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Changes of flavonoid content and antioxidant capacity in blueberries after illumination with UV-C

Chien Y. Wang [a,*], Chi-Tsun Chen [a], Shiow Y. Wang [b]

[a] Food Quality Laboratory, Plant Sciences Institute, ARS, US Department of Agriculture, Beltsville, MD 20705-2350, United States
[b] Genetic Improvement of Fruits and Vegetables Laboratory, Plant Sciences Institute, ARS, US Department of Agriculture, Beltsville, MD 20705-2350, United States

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ABSTRACT

The levels of flavonoids in blueberries (*Vaccinium corymbosum* L.) were found to increase after illumination with UV-C. Phytochemicals affected included resveratrol, myricetin-3-arabinoside, quercetin-3-galactoside, quercetin-3-glucoside, kaempferol-3-glucuronide, delphinidin-3-galactoside, cyanidin-3-galactoside, delphinidin-3-arabinoside, petunidin-3-galactoside, petunidin-3-glucoside, petunidin-3-arabinoside, malvidin-3-galactoside, malvidin-3-arabinoside, and chlorogenic acid as analyzed by HPLC. Significantly higher antioxidant capacity was detected in fruit treated with 2.15, 4.30, or 6.45 kJ m^{-2} compared to the control fruit. UV-C dosage of 0.43 kJ m^{-2} also increased phenolics and anthocyanins, but to a lesser extent. The optimum doses of UV-C for enhancing phytochemical content in blueberries were 2.15 and 4.30 kJ m^{-2}. These data suggest that proper use of UV-C illumination is capable of modifying the phytochemical content of blueberries. Time course measurements of the effects of UV-C revealed that the strongest responses of fruit to UV-C treatment occurred instantly after the illumination and the effects diminished with time. Therefore, even though residual effects were evident following UV-C exposure, the best results were obtained immediately after the treatment.

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1. Introduction

In recent years, increasing attention has been paid by consumers to the health and nutritional aspects of horticultural products (Scalzo, Politi, Pellegrini, Mezzetti, & Battino, 2005). Fruits and vegetables contain high levels of biologically active components that impart health benefits beyond basic nutritional value (Larson, 1988). Blueberries are one of the richest sources of natural antioxidants among fruits and have high antioxidant capacity against peroxyl radicals, superoxide radicals, hydrogen peroxide, and singlet oxygen (Heinonen, Meyer, & Frankel, 1998; Wang & Jiao, 2000). These antioxidants are capable of acting as free radical scavengers, peroxide decomposers, singlet oxygen quenchers, enzyme inhibitors and synergists (Larson, 1988).

UV-C illumination as a postharvest treatment has proven to be beneficial in delaying postharvest fruit senescence and reducing decay in various fruits and vegetables (Allende & Artes, 2003; Erkan, Wang, & Wang, 2008; Gonzalez-Aguilar, Wang, Buta, & Krizek, 2001). A previous study (Perkins-Veazie, Collins, & Howard, 2007) has indicated that postharvest application of UV-C might be effective in stimulating the antioxidant content of blueberries. However, no information is available on the effect of UV-C illumination on specific flavonols and flavonoids in blueberries. In addition, the residual effect of UV-C exposure has not been studied and little is known about what happens after UV-C treatment. The purpose of this study was to evaluate not only the changes in antioxidant capacity but also the response of individual flavonoid compounds in blueberries exposed to different UV-C illumination dosages, and the time course change of the responses of blueberries to the UV-C treatment.

2. Materials and methods

2.1. Chemicals

2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA Inc. (Richmond, VA, USA). Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 2,2-di-(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH), and disodium fluorescein were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Acetonitrile, methanol, acetone, and water were of HPLC grade and were purchased from Baxter (Muskegon, MI, USA). All anthocyanins and aglycons were obtained from Indofine Chemical Co. Inc. (Somerville, NJ, USA). Other authentic standards were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and Fisher Scientific (Pittsburgh, PA, USA).

* Corresponding author. Tel.: +1 301 504 5981; fax: +1 301 504 5107.
E-mail address: Chienyi.Wang@ars.usda.gov (C.Y. Wang).

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2.2. Fruit sample handling and illumination with UV-C

Blueberries (*Vaccinium corymbosum* L., cv. Duke) were hand-picked from Butler's Orchard in Maryland at a commercially mature stage. Approximately 2000 fruits were harvested and sorted to eliminate damaged, shriveled, and unripe fruit, and selected for uniform size and colour. Nine hundred and ninety fruits were selected, randomized, and used for this study. The UV-C illumination device consisted of two banks (upper and lower sides) of a 15 UV-C lamp (Model UVLMS-38: 3UV EL Series UV Lamp, 8W, LW/MR/SW, Upland, CA, USA) equipped with a filter (98-0016-02, Upland, CA, USA) to have only one wavelength at 254 nm. The intensity of the UV-C lamp was determined with a Blak-Ray J-225 photometer (Ultra-Violet Products Inc., San Gabriel, CA, USA). The UV-C lamps, reflectors, and treatment area were enclosed in a wooden box covered with aluminium foil and supported by a metal frame to provide protection for the operators. Fruit were placed on a polystyrene net and illuminated with UV-C from both the upper and lower sides. Four UV-C illumination durations and dosages were applied to blueberry fruit. These durations were 1, 5, 10, and 15 min which were equal to the dosages of 0.43, 2.15, 4.30, and 6.45 kJ m^{-2}, respectively, on each side of the berries. Non-illuminated blueberries were considered as controls. Experiments were carried out in triplicates with 30 fruits for each treatment. After UV-C illumination, control and illuminated fruit were frozen in liquid nitrogen and the samples were stored at −80 °C until analysis.

2.3. Measurement of residual effect after UV-C treatment

A separate batch of blueberries were illuminated with 4.30 kJ m^{-2} UV-C and then placed at 20 °C and samples were taken at 0, 60, 180, 300, 540, and 1440 min after UV-C illumination. Likewise, experiments were carried out in triplicates with 30 fruits for each timing in each replicate. The samples were then frozen in liquid nitrogen and stored at −80 °C until analysis.

2.4. Total anthocyanin and total phenolic content

Three 5-g composite samples from 30 berries were extracted twice with 25 mL 80% acetone containing 0.2% formic acid using a Polytron (Brinkmann Instruments Inc., Westbury, NY, USA). The homogenised samples from the acetone extracts were then centrifuged at 14,000 × *g* for 20 min at 4 °C. The supernatants were transferred to vials, then stored at −80 °C, and later used for total anthocyanin and total phenol analysis. Total anthocyanin content in fruit extracts was determined using the pH differential method (Cheng & Breen, 1991). Absorbance was measured using a Shimadzu Spectrophotometer UV-160 (Shimadzu Corp., Columbia, MD, USA) at 510 and 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}]$ with a molar extinction coefficient of cyanidin-3-glucoside (29,600). Results were expressed as milligrams of cyanidin-3-glucoside equivalent per gram of fresh weight (mg/g fw). Total soluble phenolics in the berry fruit extracts were determined with the Folin–Ciocalteu reagent by the method of Slinkard and Singleton (1977) using gallic acid as the standard. Results were expressed as mg gallic acid (GA) equivalents per gram fresh weight (mg GA/g fw).

2.5. Oxygen radical absorbance capacity (ORAC) assay

Three 5-g composite samples from 30 berries were extracted twice with 25 mL 80% acetone containing 0.2% formic acid. The ORAC assay was carried out according to Huang, Ou, Hampsch-Woodill, Flanagan, and Prior (2002) using a high-throughput instrument platform consisting of a robotic eight-channel liquid handling system. A FL800 microplate fluorescence reader (Bio-Tek Instruments Inc., Winooski, VT, USA) was used with fluorescence filters for an excitation wavelength of 485 ± 20 nm and an emission wavelength of 530 ± 25 nm. The plate reader was controlled by software KC4 3.0 (revision 29) (Bio-Tek Instruments Inc., Winooski, VT, USA). Sample dilution was accomplished by a Precision 2000 automatic pipetting system managed by precision power software (version 1.0) (Bio-Tek Instruments Inc., Winooski, VT, USA). The ORAC values were determined by calculating the net area under the curve (AUC) of the standards and samples (Huang et al., 2002). The standard curve was obtained by plotting Trolox concentrations against the average net AUC of the two measurements for each concentration. Final ORAC values were calculated using the regression equation between Trolox concentration (6.25–50 μM) and the net AUC and were expressed as micromole Trolox equivalents per gram of fresh weight (Huang et al., 2002).

2.6. Hydroxyl radical scavenging capacity (·OH; HOSC) assay

Three 5-g composite samples from 30 berries were extracted twice with 25 mL of 50% acetone. The ·OH in aqueous media is generated through the Fenton reaction. The HOSC assay was conducted with acetone solutions according to a previously published protocol (*21*) with some modifications. The assay was carried out using a high-throughput instrument platform consisting of a robotic eight-channel liquid handling system and a 96-well microplate with a FL800 microplate fluorescence reader (Bio-Tek Instruments Inc., Winooski, VT, USA). Fluorescence was measured every minute for 3 h with an excitation wavelength of 485 nm and emission wavelength of 535 nm. The plate reader was controlled by software KC4 3.0 (revision 29). Sample dilution was accomplished by a Precision 2000 automatic pipetting system managed by precision power software (version 1.0) (Bio-Tek Instruments Inc., Winooski, VT, USA). Reaction mixtures consisted of 115 μL of 3.352×10^{-6} M FL prepared in 75 mM sodium phosphate buffer, 20 μL of standard or sample or blank, 25 μL of 0.1990 M H_2O_2, and 41 μL of 3.43 mM $FeCl_3$. Trolox prepared in 50% acetone at concentrations of 12.5, 25, 50, and 100 μM was used to prepare the standard curve for HOSC quantification. The HOSC values were determined by calculating the net area under the curve (AUC) of the standards and samples. The standard curve was obtained by plotting Trolox concentrations against the average net AUC of the two measurements for each concentration. Final HOSC values were calculated using the regression equation between Trolox concentration and the net AUC and were expressed as micromole Trolox equivalents (TE) per gram of fresh weight.

2.7. 2,2-Di-(4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) assay

To determine the antioxidant activity of different extracts, 2,2-di-(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH) radicals were used. In the radical form, this molecule has an absorbance at 515 nm that disappears with acceptance of an electron from an antioxidant compound to become a stable diamagnetic molecule. The method described by Cheng, Moore, and Yu (2006) was used with some modifications. A high-throughput instrument platform consisting of a robotic eight-channel liquid handling system and a microplate with a FL800 microplate UV–visible spectrometer reader (Bio-Tek Instruments Inc., Winooski, VT, USA) was utilised for this assay. The automated sample preparation was performed using a Precision 2000 instrument with an automatic pipetting system managed by precision power software (version 1.0) (Bio-Tek Instruments Inc., Winooski, VT, USA). The plate reader was controlled by software KC4 3.0 (revision 29). Three 5-g composite samples from 30 berries were extracted twice with 25 mL of 50% acetone, and 50 μL of this extract was diluted with 150 μL of 50%

acetone. Then, 40 μL of this diluted extract was used for assay. An aliquot (160 μL) of the DPPH solution (3.3 mg/50 mL 100% ethanol) was added to each well. The mixtures were shaken gently and allowed to stand for 40 min in the dark. The decrease in absorbance was measured at 515 nm against a blank (50% acetone) without extract using a FL800 microplate UV–visible spectrometer reader (Bio-Tek Instruments Inc., Winooski, VT, USA). The DPPH values were determined by calculating the endpoint of the standards (gallic acid) and samples. Final DPPH values were calculated using the regression equation between standard gallic acid concentrations (6.25–50 μM) and were expressed as micromole gallic acid equivalents per gram of fresh weight (μmol GA/g fw).

2.8. HPLC analysis of blueberry fruit flavonoids

High performance liquid chromatography (HPLC) was used to separate and determine individual anthocyanins and phenolic compounds in blueberry fruit tissue. Three 5-g composite samples from 30 berries were extracted twice with 20 mL 80% acetone containing 0.2% formic acid using a Polytron homogenizer (Brinkmann Instruments Inc., Westbury, NY, USA) for 1 min. Extracts (40 mL) were combined and concentrated to 1 mL using a Buchler Evapomix (Fort Lee, NJ, USA) in a water bath at 35 °C. The concentrated sample was dissolved in 10 mL of acidified water (3% formic acid) and then passed through a C_{18} Sep-Pak cartridge (Waters Associated, Millipore, Milford, MA, USA), which was previously activated with methanol followed by water and then 3% aqueous formic acid. Anthocyanins and other phenolics were adsorbed onto the column while sugars, acids, and other water-soluble compounds were eluted. The anthocyanins and other phenolics were then recovered with 2 mL of acidified methanol containing 3% formic acid. The methanol extract was passed through a 0.45-μm membrane filter (Millipore, MSI, Westboro, MA, USA) and 20 μL was analyzed by HPLC. The samples were analyzed using a Waters (Waters Associated, Millipore, Milford, MA, USA) HPLC system equipped with two pumps (600 E system Controller) coupled with a photodiode array detector (Waters 990 Series). Samples were injected at ambient temperature (20 °C) onto a reverse phase NOVA-PAK C_{18} column (150 × 3.9 mm, particle size 4 μm) (Waters Associated, Millipore, Milford, MA, USA) with a guard column (NOVA-PAK C_{18}, 20 × 3.9 mm, particle size 4 μm) (Sentry guard holder universal). The mobile phase was acidified water containing 2.5% formic acid (A) and acetonitrile (B). The flow rate was 1 mL/min, with a gradient profile consisting of A with the following proportions (v/v) of B: 0 min, 3%, 1–10 min, 3–6% B; 10–15 min, 6% B; 15–35 min, 6–18% B; 35–40 min, 18–20% B; 40–45 min, 20–100% B; 45–50 min, 100% B. The phenolic compounds in fruit extracts were identified by their UV spectra, recorded with a diode-array detector and by chromatographic comparison with authentic markers. Individual flavonols and anthocyanins were quantified by comparison with an external standard of chlorogenic acid, resveratrol, myricetin, quercetin, kaempferol, or cyanidin-3-galactoside. Scanning between 250 and 550 nm was performed and data were collected by the Waters 990 3-D chromatography data system.

2.9. Statistical analysis

Data were subjected to analysis of variance using NCSS (NCSS 2007, Kaysville, UT, USA). ORAC and DPPH values, total phenolics, and total anthocyanin were evaluated by the Tukey–Kramer Multiple-Comparison test. Differences at $P \leqslant 0.05$ were considered significant. Correlation coefficients were calculated using the software, Microsoft Excel (Microsoft, 2003, Roselle, IL, USA).

3. Results

3.1. Instant effect of various doses of UV-C illumination

3.1.1. Effect on total phenols, anthocyanins, and antioxidant capacities

Different doses of UV-C illumination induced different degrees of response in blueberry fruit. Samples taken immediately after UV-C illumination showed that all UV-C doses used in this study including 0.43, 2.15, 4.30, and 6.45 kJ m^{-2} increased the levels of total phenols and anthocyanins compared to control (Table 1). The increases appeared to be dose-dependent for 0.43 and 2.15 kJ m^{-2}. However, UV-C doses higher than 4.3 kJ m^{-2} did not further enhance the levels of total phenols and anthocyanins. Instead, a slight decline in these levels were observed after blueberries were exposed to 6.45 kJ m^{-2}.

Antioxidant capacities measured by ORAC were also increased by the UV-C treatment. UV-C doses at 2.15 and 4.30 kJ m^{-2} were the most effective in promoting antioxidant capacities (Table 1). The DPPH and hydroxyl radical scavenging capacities had a similar response as the oxygen radical absorbance capacities to the UV-C exposure.

3.1.2. Effect on major phenolics and flavonoid content

Major phenolics and flavonoid components in blueberries include chlorogenic acid, resveratrol, myricetin-3-arabinoside, quercetin-3-galactoside, quercetin-3-glucoside, kaempferol-3-glucoside, and kaempferol-3-glucuronide with quercetin-3-galactoside as the predominant constituent and kaempferol-3-glucoside as a minor compound (Table 2). Resveratrol and myricetin-3-arabinoside were present at moderate amounts. Treatment with a UV-C dose at 0.43 kJ m^{-2} significantly increased the amounts of myricetin-3-arabinoside, quercetin-3-galactoside, and kaempferol-3-glucoside. Exposure to 2.15 or 4.30 kJ m^{-2} increased the levels of all the major phenolics and flavonoids detected including chlorogenic acid, resveratrol, and quercetin derivatives, in addition to myricetin-3-arabinoside, quercetin-3-galactoside, and kaempferol-3-glucoside. It appears that all individual phenolic compounds were affected similarly by the UV-C treatment. UV-C doses at 2.15 or

Table 1
Effect of different doses of UV-C illumination on total phenolics, anthocyanins, and antioxidant capacities [oxygen radical absorbance capacity (ROO·; ORAC), 2,2-di-(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH) and hydroxyl radical scavenging capacity (·OH; HOSC)] in blueberries (Duke).[a,b]

Treatment (kJ m^{-2})	Total phenolics (mg/g fwt)	Anthocyanins (mg/g fwt)	ORAC (μmol TE/g fwt)	HOSC (μmol TE/g fwt)	DPPH (μmol GAE/g fwt)
0	3.12 ± 0.06	2.02 ± 0.03	40.4 ± 3.2	48.4 ± 0.6	30.5 ± 0.6
0.43	4.05 ± 0.07	2.38 ± 0.06	54.4 ± 7.6	59.7 ± 1.4	38.3 ± 0.8
2.15	4.97 ± 0.09	2.87 ± 0.04	63.2 ± 3.0	66.8 ± 2.1	40.8 ± 0.7
4.30	4.96 ± 0.11	3.11 ± 0.09	59.6 ± 2.0	79.3 ± 0.8	43.8 ± 0.9
6.45	4.72 ± 0.03	2.42 ± 0.12	54.1 ± 5.1	66.4 ± 0.7	34.6 ± 6.8
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Significance[c] Treatment	*	*	*	*	*

[a] Samples were taken immediately after UV-C illumination.
[b] Data expressed as mean ± SE ($n = 3$).
[c,*] Significant at $P \leqslant 0.05$.

Table 2
Effects of different doses of UV-C illumination on major phenolics and flavonoid content in blueberries (Duke).[a,b]

Treatment (kJ m^{-2})	Chlorogenic acid[c]	Resveratrol[d]	Myricetin-3-arabinoside[e]	Quercetin-3-galactoside[f]	Quercetin-3-glucoside[f]	Kaempferol-3-glucoside[g]	Kaempferol-3-glucuronide[g]
0	40.6 ± 4.8	13.0 ± 0.7	12.8 ± 0.2	98.7 ± 7.5	54.5 ± 1.3	2.1 ± 0.2	24.9 ± 1.2
0.43	38.4 ± 0.2	14.6 ± 2.0	15.3 ± 3.2	146.6 ± 9.7	62.9 ± 2.4	4.5 ± 0.1	29.9 ± 0.5
2.15	55.3 ± 6.8	16.2 ± 0.9	17.2 ± 0.8	177.3 ± 3.0	75.5 ± 4.2	3.3 ± 0.4	32.7 ± 2.6
4.30	45.1 ± 6.1	17.4 ± 0.2	17.2 ± 0.4	181.9 ± 2.4	83.4 ± 4.8	3.9 ± 0.9	32.3 ± 0.4
6.45	46.0 ± 5.3	17.0 ± 0.3	14.9 ± 0.9	176.2 ± 5.1	70.8 ± 1.7	3.2 ± 0.3	29.0 ± 3.3
P value	0.12053	0.03626	0.00294	0.01105	0.00171	0.03076	0.09677
Significance[h]							
Treatment	ns	*	*	*	*	*	ns

[a] Samples were taken immediately after UV-C illumination.
[b] Data expressed as mean ± SE (n = 3).
[c] Data expressed as μg of chlorogenic acid equivalents per g of fresh weight.
[d] Data expressed as μg of *trans*-resveratrol equivalents per g of fresh weight.
[e] Data expressed as μg of myricetin equivalents per g of fresh weight.
[f] Data expressed as μg of quercetin equivalents per g of fresh weight.
[g] Data expressed as μg of kaempferol equivalents per g of fresh weight.
[h,*,ns] Significant or non-significant, respectively, at $P \leqslant 0.05$.

4.30 kJ m^{-2} induced comparable quantitative increases, but no qualitative changes.

3.1.3. Effect on major anthocyanin content

Malvidin-3-galactoside was found to be the main anthocyanin in blueberries followed by malvidin-3-arabinoside, petunidin-3-galactoside, petunidin-3-glucoside, petunidin-3-arabinoside, delphinidin-3-galactoside, cyanidin-3-galactoside, and delphinidin-3-arabinoside (Table 3). The levels of malvidin-3-galactoside, delphinidin-3-galactoside, delphindin-3-arabinoside, and petunidin-3-arabinoside were increased by the UV-C exposure at 0.43 kJ m^{-2}. The higher the UV-C doses up to 2.15 kJ m^{-2}, the higher the stimulation of the individual anthocyanins detected in this study. However, a decline in the levels of most of the individual anthocyanins was found when the UV-C dose increased to 4.3 or 6.45 kJ m^{-2}. UV-C illumination increased the concentrations of all individual anthocyanins, but did not influence the ratios of the flavonols and anthocyanins.

3.2. Residual effect of UV-C illumination

3.2.1. Changes of total phenols, anthocyanins, antioxidant capacities, and hydroxyl radical scavenging capacities in blueberries after UV-C illumination

Little information is available on the residual effect of UV-C illumination. In our study, we measured the changes of total phenols, anthocyanins, antioxidant capacities, and hydroxyl radical scavenging capacities in blueberry fruit at various time intervals after

Table 3
Effects of different doses of UV-C illumination on major anthocyanin content in blueberries (Duke).[a,b,c]

Treatment (kJ m^{-2})	Delphinidin-3-galactoside	Cyanidin-3-galactoside	Delphinidin-3-arabinoside	Petunidin-3-galactoside	Petunidin-3-glucoside	Petunidin-3-arabinoside	Malvidin-3-galactoside	Malvidin-3-arabinoside
0	90.2 ± 12.7	62.4 ± 8.1	32.4 ± 0.4	164.4 ± 12.2	157.4 ± 8.5	121.2 ± 4.1	788.6 ± 14.3	370.0 ± 6.2
0.43	136.0 ± 13.6	66.7 ± 7.8	51.3 ± 0.9	156.4 ± 7.0	147.9 ± 4.5	174.1 ± 9.1	1087.7 ± 20.9	530.3 ± 7.5
2.15	199.1 ± 4.2	72.8 ± 1.1	81.6 ± 1.7	307.5 ± 0.7	218.5 ± 13.2	192.4 ± 12.1	1251.9 ± 12.1	664.8 ± 9.4
4.30	189.6 ± 3.6	76.1 ± 10.1	70.2 ± 0.4	218.8 ± 8.7	237.7 ± 6.4	186.0 ± 10.0	1237.7 ± 13.9	612.0 ± 6.8
6.45	130.3 ± 11.9	62.1 ± 1.4	34.3 ± 0.2	145.9 ± 9.9	136.5 ± 4.8	155.0 ± 9.1	1113.3 ± 16.7	544.5 ± 3.8
P value	0.0316	0.60716	<0.0001	0.0003	0.0137	0.0029	0.0013	0.00215
Significance[d]								
Treatment	*	ns	*	*	*	*	*	*

[a] Samples were taken immediately after UV-C illumination.
[b] Data expressed as mean ± SE (n = 3).
[c] Data expressed as μg of cyaniding-3-galactoside equivalents per g of fresh weight.
[d,*,ns] Significant or non-significant, respectively, at $P \leqslant 0.05$.

Table 4
Changes of total phenolics, anthocyanins, and antioxidant capacities [oxygen radical absorbance capacity (ROO$^{\cdot}$; ORAC), 2,2-di-(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH) and hydroxyl radical scavenging capacity ($^{\cdot}$OH; HOSC)] in blueberries (Duke) after being illuminated with 4.30 kJ m^{-2} UV-C and held for various lengths of time at 20 °C.[a]

Time after UV-C illumination	Total phenolic (mg/g fwt)	Anthocyanin (mg/g fwt)	ORAC (μmol TE/g fwt)	HOSC (μmol TE/g fwt)	DPPH (μmol GAE/g fwt)
0 min	4.96 ± 0.11	3.12 ± 0.09	59.6 ± 2.0	79.3 ± 0.9	43.8 ± 1.3
60 min	4.29 ± 0.14	2.65 ± 0.06	58.5 ± 2.6	75.7 ± 0.8	37.9 ± 0.8
180 min	3.76 ± 0.12	2.57 ± 0.08	47.3 ± 1.9	68.9 ± 1.4	34.7 ± 0.6
300 min	3.54 ± 0.08	2.48 ± 0.04	47.6 ± 2.8	62.4 ± 0.7	34.5 ± 0.2
540 min	3.39 ± 0.06	2.29 ± 0.05	45.4 ± 2.0	58.6 ± 1.2	33.0 ± 0.6
1440 min	3.31 ± 0.05	2.13 ± 0.04	44.1 ± 3.1	52.1 ± 0.9	32.6 ± 0.3
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Significance[b]					
Treatment	*	*	*	*	*

[a] Data expressed as mean ± SE (n = 3).
[b,*] Significant, at $P \leqslant 0.05$.

the fruit had been exposed to 4.3 kJ m^{-2} UV-C. We found that the levels of these components and capacities were stimulated immediately after UV-C illumination as described in the previous sections. However, these levels gradually declined with time (Table 4). Samples taken at 1440 min after UV-C treatment contained much lower amounts of total phenols and anthocyanins than those taken immediately after the illumination. The decreases were not linear over time. The greatest decline occurred within the first 180 min following UV-C exposure and then gradually tapered off. After 1440 min, the concentrations of phenolic compounds were back to levels comparable to those of the initial samples (Table 1).

3.2.2. Changes of major phenolics and flavonoid content in blueberries after UV-C illumination

The main phenolics, chlorogenic acid, increased markedly immediately following UV-C illumination (Table 5). However, it declined steadily with time. After 1440 min following UV-C treatment, the amount of chlorogenic acid in treated samples decreased to a level comparable to those of the non-illuminated samples (Table 2). The major flavonoids such as quercetin-3-galactoside, myricetin-3-arabinoside, and resveratrol reacted to the UV-C treatment in a similar way. Residual effects of UV-C were evident from the change of levels of these flavonoids after the illumination, but the effects weakened with time.

3.2.3. Changes of major anthocyanins content in blueberries after UV-C illumination

Similar to phenolics and flavonoids, major anthocyanins increased substantially right after UV-C exposure (Table 6). Although there were obvious residual effects after UV-C treatment, these effects diminished with time. This phenomenon was especially noticeable in malvidin-3-galactoside, malvidin-3-arabinoside, petunidin-3-glucoside, petunidin-3-galactoside, delphindin-3-galactoside, and cyanidin-3-galactoside.

4. Discussion

Anthocyanins and phenolic compounds are largely responsible for the antioxidant capacity in plant tissues (Larson, 1988). Natural anthocyanins are glycosides which release aglycone forms (anthocyanidins) by hydrolysis. Anthocyanins have been reported to help reduce damage caused by free-radical activity such as low-density lipoprotein oxidation, platelet aggregation, and endothelium-dependent vasodilation of arteries (Heinonen et al., 1998). The increases of anthocyanins, phenolics, and antioxidant capacities in UV-C irradiated blueberries may allow for additional quenching of active oxygen species. Enhancement of anthocyanin levels by UV-C treatment also has been reported in strawberries and sweet cherries (Baka, Mercier, Corcuff, Castaigne, & Arul, 1999; Kataoka, Beppu, Sugiyama, & Taira, 1996). Fruit undergoing UV-C (9.2 kJ m^{-2}) and heat treatment (45 °C) retained better fruit quality and antioxidant activity than control fruit in boysenberries (Vicente et al., 2004). In grapes, accumulation of anthocyanin occurred in the skins when berry sections were exposed to UV irradiation (Kataoka, Sugiyama, & Beppu, 2003).

Stimulation of the biosynthesis of phenolic compounds in epicarp and mesocarp cells and biochemical reinforcement of the cell wall through lignification and suberization induced by UV-C treatment have been reported in tomatoes (Charles, Goulet, & Arul, 2008). These modifications have been implicated in the increased

Table 5
Changes of major phenolics and flavonoid content in blueberries (Duke) after being illuminated with 4.3 kJ m^{-2} UV-C and held for various lengths of time at 20 °C.[a]

Time after UV-C illumination	Chlorogenic acid[b]	Resveratrol[c]	Myricetin-3-arabinoside[d]	Quercetin-3-galactoside[e]	Quercetin-3-glucoside[e]	Kaempferol-3-glucoside[f]	Kaempferol-3-glucuronide[f]
0 min	45.1 ± 6.1	17.4 ± 0.2	16.7 ± 1.6	181.9 ± 2.4	83.4 ± 4.8	3.9 ± 0.9	32.3 ± 0.3
60 min	44.6 ± 2.7	16.4 ± 1.1	14.6 ± 0.3	171.1 ± 9.5	78.9 ± 8.9	2.6 ± 0.6	28.6 ± 2.4
180 min	42.1 ± 2.4	16.9 ± 2.1	12.2 ± 1.4	152.7 ± 6.1	71.5 ± 8.6	2.3 ± 0.8	26.8 ± 2.2
300 min	42.8 ± 3.2	15.2 ± 0.3	10.8 ± 0.8	134.6 ± 1.8	63.6 ± 0.3	2.2 ± 0.3	25.1 ± 0.8
540 min	41.7 ± 2.1	13.8 ± 0.8	9.2 ± 0.6	117.9 ± 6.3	60.2 ± 5.8	2.1 ± 0.6	24.9 ± 3.1
1440 min	40.8 ± 0.4	13.6 ± 0.2	8.7 ± 1.3	109.4 ± 1.2	57.1 ± 1.7	2.3 ± 0.7	25.7 ± 2.8
P value	0.0035	0.0429	0.0058	0.0009	0.0163	0.0398	0.0221
Significance[g]							
Treatment	*	ns	*	*	*	ns	*

[a] Data expressed as mean ± SE ($n = 3$).
[b] Data expressed as μg of chlorogenic acid equivalents per g of fresh weight.
[c] Data expressed as μg of *trans*-resveratrol equivalents per g of fresh weight.
[d] Data expressed as μg of myricetin equivalents per g of fresh weight.
[e] Data expressed as μg of quercetin equivalents per g of fresh weight.
[f] Data expressed as μg of kaempferol equivalents per g of fresh weight.
[g,*,ns] Significant or non-significant, respectively, at $P \leqslant 0.05$.

Table 6
Changes of major anthocyanins in blueberries (Duke) after being illuminated with 4.30 kJ m^{-2} UV-C and held for various lengths of time at 20 °C.[a,b]

Time after UV-C illumination	Delphinidin-3-galactoside	Cyanidin-3-galactoside	Delphinidin-3-arabinoside	Petunidin-3-galactoside	Petunidin-3-glucoside	Petunidin-3-arabinoside	Malvidin-3-galactoside	Malvidin-3-arabinoside
0 min	189.6 ± 3.6	76.1 ± 10.1	70.2 ± 0.4	218.8 ± 13.6	237.7 ± 6.4	186.0 ± 9.6	1237.7 ± 19.9	612.0 ± 2.8
60 min	181.4 ± 9.3	68.9 ± 7.2	67.4 ± 3.2	197.8 ± 17.1	198.5 ± 4.3	187.4 ± 11.6	1204 ± 14.2	561.3 ± 0.3
180 min	170.8 ± 7.6	65.3 ± 3.1	65.6 ± 0.8	185.6 ± 12.6	189.3 ± 12.2	183.8 ± 6.2	1054.1 ± 12.2	463.4 ± 7.0
300 min	162.7 ± 3.2	66.3 ± 8.6	54.3 ± 1.6	180.8 ± 8.2	172.1 ± 8.4	171.2 ± 7.8	986.4 ± 10.2	459.1 ± 7.7
540 min	156.9 ± 4.5	64.8 ± 7.8	51.7 ± 2.8	169.8 ± 5.6	163.9 ± 4.1	165.6 ± 3.6	871.2 ± 15.3	447.4 ± 8.9
1440 min	147.2 ± 5.8	63.2 ± 6.9	46.5 ± 1.1	167.2 ± 7.8	166.9 ± 9.6	152.4 ± 2.7	861.1 ± 6.2	385.7 ± 4.5
P value	<0.0001	0.0762	<0.0001	0.0050	0.0014	<0.0001	<0.0001	<0.0001
Significance[c]								
Treatment	*	ns	*	*	*	*	*	*

[a] Data expressed as mean ± SE ($n = 3$).
[b] Data expressed as μg of cyaniding-3-galactoside equivalents per g of fresh weight.
[c,*] Significant, at $P \leqslant 0.05$.

disease resistance in UV-C treated tomato fruit. The beneficial effect of UV-C has also been linked to the activation of phenylalanine ammonia lyase (Nigro, Ippolito, Iattanzio, Di-Venere, & Salerno, 2000; Pan, Vicente, Martinez, Chaves, & Civello, 2004) and the accumulation of phytoalexins (Mercier, Arul, & Julien, 1993; Rodov, Ben-Yehoshua, Kim, Shapiro, & Ittah, 1992). In our study, we have found that UV-C illumination also enhanced levels of flavonoids and other antioxidant compounds.

UV-C exposure may act by inducing stress in plants. Increases in flavonoids and antioxidant capacities as described in this study might be part of the defense mechanism produced by blueberries in reacting to stress induced by UV-C illumination. The enrichment of stilbenes, such as resveratrol, in grapes in response to stress induced by postharvest UV-C irradiation has been described (Cantos, Espín, & Tomás-Barberán, 2002). The biological activity of resveratrol as an anticarcinogenic and an antioxidant has been reported (Jang et al., 1997). The increases of total phenols and anthocyanins in blueberries by UV-C illumination in our study appeared to be dose-dependent at lower doses (0.43–2.15 kJ m^{-2}). However, higher doses (4.30–6.45 kJ m^{-2}) tended to suppress these increases. This phenomenon has also been reported in strawberries where high doses of UV-C exposure was thought to cause too much stress and possibly resulted in injury (Baka et al., 1999).

Previous studies have showed that UV-C exposure retarded fungal growth in various commodities, and consequently reduced decay development in several fruits and vegetables after harvest (Stevens et al., 1996; Wilson et al., 1997). UV-C light has been considered as a germicidal agent and the use of this treatment has been demonstrated to directly inhibit microbial growth (Allende & Artes, 2003; Stevens et al., 1996). UV-C may also retard the mold development by enhancing the resistance of plant tissues against pathogenic attack (Droby et al., 1993). Thus, adequate UV-C treatment can be beneficial and have practical impact for commercial practices in terms of reducing the spoilage of fresh produce. Any other benefits obtained from UV-C treatment would be additive. Even though our study showed that the effect of UV-C on antioxidant capacities, phenolics, and anthocyanins might be short-term in blueberries, the responses of other commodities or the effect using different dosages could be different. Further investigations may also be warranted to explore if combining UV-C illumination with other postharvest techniques would have a long lasting effect in increasing the levels of antioxidant activity and potential health-promoting nutraceutical compounds.

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