:29.9:0.1, v/v) solvent were added and sonicated with homogenized samples for 30 min in a centrifuge tube. Later, tubes were kept at ambient temperature for 2 h. The tubes were centrifuged for 50 min at 4°C at 10.000×g. The supernatant was collected for two different antioxidant capacity analysis as described below.

Ferric reducing antioxidant power (FRAP): The FRAP assay protocol was modified from Benzie and Strain (1996). The FRAP reagent was a combination of 300 mM acetate buffer (pH 3.6) with 10 mM 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ), 40 mM HCl, and 20 mM ferric chloride ($FeCl_3 \cdot H_2O$) in the respective ratios, 10:1:1. The 10 μ l of serial dilution of the supernatant was blended with 290 μ l of FRAP reagent in a multi-well plate (Fisher Scientific, Fairlawn, NJ). The sample mixtures were incubated for 10 min at 37°C. After incubation, the absorbance levels of samples were read at an absorbance of 595 nm with a microplate reader spectrometry (Spectra Max Gemini XPS, Molecular Devices, Sunnyvale, CA) at 1 min intervals for 4 min. Ferrous sulfate ($FeSO_4 \cdot 7H_2O$) were prepared as standards (0.1 mM-1.0 mM). The results were expressed as mmole of Fe^{2+} /g.

DPPH free radical scavenging activity: The DPPH assay protocol was modified from Brand-Williams et al. (1995). Briefly, 80 μ l of serially diluted extracts were mixed with 220 μ l of 0.1 M DPPH radical solution, dissolved in 80% methanol. The mixtures were agitated and incubated at ambient temperature for 30 min in dark. Absorbances at 517 nm were measured with the microplate reader used in FRAP assay. Scavenging DPPH levels were calculated according to the equation below.

$$DPPH(\%) = \left(\frac{A_{517} \text{ of control} - A_{517} \text{ of sample}}{A_{517} \text{ of control}} \right) \times 100 \quad (1)$$

Determination of Antioxidant Enzyme Activity

For superoxide dismutase (SOD) activity, 3 g of fresh blueberries were homogenized in 5 to 10 ml of 20 mM of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.2) with 1 mM ethylenediaminetetraacetic acid (EDTA), 210 mM mannitol, and 70 mM sucrose per gram blueberry sample. Similarly, 3 g of fresh blueberries were homogenized in 5 to 10 ml of buffer (pH 7.5), which was a mixture of 50 mM Tris-HCl, 5 mM EDTA, and 1 mM dithiothreitol (DTT), per gram blueberry sample for glutathione peroxidase (GPx) activity. The homogenates for SOD and GPx were centrifuged for 5 min at 1,500×g and for 15 min at 4°C 10,000×g, respectively. The supernatants of centrifuged samples were collected and stored on ice.

Superoxide dismutase (SOD) activity: The Superoxide Dismutase Assay Kit (Cayman Chemical Company, Ann Arbor, Michigan, USA) was used to determine the SOD enzyme activity of blueberries. Briefly, 10 µl of serially diluted extracts and 200 µl of the diluted tetrazolium salt solution (radical detector) were mixed in a multiwell plate. The reaction was initiated by the addition of Xanthine Oxidase (20 µl) to all used wells. The multiwell plate was shaken for a few seconds to mix before 20 min incubation at room temperature. The absorbance was read at 460 nm using a micro-plate reader spectrometry (Spectramax 340³⁸⁴, Molecular Devices Inc., Sunnyvale, CA). The absorbance of standard A was divided by itself and the absorbances of all the other standards and samples, obtaining the standard linearized rates (RT). Following this step, the standard LR values were plotted as a function of the final SOD Activity values, provided by the manufacturer, to make a standard curve. The SOD levels of blueberries were calculated using the equation below:

$$\text{SOD} = \left[\left(\frac{\text{SLR} \cdot y - I}{S} \right) \times \frac{0.23 \text{ ml}}{0.01 \text{ ml}} \right] \times \text{SD} \quad (2)$$

Where,

SOD : SOD (U/ml),

SLR : Sample LR,

I : Intercept,

S : Slope,

SD : Sample dilution

Since our samples were prepared as weight (grams), obtained results were converted to be expressed as U/g of samples.

Glutathione peroxidase (GPx) activity: The Glutathione Peroxidase Assay Kit (Cayman Chemical Company, Ann Arbor, Michigan, USA) was used for the determination of the GPx activity of blueberries. Briefly, 20 µl of serial dilution of extracts, 100 µl of the Assay Buffer (50 mM Tris-HCl with 5 mM EDTA, pH 7.6), and 50 µl of the Co-Substrate Mixture (including lyophilized powder of NADPH, glutathione, and glutathione reductase) were mixed in the wells of a multi-well plate. The reaction was initiated by addition of cumene hydroperoxide (20 µl) to all the used wells. The absorbance was read once every minute at 340 nm using the same micro-plate reader to obtain at least 5 different time points. The change in absorbance (ΔA_{340}) per min

was determined by the selection of two points on the linear portion of the curve and using the following equation:

$$\Delta A_{340} = \frac{[A_{340}(\text{Time 2}) - A_{340}(\text{Time 1})]}{[\text{Time 2}(\text{min}) - \text{Time 1}(\text{min})]} \quad (3)$$

Time 1 and Time 2 mean the two different time periods, expressed as minutes.

And GPx activity was determined with the equation below, which was formed by the manufacturer:

$$\text{GPx} = \frac{(\Delta A_{340}/\text{min})}{0.00373 \mu\text{M}^{-1} \times 0.19 \text{ ml} / 0.02 \text{ ml} \times \text{SD}} \quad (4)$$

Where,

GPx : GPx activity (nmol/min/ml),

SD : Sample dilution

Since our samples were prepared as weight (grams), obtained results were converted to be expressed as nmol/min/g of samples.

Statistical Analysis

The collected data were analyzed using the Statistical Analysis System (SAS[®] Institute Inc., Cary, NC, USA) version 9.1. Statistical significance on each group of microbial load, antioxidant capacity and antioxidant enzyme activity was determined with Analysis of Variance (one-way ANOVA) and Tukey's HSD test. The data at each treatment time were the average of triplicates with standard deviation. The significance level was set as $P \leq 0.05$.

Results and Discussion

Microbiological Analysis

Total Aerobic Plate Count (TAPC) population of control and PUV treated blueberries were shown as log CFU/g in Table 1. The TAPC population of PUV treated blueberries ranged from 6.96±0.42 to 8.92±0.77 log CFU/g (Table 1). Non-significant reductions ($P > 0.05$) were observed in PUV treated blueberries for 30 and 60 s. Significant reductions were observed for PUV treated blueberries for 90 s and 120 s ($P \leq 0.05$). The population of total aerobic microorganisms reduced 1.97 and 1.72 log CFU/g for 90 s and 120 s treated samples, respectively. The yeast and mold population of control and PUV treated blueberries as log CFU/g was shown in Table 1. The yeast and mold population of PUV treated blueberries ranged from 6.52±0.20 to 7.81±0.61 as log CFU/g (Table 1). PUV treatment for 30 and 60 s did not significantly reduce the population of yeast and mold ($P > 0.05$). Similar to the population of TAPC, decrease in population of yeast and mold was significant and 1.29 log CFU/g for 90 s and 1.17 log CFU/g for 120 s treated blueberries ($P \leq 0.05$).

The reducing effect of PUV light on microbial load and pathogens on various fruit and vegetables were reported, previously (Bialka and Demirci, 2007; Bialka et al., 2008; Manzocco et al., 2011; Hsu and Moraru, 2011; Oms-Oliu et al., 2010; Williams et al., 2012). Pulsed UV treatment was more effective after 90 s exposure time on

Total Aerobic Plate Count (TAPC) of fresh blueberries. A linear correlation was calculated between PUV treatment time and log CFU of TAPC indicating that PUV exposure time was the major factor of inactivation (Figure 1: R² = 0.85). Similar to result in our study, Manzocco et al. (2011) observed reductions ranged from up to 2.14 log CFU/g when cutting melon under UV-C light at different dosages and times (1, 5, and 10 min). This may indicate that shorter periods of PUV exposure are more effective than UV-C. The PUV light reduced *Salmonella* population as 1.35 log CFU/g on tomato surface after 60 s

PUV exposure at 6.35 cm from the strobe (Williams et al., 2012). The population of TAPC after PUV treatment in our study supports that 60 s of exposure may not be enough to cause significant log reductions if microbial concentration on fruit surface is in high level. The amount of inactivated Total Yeast and Mold had a linear correlation with PUV exposure time (Figure 1: R² = 0.88). Similar to inactivation of TAPC, PUV treatments for 90 s and 120 s were more effective on reducing yeast and mold population in fresh blueberries.

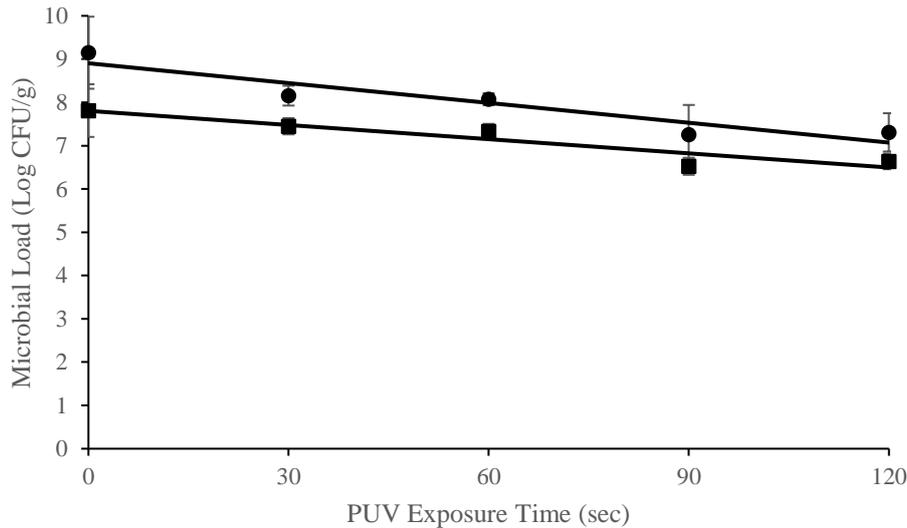


Figure 1 Linear regression lines of Total Aerobic Plate Count (TAPC) (●) and Total Yeast and Mold count (■) for PUV light treated fresh blueberries (n=3)

Table 1 Total Aerobic Plate Count (TAPC) and Total Yeast and Mold (TYMC) population of blueberries after PUV light treatment

Treatment	Log CFU*/g fresh weight ± SD	
	TAPC	TYMC
Control	8.92 ± 0.77 ^a	7.81 ± 0.61 ^a
PUV 30 s	8.10 ± 0.29 ^{a,b}	7.45 ± 0.19 ^a
PUV 60 s	8.03 ± 0.17 ^{a,b}	7.33 ± 0.17 ^a
PUV 90 s	6.96 ± 0.42 ^b	6.52 ± 0.20 ^b
PUV 120 s	7.21 ± 0.50 ^b	6.64 ± 0.18 ^b

Means (in columns) with the different letter is significantly different (P≤0.05). *CFU: Colony forming unit

Antioxidant Capacity Analysis

Ferric reducing antioxidant power (FRAP): The effects of PUV light on antioxidant capacity in fresh blueberries were shown in Table 2. The FRAP values observed among PUV treated and control blueberries ranged between 22.5±3.4 to and 27.5±13.9 mmole of Fe²⁺/g fresh weight. The FRAP values of PUV treated blueberries for up to 90 s did not show significant difference (P>0.05), as compared to control samples (Table 2). Blueberries treated for 60 s and 90 s had higher FRAP than the control group. Treatment for 30 and 120 s reduced the FRAP, but only blueberries for 120 s had significant decrease compare to untreated samples (P≤0.05).

FRAP assay showed no increasing effect on antioxidant capacity when blueberries were PUV treated

for 30 s and 120 s compared to untreated samples. However, significant increase was observed when the exposure time was 60 s and 90 s (P≤0.05). This increase may be originated from the UV light. It was claimed that UV light accelerates the synthesis of secondary metabolites and therefore antioxidant capacity in fresh fruits and vegetables (Allothman et al., 2009; Lamikanra et al., 2005; Perkins-Veazie et al., 2008). For the measurement of antioxidant capacity of a sample, more than one assay should be applied since single method is insufficient to determine various actions of different antioxidants (Dudonné et al., 2009). The results for applied antioxidant capacity measurement assays in here was incompatible with findings by Rock et al. (2015). PUV treated fresh blueberries had lower antioxidant capacity at 90 and 120 s exposure based on ORAC

analysis (Rock et al., 2015). This proves the importance of the use of different assays methods for antioxidant capacity measurements. UV-C light application increased FRAP values, relatively antioxidant capacity, compared to fresh Collin and Bluecrop blueberries (Perkins-Veazie et al., 2008). The dosage of UV-C light was the focal point of this research and different dosages were applied to blueberries. The FRAP values reduced in 'Collins' as dosage was changed from 0 or 1 kJ/m² while they increased in 'Bluecrop' as dosage 4 kJ/m² was compared to 0 kJ/m². The activation of antioxidants might be related to the UV-C exposure dosage, cultivar type, and the individual flavonoid changes in blueberries (Perkins-Veazie et al., 2008).

DPPH free radical scavenging activity: The scavenged (α -diphenyl- β -picrylhydrazyl) DPPH radical values (expressed as percentage) of PUV treated and control fresh blueberries were shown in Table 2. The DPPH values obtained among blueberries between 83.15 \pm 4.48 and 84.99 \pm 0.76% (Table 2). There were no significant differences observed between DPPH values of control and any PUV treated blueberries ($P > 0.05$).

Huang et al. (2012) reported that blueberries scavenged 96.96% of DPPH radicals. In our study, PUV treated blueberries for different time periods was not different from control group, but scavenged DPPH radicals on average of 84%. The diversity of cultivar, variety, maturity, environmental conditions, and/or method steps may have caused this difference. Fukumoto and Mazza (2000) claimed that using DPPH assay gives similar results to those of an oxidation method. However, the comparisons are not quantitative and the compound's structural conformation of phenolics influences the reaction with DPPH radical. The antioxidant capacity values might have showed similar results because of this structural conformation.

Antioxidant Enzyme Analysis

Superoxide dismutase (SOD) activity: SOD activity of control and PUV treated blueberries as U/g fresh weight

was shown in Table 3. The SOD activity results observed here ranged from 17.8 \pm 5.5 to 28.7 \pm 14.9 U/g fresh weight (Table 3). A significant difference was observed only in blueberries PUV treated for 30 s compared to untreated blueberries ($P \leq 0.05$).

The SOD activity was significantly reduced when the blueberries were PUV treated for 30 s in this study. Erkan et al. (2008) observed that the SOD activities of UV treated strawberries extracts were higher than those of control fruit. The SOD activity levels of the other PUV treatment times (60, 90, and 120 s) were not significantly different than the control group in this study ($P > 0.05$). The initial decrease may be a response to the oxidative stress of UV light on enzymes. However, SOD was activated with exposure time, probably, as a result of PUV light induced oxidative stress, which triggered antioxidant enzyme system.

Glutathione peroxidase (GPx) activity: GPx activities of control and PUV treated blueberries as nmol/min/g fresh weight was shown in Table 3. The GPx activity results obtained from PUV treated blueberries ranged from 40.1 \pm 9.0 to 116.0 \pm 29.4 nmol/min/g fresh weight (Table 3). PUV treated blueberries for 30 s and 90 s were not significantly different ($P > 0.05$) than untreated blueberries (control). Blueberries treated for 60 s (79.8 \pm 10.3 nmol/min/g) had almost two times higher GPx activity than 30 and 90 s treatments. Treatment for 120 s was 46 nmol/min/g more than untreated blueberries ($P \leq 0.05$).

Although the GPx activity of PUV light treated and untreated groups showed different responses to different exposure times, the longest PUV light treated blueberries showed the highest GPx activity. This high response to long PUV light treatment may be occurred in the demand of additional antioxidant enzymes when a limitation happened to the other antioxidant defense systems, such as polyphenols, against oxidative stress caused by PUV light treatment. Erkan et al. (2008) showed that the GPx activity was the highest when samples were treated with UV light for the longest time (10 min) as well.

Table 2 Antioxidant capacity of PUV light treated blueberries expressed as the Ferric Reducing Antioxidant Power (FRAP) and the DPPH Free Radical Scavenging Activity

Treatment	FRAP (mmole of Fe ²⁺ /g fresh weight) \pm SD	DPPH (%) \pm SD
Control	23.4 \pm 3.5 ^{a,b}	84.6 \pm 1.8 ^a
PUV 30 s	22.8 \pm 3.1 ^{a,b}	85.0 \pm 0.8 ^a
PUV 60 s	26.7 \pm 3.8 ^{a,b}	83.2 \pm 4.5 ^a
PUV 90 s	27.5 \pm 13.9 ^a	84.1 \pm 2.4 ^a
PUV 120 s	22.5 \pm 3.4 ^b	83.3 \pm 2.8 ^a

Means (in columns) with the different letter is significantly different ($P \leq 0.05$).

Table 3 Superoxide dismutase (SOD) and Glutathione peroxidase (GPx) activity of PUV light treated and control fresh blueberries

Treatment	SOD U*/g fresh weight \pm SD	GPx nmol/min/g fresh weight \pm SD
Control	25.9 \pm 7.7 ^a	51.4 \pm 7.8 ^c
PUV 30 s	17.8 \pm 5.5 ^b	40.7 \pm 14.3 ^c
PUV 60 s	19.6 \pm 5.6 ^{a,b}	79.8 \pm 10.3 ^b
PUV 90 s	20.5 \pm 7.4 ^{a,b}	40.1 \pm 9.0 ^c
PUV 120 s	28.6 \pm 14.9 ^a	116.0 \pm 29.4 ^a

Means (in columns) with the different letter is significantly different ($P \leq 0.05$). *One unit is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.

Conclusions

In conclusion, the disinfection effect of PUV light was observed in natural microbial biota of fresh blueberries. Thus, PUV light might have increasing effects on antioxidant capacity of fresh blueberries when application is used in appropriate conditions. Antioxidant capacity did not have important changes after PUV treatment based on DPPH, but not FRAP analysis. This indicates the importance the use of more than one methodology in antioxidant capacity tests. Antioxidant enzyme (SOD and GPx) activities in blueberries increased as different responses. Other fresh fruits rich in secondary metabolites can be conducted with various times and/or distances away from PUV lamp to observe enhancing effect on antioxidant capacity and antioxidant enzyme activity as future research.

Acknowledgements

The authors thank Wade Yang for his helpful discussion and recommendations. We also acknowledge the technical assistance of Cheryl Rock.

References

- Allothman M, Bhat R, Karim AA. 2009. Effects of radiation processing on phytochemicals and antioxidants in plant produce. *Trends Food Sci. Technol.*, 20(5): 201–212. DOI: 10.1016/j.tifs.2009.02.003
- Ballester AR, Lafuente MT, González-Candelas L. 2006. Spatial study of antioxidant enzymes, peroxidase and phenylalanine ammonia-lyase in the citrus fruit–*Penicillium digitatum* interaction. *Postharvest Biol. Technol.*, 39(2): 115–124. DOI: 10.1016/j.postharvbio.2005.10.002
- Benzie IFF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.*, 239(1): 70–76. DOI: 10.1006/abio.1996.0292
- Bialka KL, Demirci A. 2007. Decontamination of *Escherichia coli* O157:H7 and *Salmonella enterica* on blueberries using ozone and pulsed UV-light. *J. Food Sci.*, 72(9): 391–396. DOI: 10.1111/j.1750-3841.2007.00517.x
- Bialka KL, Demirci A, Puri VM. 2008. Modeling the inactivation of *Escherichia coli* O157:H7 and *Salmonella enterica* on raspberries and strawberries resulting from exposure to ozone or pulsed UV-light. *J. Food Eng.*, 85(3): 444–449. DOI: 10.1016/j.jfoodeng.2007.08.007
- Brand-Williams W, Cuvelier ME, Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. *Lebensm. Wiss u-Technol.*, 28(1): 25–30. DOI: 10.1016/S0023-6438(95)80008-5
- Chung S-Y, Yang W, Krishnamurthy K. 2008. Effects of Pulsed UV-light on peanut allergens in extracts and liquid peanut butter. *J. Food Sci.*, 73(5): 400–404. DOI: 10.1111/j.1750-3841.2008.00784.x
- Demirci A, Panico L. 2008. Pulsed ultraviolet light. *Food Sci. Technol. Int.*, 14(5): 443–447. DOI: 10.1177/1082013208098816
- Duarte-Molina F, Gomez PL, Castro MA, Alzamora SM. 2016. Storage Quality of Strawberry Fruit Treated by Pulsed Light: Fungal Decay, Water Loss and Mechanical Properties. *Innovative Food Science and Emerging Technologies*, 34: 267-274. DOI: 10.1016/j.ifset.2016.01.019
- Dudonné S, Virtac X, Coutière P, Woillez M, Mérillon J-M. 2009. Comparative Study of Antioxidant Properties and Total Phenolic Content of 30 Plant Extracts of Industrial Interest Using DPPH, ABTS, FRAP, SOD, and ORAC Assays. *J. Agric. Food Chem.*, 57(5): 1768–1774. DOI: 10.1021/jf803011r
- Erkan M, Wang SY, Wang CY. 2008. Effect of UV treatment on antioxidant capacity, antioxidant enzyme activity and decay in strawberry fruit. *Postharvest Biol. Technol.*, 48(2): 163–171. DOI: 10.1016/j.postharvbio.2007.09.028
- Fukumoto LR, Mazza G. 2000. Assessing antioxidant and prooxidant activities of phenolic compounds. *J. Agric. Food Chem.*, 48(8): 3597–3604. DOI: 10.1021/jf000220w
- Hsu L, Moraru C. 2011. A numerical approach for predicting volumetric inactivation of food borne microorganisms in liquid substrates by pulsed light treatment. *J. Food Eng.*, 105(3): 569–576. DOI: 10.1016/j.jfoodeng.2011.03.025
- Huang W-y, Zhang H-c, Liu W-x, Li C-y. 2012. Survey of antioxidant capacity and phenolic composition of blueberry, blackberry, and strawberry in Nanjing. *J. Zhejiang Univ-Sci. B. (Biomed & Biotechnol.)*, 13(2): 94–102. DOI: 10.1631/jzus.B1100137
- Lacan D, Baccou J-C. 1998. High levels of antioxidant enzymes correlate with delayed senescence in non-netted muskmelon fruits. *Planta*, 204(3): 377–382.
- Lamikanra O, Bett-Garber K, Ingram D, Watson M. 2005. Use of mild heat pre-treatment for quality retention of fresh-cut canteloupe melon. *J. Food Sci.*, 70(1): 53–57. DOI: 10.1111/j.1365-2621.2005.tb09020.x
- Lemoine ML, Chaves AR, Martinez GA. 2010. Influence of combined hot air and UV-C treatment on the antioxidant system of minimally processed broccoli (*Brassica oleracea* L. var. *Italica*). *LWT-Food Science and Technology*, 43: 1313-1319. DOI: 10.1016/j.lwt.2010.05.011
- Lushchak VI. 2014. Free radicals, reactive oxygen species, oxidative stress and its classification. *Chem. Biol. Interact.*, 224: 164–175. DOI: 10.1016/j.cbi.2014.10.016
- Manzocco L, Pieve SD, Maifreni M. 2011. Impact of UV-C light on safety and quality of fresh-cut melon. *Innov. Food Sci. Emerg. Technol.*, 12: 13–17. DOI: 10.1016/j.ifset.2010.11.006
- Molan AL, Lila MA, Ravindran G. 2010. Blueberries: Genotype-dependent variation in antioxidant, free-radical scavenging, and prebiotic activities. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology A. Méndez-Vilas (Ed.):* 427-434.
- Oms-Oliu G, Aguiló-Aguayo I, Martín-Belloso O, Soliva-Fortuny R. 2010. Effects of pulsed light treatments on quality and antioxidant properties of fresh-cut mushrooms (*Agaricus bisporus*). *Postharvest Biol. Technol.*, 56(3): 216–222. DOI: 10.1016/j.postharvbio.2009.12.011
- Prior RL. 2015. Oxygen radical absorbance capacity (ORAC): New horizons in relating dietary antioxidants/bioactives and health benefits. *J. Funct. Foods*, 18: 797–810. DOI: 10.1016/j.jff.2014.12.018
- Perkins-Veazie P, Collins JK, Howard L. 2008. Blueberry fruit response to postharvest application of ultraviolet radiation. *Postharvest Biol. Technol.*, 47(3): 280–285. DOI: 10.1016/j.postharvbio.2007.08.002
- Rock C, Güner S, Yang W, Gu L, Percival S., Salcido E. 2015. Enhanced Antioxidant Capacity of Fresh Blueberries by Pulsed Light Treatment. *J. Food Res.*, 4(5): 89–97. DOI: 10.5539/jfr.v4n5p89

- Tabart J, Kevers C, Pincemail J, Defraigne J-O, Dommes J. 2009. Comparative Antioxidant Capacities of Phenolic Compounds Measured by Various Tests. *Food Chem.*, 113(4): 1226–1233. DOI: 10.1016/j.foodchem.2008.08.013
- Tournas VH, Katsoudas E. 2005. Mould and yeast flora in fresh berries, grapes and citrus fruits. *Int. J. Microbiol.*, 105(1): 11–17. DOI: 10.1016/j.ijfoodmicro.2005.05.002
- Wang BC, He R, Li ZM. 2010. The stability and antioxidant activity of anthocyanins from blueberries. *Food. Technol. Biotechnol.*, 48(1): 42–49.
- Williams LL, Yang WW, English T, English N, Johnson JU, Rababah T, Khatiwada J. 2012. Disinfection of *Salmonella* spp. on tomato surface by pulsed ultraviolet light and selected sanitizers. *Int. J. Food Eng.*, 8(1): Article 21. DOI: 10.1515/1556-3758.2063