Effect of postharvest UV-B or UV-C irradiation on phenolic compounds and their transcription of phenolic biosynthetic genes of table grapes

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Abstract Ultraviolet (UV) irradiation has been related to the extension shelf-life and maintenance of postharvest quality in fruits. However, the comparison of UV-B and UV-C treatment on the biosynthesis of phenolic compounds of grape remain unclear. This study provides a comparison on the mechanism of phenolic secondary metabolism at the same dose of 3.6 kJ m^{-2} UV treatment. Total phenolic compounds, total flavonoid, total flavanol, and total anthocyanin content and antioxidant activities of grapes after UV-C treatments were higher than those of the control and UV-B treatment. Among the evaluated parameters of individual phenolic compounds, the content of *trans*-resveratrol showed the highest percentage increase after the UV application. The transcriptions of *PAL*, *CHS*, *F3H*, *LAR*, *ANS* and *STS* were higher in grapes treated by UV-C than in those treated by UV-B. The *CHS*, *LAR*, *ANS* and *STS* genes were more induced in UV-B treatment than in control group. The same applied dose of UV-B or UV-C irradiation have different impact on gene expression and phenolic metabolites synthesis. The UV-C irradiation

stimulated a higher gene expression of the phenolic compounds biosynthesis and also induced a greater accumulation of these metabolites at the same applied dose.

Keywords Grape · Ultraviolet irradiation · Phenolic compounds · Antioxidant activity

Introduction

Grapes are one of the most consumed fruit worldwide due to the diversity of grape processing and consumption. Phenolics in grapes include two classes: flavonoids, such as flavanols and anthocyanins; non-flavonoids, comprising hydroxycinnamic, hydroxybenzoic acids and stilbenes. The benefits of consumption of grapes have been associated with the abundant polyphenol content. The quality of grape is determined by the phenolic compounds. Moreover, these phenolic compounds have many health benefits, such as antioxidant, anti-microbial, and anticarcinogenic characteristics (Giovinazzo and Grieco 2015).

Ultraviolet (UV) irradiation is a viable physical technique in postharvest fruits for commercial application. Many studies have reported that UV irradiation induces the synthesis and the accumulation of phenolic compounds in fruit and vegetables. Darre et al (2017) observed that UV-B irradiation could increase antioxidant capacity, reduced weight loss, and delayed yellowing in broccoli florets. Pinheiro et al. (2015) indicated that UV-C irradiation increased the content of total phenolic compounds in tomato during storage. However, Marais et al. (2001) reported that UV-B irradiation did not induce anthocyanin accumulation in European pears. There are some opposite results in previous studies on the effects of UV irradiation on phenolic biosynthesis. Therefore, the effect of

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postharvest UV irradiation on phenolic metabolism has become a prominent issue. In addition, the effect of postharvest UV irradiation on changes of individual phenolic compounds and changes of phenolic biosynthetic key gene expression in grapes remain unclear.

Little is known about whether the expression of key structural genes in phenolic metabolism is regulated by different UV irradiation at the same applied dose in grapes. In addition, it is still unknown whether there are different response mechanisms to two types of UV irradiation (UV-B or UV-C). In order to answer these questions, our research was to investigate the effect of postharvest UV-B or UV-C irradiation on phenolic compounds and antioxidant capacity. In addition, the chemical and molecular mechanism of relevant secondary metabolism was studied. The genes expression involved in the biosynthesis of these compounds were also studied, which may explain how the different types of UV irradiation affect the phenolic metabolism at molecular level.

Materials and methods

Plant materials and ultraviolet irradiation treatment

Grapes (*V. vinifera* × *V. labrusca* cv. Summer Black) were purchased from the commercial vineyard in the Hefei district of Anhui province (north latitude 31.86, east longitude 117.27) in July 2016. The grape samples were washed and dried at room temperature for a short time.

Grape berries were divided into three groups, and each group has 80–100 grapes. Control group, grapes were not exposed to UV irradiation and placed in dark at 23 ± 2 °C with relative humidity of 95% during 3 days storage; UV-B group, grapes were exposed to 1.8, 3.6, 7.2 kJ m⁻² UV-B irradiation; UV-C group, grapes were exposed to same dose of 1.8, 3.6, 7.2 kJ m⁻² UV-C irradiation.

Grapes were treated with UV-B or UV-C inside the UV radiation device (30 W, Philips). The distance of the UV lamp from the fruits was adjusted to obtain the required intensity (0.527 mW cm⁻²) by using a portable digital radiometer (TAINA TN-2254, TAINA Co., Ltd., Taiwan, China). The exposure time was altered until the grapes received a radiation dose of 1.8, 3.6, 7.2 kJ m⁻² (Crupi et al. 2013). Each grape was kept under light source for half the time, rotated at 180°, and subjected to the additional time in order to reach to the applied dose. Following UV treatment, grapes were stored under the same storage condition as control group. Grapes were collected after 3 days of storage, frozen in liquid nitrogen and kept at -80 °C for the following analysis.

Chemicals

The standard gallic acid, protocatechuic acid, caffeic, *trans*-ferulic, chlorogenic, naringenin, quercetin, quercetin 3-glucoside, rutin, catechin, epicatechin, epicatechin gallate, *trans*-resveratrol, *trans*-piceid, Folin-Ciocalteu reagent, 4-dimethylaminocinnamaldehyde (DMACA), DPPH reagent, and ABTS reagent were purchased from Sigma Chemical Co. (St. Louis, USA).

Fruit quality

Firmness of grape was determined by performing a texture analyzer (TA-XT2i, Stable Micro Systems, Guildford, UK) with a 2 mm diameter probe at a speed of 2 mm s⁻¹ on the skin of each sample. Firmness was expressed as the maximum penetration force (N). According to the previous methods described by the AOAC (1995), total titratable acidity, total soluble solids, and pH were also analyzed.

Surface color of grapes was measured using a chromometer (Chroma Meter WSC-S, Shanghai Precision and Scientific Instrument Co. Ltd., Shanghai, China), and the parameters (*L**, *a** and *b** values) were obtained by the CIE color system.

Ascorbic acid and chlorophyll

Ascorbic acid was determined according to the method described by Liu et al. (2011). The sample was added to 20 mL of buffer solution containing 4 g L⁻¹ anhydrous sodium acetate and 1 g L⁻¹ oxalic acid. The fruit extract was titrated with a calibrated DPIP solution and the titration was repeated using 0.10 g L⁻¹ ascorbic acid solution. Result was expressed as mg/100 g of fresh weight (FW) basis.

The content of total chlorophyll was measured according to the method previously described by Wang et al. (2017) with a slight modification. 1 g of grape sample was ground and extracted in 80% acetone. The supernatant measured at 663 and 645 nm. The chlorophyll content was expressed as mg/100 g of fresh weight (FW) basis.

PPO and POD activity

PPO activity and POD activity was measured according to the method of Wang et al. (2014) with some modifications. For PPO activity assay, 2.0 g of grape sample was homogenized with 10 mL of 0.1 mol L⁻¹ phosphate buffer, including 5% polyvinylpyrrolidone. The mixture was centrifuged at 5000g for 10 min at 4 °C. The reaction mixture contained 0.5 mL supernatant, 0.1 mol L⁻¹ buffered substrate and 0.5 mol L⁻¹ catechol. The absorbance

of the mixture was obtained at 398 nm. The results were expressed as U/min/g FW.

For POD activity assay, 2.0 g of grape sample was homogenized in 10 mL of 0.05 mol L⁻¹ phosphate buffer. The mixture was centrifuged at 5000g for 10 min at 4 °C. The reaction mixture contained 0.1 mL of supernatant, 0.05 mol L⁻¹ buffered substrate, and 0.04 mol L⁻¹ guaiacol. The absorbance was measured at 460 nm. The results were expressed as U/min/g FW.

Extractions of phenolic compounds

A cold ethanol extract was prepared according to the method described by Crupi et al. (2013), with some modifications. Equal amounts of grape skin were extracted with 10 mL of 70% (v/v) ethanol containing 1% hydrochloric acid for 60 min at 20 °C and subjected to ultrasonic treatment (SK3310HP, KUDOS, China) for 5 min. The power of ultrasonic treatment was 180 W. The mixture was centrifuged (Centrifuge 5418R, Eppendorf, Germany) at 5000g for 10 min at 4 °C, and the residue was re-extracted twice as described above. Then, the three supernatants were combined, and the extract has been filtered through 0.45 µm filters and stored at -20 °C till further analysis.

Total phenolic compounds, total flavonoid, total flavanol, and total anthocyanin of extracts

Total phenolic content in grape extracts was measured by the Folin–Ciocalteu colorimetric method (Farhadi et al. 2016), with some modifications. 50 µL of grape extracts was mixed to 1.0 mL of the Folin–Ciocalteu reagent. After stand for 10 min, 0.5 mL of 7.5% Na₂CO₃ solution was added to the mixture. After 30 min at room temperature, the absorbance of mixture was recorded at 765 nm using a spectrophotometer (UV-vis, Evolution 201, thermo fisher). Gallic acid (GAE) was employed as a calibration standard and results were expressed as mg GAE per g of grape berries fresh weight (FW).

According to the methods described by Farhadi et al. (2016), total flavonoid content was measured with some modifications. 50 µL of extracts was added to 950 µL of deionized water. Then, 75 µL of 5% NaNO₂ solution was added to this dilution. After standing for 5 min, 150 µL of 10% AlCl₃·6H₂O solution and 500 µL of 1 mol L⁻¹ NaOH were added. After 5 min, this mixture was added to 3.0 mL with deionized water. The absorbance was recorded at 510 nm. Rutin was employed as a calibration standard and results were expressed as mg rutin (RE) equivalents per g of grape berries FW.

Total flavanol content was measured by the 4-dimethylaminocinnamaldehyde (DMACA Katalinic et al. 2010). The DMACA solution contained 0.1%

DMACA in a dilution of HCl:methanol (1:10, v/v). 10 µL of extracts was diluted with 190 µL of methanol. Then, 1 mL of DMACA solution was added to the dilution of extracts. The absorbance was recorded at 510 nm. Total flavanols content was calculated by (+)-catechin (CAT) as a standard. The results are expressed in mg CAT equivalents per g of grape berries FW.

The total anthocyanin content was determined by using a pH differential method (Farhadi et al. 2016). The anthocyanin content in extracts was expressed as Cy-3-glc equivalents.

Analysis of individual phenolic compounds by HPLC–ESI–MS

Individual phenolic compounds were analysed according to He et al. (2010), slightly modified by HPLC–ESI–MS technologies (HPLC–LTQ Orbitrap XL ETD, Thermo Fisher). The separation was performed on hypersil Gold C18 column (150 mm × 2.1 mm i.d; 3 µm particle size, Thermo), using injection volume of 10 µL and flow-rate of 0.3 mL min⁻¹. The mobile phase A (0.1% formic acid, v/v) and a mobile phase B (acetonitrile), using a gradient program as follows: from 5 to 18% B (10 min), from 18 to 98% B (5 min), 98% isocratic (15 min), from 98 to 5% (1 min), 5% B (9 min). MS–ESI was used in negative mode with a capillary voltage of -35 V. Nitrogen was used both as sheath gas at flow rates of 30 arb, and as Aux gas at 10 arb. The capillary temperature was 275 °C. The scan range was 50–1000 m/z.

Antioxidant activity

DPPH radical scavenging activity of the extract was determined according to the method described by Sharma and Bhat with slight modification (Sharma and Bhat 2009). 0.1 mL of extract was added to 2.9 mL of 6 × 10⁻⁵ mol L⁻¹ DPPH methanolic solution. After incubated in dark for 30 min, the extracts was measured at 517 nm. The results was calculated by using the relationship: $I(\%) = (A_{DPPH} - A_{sample}) / A_{DPPH} \times 100$, where A_{DPPH} is the absorption of the DPPH control solution against the blank of solvent, and A_{sample} is the absorption of the extract against the blank of sample solution.

The free radical-scavenging activity was determined by ABTS radical cation decolourization assay described by Re et al. (1999). The ABTS⁺ reaction solution was prepared by mixing 7 µmol L⁻¹ ABTS solution with 2.45 µmol L⁻¹ potassium persulfate and kept for 12–16 h at room temperature. Then, the reaction solution was diluted to an absorbance of 0.7 ± 0.02 at 734 nm as the test solution. 100 µL of extracts was added to 3 mL of ABTS reaction

solution. After incubation for 6 min, the absorbance was determined at 734 nm. The calculation method was the same as DPPH radical-scavenging activity.

RNA extraction and quantitative PCR

Total RNA was extracted from grape skin using Plant RNA Extraction Kit (Takara, Dalian, China) according to the instruction of the manufacturer. The synthesis of cDNA was processed by Takara PrimeScript RT reagent Kit (Takara, Dalian, China). The qPCR reaction parameters were according to the manufacturer's instructions. The genes were chosen on account of the phenolic biosynthesis pathway, which mainly affected by the UV treatments. The following primers for phenylalanine ammonia lyase (*PAL*), chalcone synthase (*CHS*), flavanone 3-hydroxylase (*F3H*), anthocyanidin synthase (*ANS*), leucoanthocyanidin reductase (*LAR*), stilbene synthase (*STS*) and the control endogenous actin were used (Table S1) (Samuelian et al. 2009). The relative quantitation of gene expression between control and UV treatment was calculated using the Ct (Cycle threshold) value (Livak and Schmittgen 2001).

Statistical analysis

Statistical analysis was processed by SPSS 11.5 software (Chicago, USA). Data was presented as mean $\pm$ SD. The statistical difference was set at a significance level of $p < 0.05$. The homogeneity of variances was tested before the standard *t* test processing.

Results and discussion

Effect of postharvest UV irradiation on quality of grape

We initially evaluated the changes in grape treated at three distinct dose (1.8, 3.6, 7.2 kJ m⁻²) and with two different types UV (UV-B or UV-C) after storage for 3 days. In this study, grape fruit gradually softened when stored at shelf temperature. However, UV treatments effectively inhibited the softening compared with the control after storage for 3 days. Table 1 showed that application of UV-B or UV-C treatment at 3.6 kJ m⁻² was the most effective for keeping in firmness of grape. And the difference between UV-B and UV-C treatment was not significant ($p > 0.05$). The process of grape fruit softening may be connected with the increase of free radicals and reduction in activities of cell wall degrading enzymes (Barka et al. 2000).

Table 1 also showed the effect of the UV-B or UV-C treatment on different chemical parameters of grapes.

There were no significant changes of the quality such as pH, titratable acidity, and total soluble solids (TSS) content after the postharvest UV application. However, the TSS content in all treatments decreased during storage for 3 days. Similar results were obtained by Pinto et al. (2016), who indicated that the UV light treatment did not observe different effect on organic acids and sugars in grapes. George et al. (2016) also indicated that UV-C irradiation had no adverse effects on the quality of mangoes.

Surface color of grape is an important factor that can affect quality of fruit. As shown in Table 2, grape skin lightness (L^*) decreased during storage in all treatment. For UV-B or UV-C treatment at dose of 3.6 kJ m⁻², L^* value of grapes slowly decreased and significant difference was noticed after 3 days of storage compared with the control. The grape fruit exposed to does at 7.2 kJ m⁻² UV treatment exhibited significantly lower lightness values than the control. There was no significant difference in L^* value between the UV-B and UV-C treatment. No significant differences showed in a^* and b^* values which indicated that the UV-B or UV-C treatment did not lead to a negative effect in the color change of grape. Little changes were observed in color of grape berries during 3 days of storage. However, during long-term storage, berry browning may occur. A similar result has been reported for pineapple (Sari et al. 2016).

Effect of postharvest UV treatment on ascorbic acid and total chlorophyll content

As shown in Table 1, the ascorbic acid content presented decreasing trend in storage period in all treatments. However, the content of ascorbic acid in grape treated by UV-B or UV-C was higher than that in the control. Ascorbic acid content in UV-C treated fruit (5.68 mg/100 g) was higher than that in untreated fruit (5.11 mg/100 g) 3 days of storage. The exposure to UV-C irradiation causes stress in plant tissues, which stimulates the biosynthesis of defensive secondary metabolites with antimicrobial and antioxidant activity. In addition, the present results were similar with previous findings for pineapple (Sari et al. 2016).

To obtain further information regarding the role of UV treatment dose on grape color, the changes on total chlorophyll content were also determined (Table 1). During 3 days of storage, the chlorophyll content significantly decreased regardless of the treatment applied. Treatment with UV-B or UV-C at 7.2 kJ m⁻² induced a higher loss than the control. The effective UV-B or UV-C treatment at 3.6 kJ m⁻² retained higher chlorophyll content than the control. The previous work indicated that UV treatment could modulate the activity of chlorophyll degrading enzymes (Aiama et al. 2009). Darre et al. (2017) also suggested that UV-B irradiation at dose of 4 kJ m⁻²

Table 1 Effect of UV-B or UV-C treatment on firmness, total soluble solids content, total titratable acidity, pH, total ascorbic acid content, and total chlorophyll content of grape fruit during storage

Days after storage	Control	UV-B dose			UV-C dose		
	0 kJ m ⁻²	1.8 kJ m ⁻²	3.6 kJ m ⁻²	7.2 kJ m ⁻²	1.8 kJ m ⁻²	3.6 kJ m ⁻²	7.2 kJ m ⁻²
Firmness (N)							
0 days	6.12 ± 0.32	6.12 ± 0.32	6.12 ± 0.32	6.12 ± 0.32	6.12 ± 0.32	6.12 ± 0.32	6.12 ± 0.32
3 days	4.18 ± 0.21 c	4.78 ± 0.12 b	5.21 ± 0.11 a	4.29 ± 0.11 c	4.83 ± 0.12 b	5.46 ± 0.13 a	4.11 ± 0.15 c
Total soluble solids content (%)							
0 days	21.54 ± 0.77	21.54 ± 0.77	21.54 ± 0.77	21.54 ± 0.77	21.54 ± 0.77	21.54 ± 0.77	21.54 ± 0.77
3 days	18.56 ± 0.83 a	19.32 ± 0.75 a	19.20 ± 1.20 a	16.32 ± 0.67 b	17.89 ± 0.48 a	19.22 ± 1.31 a	16.11 ± 0.78 b
Total titratable acidity (g/kg)							
0 days	9.78 ± 0.67	9.78 ± 0.67	9.78 ± 0.67	9.78 ± 0.67	9.78 ± 0.67	9.78 ± 0.67	9.78 ± 0.67
3 days	10.3 ± 1.16 a	9.77 ± 0.67 a	10.08 ± 0.97 a	9.89 ± 0.78 a	10.56 ± 0.95 a	10.47 ± 0.97 a	10.67 ± 0.72 a
pH							
0 days	3.40 ± 0.01	3.40 ± 0.01	3.40 ± 0.01	3.40 ± 0.01	3.40 ± 0.01	3.40 ± 0.01	3.40 ± 0.01
3 days	3.43 ± 0.03 a	3.51 ± 0.04 a	3.41 ± 0.07 a	3.45 ± 0.03 a	3.51 ± 0.05 a	3.50 ± 0.04 a	3.41 ± 0.04 a
Ascorbic acid (mg/100 g FW)							
0 days	7.21 ± 0.23	7.21 ± 0.23	7.21 ± 0.23	7.21 ± 0.23	7.21 ± 0.23	7.21 ± 0.23	7.21 ± 0.23
3 days	5.11 ± 0.14 b	4.86 ± 0.15 b	5.66 ± 0.17 a	4.06 ± 0.21 c	4.77 ± 0.22 b	5.68 ± 0.12 a	3.89 ± 0.27 c
Total chlorophyll content (mg/100 g FW)							
0 days	3.11 ± 0.12	3.11 ± 0.12	3.11 ± 0.12	3.11 ± 0.12	3.11 ± 0.12	3.11 ± 0.12	3.11 ± 0.12
3 days	1.56 ± 0.13 bc	1.87 ± 0.15 b	2.55 ± 0.12 a	1.24 ± 0.14 c	1.72 ± 0.15 b	2.47 ± 0.22 a	1.32 ± 0.21 c

Values followed by different lowercase letters in the same row are significantly different from each other ($p < 0.05$). Data were expressed by mean ± SD

Table 2 Effect of UV-B or UV-C treatment on the color of grape fruit during storage

Days after storage	Control	UV-B dose			UV-C dose		
	0 kJ m ⁻²	1.8 kJ m ⁻²	3.6 kJ m ⁻²	7.2 kJ m ⁻²	1.8 kJ m ⁻²	3.6 kJ m ⁻²	7.2 kJ m ⁻²
L*							
0 days	25.43 ± 0.25	25.43 ± 0.25	25.43 ± 0.25	25.43 ± 0.25	25.43 ± 0.25	25.43 ± 0.25	25.43 ± 0.25
3 days	20.78 ± 0.25 b	20.34 ± 0.12 b	22.35 ± 0.12 a	18.85 ± 0.15 c	21.65 ± 0.15 b	23.41 ± 0.15 a	18.67 ± 0.13 c
a*							
0 days	6.55 ± 0.32	6.55 ± 0.32	6.55 ± 0.32	6.55 ± 0.32	6.55 ± 0.32	6.55 ± 0.32	6.55 ± 0.32
3 days	6.32 ± 0.28 a	6.55 ± 0.34 a	6.53 ± 0.21 a	6.57 ± 0.45 a	6.54 ± 0.37 a	6.55 ± 0.21 a	6.47 ± 0.31 a
b*							
0 days	1.55 ± 0.11	1.55 ± 0.11	1.55 ± 0.11	1.55 ± 0.11	1.55 ± 0.11	1.55 ± 0.11	1.55 ± 0.11
3 days	1.46 ± 0.13 a	1.52 ± 0.11 a	1.54 ± 0.15 a	1.61 ± 0.12 a	1.53 ± 0.13 a	1.56 ± 0.12 a	1.48 ± 0.13 a

Values followed by different lowercase letters in the same row are significantly different from each other ($p < 0.05$). Data were expressed by mean ± SD

delayed broccoli yellowing and improved chlorophyll retention. Thereafter, the increase of chlorophyll content might be related to their role as a protective function against oxidative damage from ROS brought about by UV treatment.

Effect of postharvest UV treatment on PPO and POD activity

As shown in Table 3, PPO and POD activities increased gradually during 3 days of storage. UV-B or UV-C treatment significantly enhanced the activities of PPO and POD. PPO and POD activities treated by UV-B or UV-C

Table 3 Effect of UV-B or UV-C treatment on PPO and POD activity of grape fruit during storage

Days after storage	Control	UV-B dose			UV-C dose		
	0 kJ m ⁻²	1.8 kJ m ⁻²	3.6 kJ m ⁻²	7.2 kJ m ⁻²	1.8 kJ m ⁻²	3.6 kJ m ⁻²	7.2 kJ m ⁻²
PPO (U/min/g FW)							
	8.25 ± 0.21	8.25 ± 0.21	8.25 ± 0.21	8.25 ± 0.21	8.25 ± 0.21	8.25 ± 0.21	8.25 ± 0.21
	9.52 ± 0.32 d	10.34 ± 0.88 cd	13.85 ± 0.26 b	14.23 ± 0.23 a	11.23 ± 0.21 c	14.78 ± 0.23 a	13.39 ± 0.38 b
POD (U/min/g FW)							
	5.12 ± 0.17	5.12 ± 0.17	5.12 ± 0.17	5.12 ± 0.17	5.12 ± 0.17	5.12 ± 0.17	5.12 ± 0.17
	5.87 ± 0.12 d	7.54 ± 0.32 c	9.56 ± 0.18 a	9.89 ± 0.21 a	8.41 ± 0.11 b	10.12 ± 0.43 a	9.67 ± 0.22 a

Values followed by different lowercase letters in the same row are significantly different from each other ($p < 0.05$). Data were expressed by mean ± SD

irradiation at dose of 1.8 kJ m⁻² increased slightly in grape fruit. In addition, UV-B or UV-C irradiation at dose of 7.2 kJ m⁻² significantly increased the PPO and POD activities compared with that in the control. A similar result has been reported for strawberry (Jin et al. 2017). Thus, our results suggested that the increased activities of defense enzymes might be one part of the protective mechanism induced by UV-B or UV-C irradiation in grape fruit.

Effect of postharvest UV irradiation on total phenolic compounds, total flavonoids, total flavanols, and total anthocyanins content of grape

UV irradiation has shown many regulatory effects on fruit and vegetables, and in particular on inducing the accumulation of secondary metabolites. On the basis of the observation that UV-B or UV-C treatment delayed the senescence of grape fruit, we hypothesized that UV-B or UV-C treatment at dose of 3.6 kJ m⁻² seemed to be the most effective in extending the postharvest life and improving the quality of grape fruit. Therefore, the UV-B or UV-C treatment at 3.6 kJ m⁻² was selected to further study the phenolic compounds content along with the expression of phenolic biosynthesizing genes. Compared with control group, the total phenolic compounds content was greatly affected by UV-C treatment, whereas UV-B showed a slight increase in total phenolic content (Fig. 1a). In our study, there was greater accumulation of phenolic compounds in grapes derived from the effect of UV-C irradiation. This effect could be explained by the protective mechanism. UV irradiation induced physiological stress and enhanced flavonol accumulation, which could be explained as a protective regulatory response through increasing antioxidant capacity (Del-Castillo-Alonso et al. 2016). The application of UV-C irradiation was also observed in tomato. UV-C treated tomato showed an increase of total phenolic content compared with those in untreated tomato during storage days. The increase of total

phenolic content after UV-C treatments could be considered an adaptation mechanism of tomato due to UV stress (Pinheiro et al. 2015).

The total flavonoid changes were shown in Fig. 1b. In our study UV-B irradiation did not show any differences compared with the control group at the applied dose. The total flavonoid content increased by UV-C irradiation compared with the control group in the present study. Similar results in regard to UV-C treatment have been reported in previous reports. Park and Kim (2015) reported that UV-C treatment at 2 kJ m⁻² increased total phenolic compounds and flavonoid content in garlic. Liu et al. (2012) also indicated that UV-C treatment at 4 kJ m⁻² increased the antioxidant activity and induced the accumulation of total flavonoids in tomato.

In this study, the flavanols content slightly increased by UV-B irradiation. Moreover, the flavanols content significantly increased by the application of UV-C compared with control and UV-B group (Fig. 1c). Wang et al. (2009) indicated that blueberries treated with UV-C significantly increased the amount of quercetin-3-*O*-galactoside. Wen et al. (2015) also indicated that UV-C treatment for 30 min promoted the accumulation of flavanol polyphenol in grapes.

The health benefits of grape may be for the high concentration of anthocyanins. In this study the anthocyanin content significantly increased by the UV-C application after postharvest treatment. Similarly, the UV-B treatment increased the anthocyanin content (Fig. 1d). Xu et al. (2016) indicated that UV-C irradiation at 6 kJ m⁻² stimulated the accumulation of flavonols and increased the anthocyanin in blueberry. Nguyen et al. (2017) also indicated that UV-B irradiation at the same dose of 6 kJ m⁻² stimulates an increase in anthocyanin biosynthesis in blueberry.

Among the evaluated parameters, the UV-C application greatly increased the content of these phenolic compounds

Fig. 1 Contents of total phenolic compounds (a), total flavonoid (b), total flavanol (c), total anthocyanin (d), DPPH (e) and ABTS (f) scavenging rate of ‘Summer Black’ grape berries with postharvest UV-B or UV-C irradiation. Bars represent mean and standard deviation. Values followed by different lowercase letters are significantly different from each other ($p < 0.05$)

after postharvest treatment. However, the UV-B treatment slightly increased at the same applied dose.

Antioxidant capacity

Although the different methods, such as DPPH and ABTS, have been used to assess the antioxidant activity of the grapes, the results were similar. Compared with the control group, the antioxidant activity measured by the DPPH assay in UV-C treated grapes greatly increased. Meanwhile, the UV-B treatment did not show any differences compared with the control (Fig. 1e). The activities determined by the ABTS assay in the UV-C treatments were also much higher than that in both the control and UV-B treatment (Fig. 1f).

The effect of UV-C on the increase of the antioxidant capacity may have attributed to the results of the accumulation of phenolic compounds. As it previously reported, the UV-C treatment had positive effect on the antioxidant activity in grapes (Marti et al. 2014). Our result showed a

positive correlation between total phenolic compounds and the antioxidant capacity.

Analysis of individual phenolic compounds

As the total phenolic content could not provide an overall picture of the quantification of the phenolic constituents present in the selected grape extracts, the identification of 14 individual phenolic compounds was measured by HPLC–ESI–MS. The results of identification of six classes of hydroxybenzoic acids (gallic acid, protocatechuic acid), hydroxycinnamic acids (caffeic, *trans*-ferulic, chlorogenic), flavanones (naringenin), flavonols (quercetin, quercetin 3-glucoside, rutin), flavan-3-ols (catechin, epicatechin, epicatechin gallate), and individual stilbene (*trans*-resveratrol, *trans*-piceid) in grape skin extracts are presented in Table 4. The major individual phenolic compounds in grapes increased when treated by UV-B or UV-C treatment. This increase was especially distinct in the UV-C treatment.

Di Lecce et al. (2014) found that the most abundant phenolic compounds in grape skins were flavan-3-ols, which was in accordance with our study (Table 4). Catechin was the main flavan-3-ols in the skins of grapes. Moreover, the content of catechin in UV-C group was almost 1.9-fold higher than in control group, and nearly 1.2-fold higher than in UV-B group.

Among flavonols quantified in all group, Quercetin 3-glucoside was the most abundant in ‘Summer Black’ table grape. The content of Quercetin 3-glucoside in UV-C group was around 1.5-fold and 1.3-fold higher than in the control and UV-B group, respectively. It was found that the different effect of UV treatment on the content of flavonols in grapes was in agreement with the previous results (Zhang et al. 2013).

With regards to stilbenic compounds, the content of *trans*-resveratrol in UV-C group was nearly 4.6-fold and 3.7-fold higher than in the control and UV-B group, respectively. Among the evaluated parameters of phenolic compounds, the content of *trans*-resveratrol showed the highest percentage increase after the postharvest application. Wang et al. (2010) also showed that UV-C treatment promoted the biosynthesis of resveratrol. The presence of hydroxycinnamic acids (caffeic, *trans*-ferulic, chlorogenic) was characteristic for grape skins. Among two quantified

hydroxybenzoic acids, gallic acid and protocatechuic acid were also found in all treatment groups (Table 4).

Expression levels of phenolic compounds biosynthetic genes

The relative expression of the genes involved in the phenolic synthesis in response to UV irradiation was examined in grapes. As is showed in Fig. 2, the postharvest UV-C treatment induced a significant effect on the relative gene expression in the biosynthetic pathway of phenolic compounds, such as *PAL*, *CHS*, *F3H*, *LAR*, *ANS* and *STS*. The application of postharvest UV-B only could induce higher levels of the gene encoding namely *CHS*, *ANS*, *LAR* and *STS*. More specifically, the expression of *PAL* in UV-C group was up-regulated nearly at 3-fold higher than the control group. Additionally, the expression of *CHS* in UV-C group was almost up-regulated at 8-fold and 4-fold higher than the control group and UV-B group. This effect was in accord with the results by Zhang et al. (2012), who indicated UV irradiation induced the phenolic compounds accumulation in grapes by up-regulated the structural genes expression.

Numerous evidences have clearly indicated that UV application in other plant promoted the flavonoids biosynthesis such as onion (Rodrigues et al. 2010). This

Table 4 The content of individual phenolic compounds in the extracts

		Control	UV-B	UV-C
	<i>Hydroxybenzoic acids</i>			
1	Gallic	11.23 ± 0.39 a	13.37 ± 0.88 b	16.33 ± 1.38 c
2	Protocatechuic	2.26 ± 0.51 a	3.72 ± 0.24 b	4.63 ± 0.48 c
	<i>Hydroxycinnamic acids</i>			
3	Caffeic	6.17 ± 0.24 a	6.79 ± 0.28 a	11.97 ± 0.76 b
4	<i>trans</i> -Ferulic	5.63 ± 0.26 a	5.88 ± 0.36 a	5.77 ± 0.29 a
5	Chlorogenic	7.12 ± 0.73 a	7.58 ± 1.41 a	12.48 ± 2.18 b
	<i>Flavanons</i>			
6	Naringenin	1.95 ± 0.16 a	3.26 ± 0.74 b	3.67 ± 0.89 b
	<i>Flavonols</i>			
7	Quercetin	8.71 ± 0.88 a	9.24 ± 0.69 a	12.72 ± 1.41 b
8	Quercetin 3-glucoside	15.36 ± 1.83 a	17.49 ± 1.861 b	24.33 ± 1.76 c
9	Rutin	10.55 ± 0.47 a	10.06 ± 1.04 a	15.18 ± 1.59 b
	<i>Flavan-3-ols</i>			
10	Catechin	48.25 ± 6.62 a	74.38 ± 5.50 b	93.59 ± 4.23 c
11	Epicatechin	25.53 ± 3.55 a	31.63 ± 1.74 b	39.15 ± 2.45 c
12	Epicatechin gallate	9.65 ± 0.51 a	10.15 ± 0.89 a	12.59 ± 0.76 b
	<i>Stilbene</i>			
13	<i>trans</i> -resveratrol	20.48 ± 1.31 a	25.30 ± 1.19 b	94.90 ± 7.2 c
14	<i>trans</i> -piceid	17.87 ± 1.53 a	23.51 ± 1.27 b	47.75 ± 3.06 c

The results are expressed as $\mu\text{g g}^{-1}$ of the fresh weight sample. Values followed by different lowercase letters in the same row are significantly different from each other ($p < 0.05$). Data were expressed by mean ± SD

Fig. 2 Relative expression of gene transcript accumulation of the biosynthesis pathway of phenolic compounds: phenylalanine ammonia lyase (*PAL*), chalcone synthase (*CHS*), flavanone 3-hydroxylase (*F3H*), Leucoanthocyanidin reductase (*LAR*) anthocyanidin synthase (*ANS*), and stilbene synthase (*STS*) in grapes treated by UV-B or UV-C irradiation. Bars represent mean and standard deviation. Values followed by different lowercase letters are significantly different from each other ($p < 0.05$)

study reveals that not all key genes are enhanced by UV-B irradiation. However, UV-C irradiation exhibited the same expression elevation in accord with the increase of phenolic compounds. UV-C irradiation seemed to have more significant response to genes expression than UV-B irradiation at the same applied dose. Previous study indicated that when fruits and vegetables are treated with extra UV, it produce some protective products such as flavonoids to protect itself from UV damage (Kolb et al. 2001).

Many previous reports indicated the effect of UV irradiation on the flavonoid metabolism, particularly anthocyanin biosynthesis in the fruit (Zhang et al. 2012). In our study, the same dosage of UV-B and UV-C irradiation were applied, and their impact on gene expression and phenolic metabolites synthesis was detected to be different. It is predicted that UV-B irradiation shows less damaging so as not to cause the protective response at a same given dosage of UV-B and UV-C irradiation. Therefore, UV-C irradiation which has shorter wavelength irradiating grapes

would assuredly benefit the content of phenolic compounds in the table grapes.

Conclusion

There were differences in the response of grapes to the UV-B or UV-C treatment. The synthesis of phenolic compounds and associated antioxidant activities were stimulated by the postharvest UV-B or UV-C treatment. UV-C irradiation induced the higher the expression of genes related to the accumulation of phenolic compounds, and caused the higher accumulation of phenolic metabolites compared with UV-B irradiation at the same applied dose. The grapes treated by UV-C showed antioxidant activity higher than that treated by UV-B irradiation. It can be inferred that UV-C irradiation could change the content of individual phenolic compounds and improve maintenance of postharvest fruit nutritional quality. Moreover, the understanding of differential types of UV irradiation in the

phenolic secondary metabolism pathway will be beneficial to further study on the biosynthesis of diverse phenolic compounds.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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