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Effect of UV-C Irradiation on Phenolic Composition of 'Rishbaba' Table Grape (*Vitis vinifera* cv. Rishbaba)

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Abstract

The effect of postharvest UV-C irradiation on phenolic compounds accumulation was investigated in berries of 'Rishbaba' table grape (*Vitis vinifera* cv. Rishbaba). Grape clusters were harvested at mature stage and irradiated with UV-C using fluorescent germicidal lamp (30 W, 90 cm) with a peak emission at 254 nm for 0 (control), 5 and 10 min. UV-C treatment had significant effect on individual phenolic compounds. Catechin, epicatechin, procyanidin B2, quercetin 3-galactoside, quercetin 3-rhamnoside, chlorogenic acid and total polyphenols increased with UV-C dosage, but procyanidin B1 and cyanidin 3-galactoside decreased. Positive correlation was observed between UV-C treatment and individual phenolics except procyanidin B1 and cyanidin 3-galactoside. In conclusion, UV-C irradiation increased phenolic compounds of 'Rishbaba' table grape and its nutritional value.

Keywords: Phenolic compounds, Postharvest, 'Rishbaba' table grape, UV-C irradiation

Introduction

The general role of phenolic compounds in plant physiology and allelopathy has been known for many years and is the subject of recently published books (Feuchet and Treutter 1999). During the past few years, these secondary metabolites, which occur abundantly in plant foods, have been discovered by human nutritionists, to be beneficial components of functional food. A protective role of diseases from fruit and vegetable consumption is generally attributed to their vitamin C and E, flavonoids, carotenoids, lycopene and dietary fiber constituents (Steinmetz and Potter 1996). Many dietary phenolic compounds derived from plants have a stronger *in vitro* antioxidant activity on a molar basis than the classic antioxidant vitamins such as vitamin C and E (Rice-Evans *et al.* 1997). Phenolic compounds, especially flavonoids and phenolic acids are also involved in the quality characteristics of fresh fruits and its processed products, like texture, color and taste, e.g. bitterness and astringency (Lea and Timberlake 1974; Lidster *et al.* 1986; Lancaster 1992). Phenolic compounds have been reported to show a number of health beneficial properties such as

antioxidant capacity (Kanner *et al.* 1994), inhibition of low density lipoprotein (LDL) oxidation (Teissedre *et al.* 1996; Meyer *et al.* 1997), etc. In fact, a high intake of sources rich in phenolics such as fruits and vegetables have been correlated with the low incidence of cardiovascular diseases and some types of cancer (Bazzano *et al*. 2002; Flood *et al.* 2002). Grapes and derived products are important dietary sources with high polyphenolic content (Teissedre *et al.* 1996).

In the past few years, epidemiological, clinical and in vitro studies have shown the role of grapes, especially red grapes, in preventing cardiovascular disease mortality (Frankel *et al.* 1993; Coa *et al.* 1998b). Antioxidant and anticarcinogenic phenolic compounds present in grapes seem to be responsible for these activities.

Irradiation of plant tissues with UV light has some important effects on phenolic metabolism. UV light irradiation seems to be associated with an increase in the enzymes responsible of flavonoid biosynthesis, as these compounds can act as UV screens preventing the UV induced damage in the genetic material of plant cells. UV light also produces an abiotic stress in plant tissues and affects plant phenolic metabolites in different ways. It can induce postharvest anthocyanin biosynthesis in apple (Reay and Lancaster 2001; Marais *et al.* 2001), cherries (Arakawa 1993; Kataoka *et al*. 1996) and grapes (Cantos *et al.* 2000; Cantos *et al.* 2001). UV-B is associated with flavonoid biosynthesis in parsley (Eckeykaltenbach *et al.* 1993) and with the protection of UV-B induced damage in apple (Kootstra 1994) and maize (Stapleton and Walbot 1994). In addition, it has been demonstrated that *Arabidopsis mutans* lacking phenolic sunscreens exhibit an enhanced UV-B induced injury and oxidative damage (Lois and Buchanan 1994; Landry *et al.* 1995).

One of the traditional claims in proper dietary habits is the increase in the intake of fruits and vegetables (Tavani and La-Vecchia 1995; Coa *et al.* 1998a; Lampe 1999; Liu *et al*. 2000). Modern way of life usually involves the lack of suitable intake of rich sources of phenolic compounds such as fruits and vegetables. Moreover, some parts of the population (especially children) are not often open to the inclusion of these sources in their dietary habits.

Therefore, the aim of the present study was to evaluate the effect of postharvest UV-C irradiation on the phenolic composition of red 'Rishbaba' grapes.

Material and Methods

Chemicals and solvents

Phenolic standards were purchased from different manufacturers. Gallic acid, chlorogenic acid, *p*-coumaric acid, (+)-catechin, (-)-epicatechin, quercetin and sodium carbonate (Na_2CO_3) were purchased from Sigma Chemical Co. (St. Louis, MO); quercetin 3-galactoside, quercetin 3-glucoside and quercetin 3-rhamnoside were from Fluka Chemie GmbH (Buchs, Switzerland); procyanidins B1 and B2, phloridzin, and cyanidin 3-galactoside were from Indofine Chemical Co. (Hillsborough, NJ). All other solvents were of HPLC grade and were purchased from Caledon Laboratories Ltd. (Georgetown, ON).

Plant material

'Rishbaba' grapes (*Vitis vinifera* cv. Rishbaba) which have red color, were harvested at mature stage from a commercial orchard near Urmia, Iran, and transported to the laboratory, where they were treated the same day.

UV-C irradiation

Grape clusters were irradiated from above with UV-C radiation using fluorescent germicidal lamp (30 W, 90 cm) with a peak emission at 254 nm according to Hemmaty *et al.* (2007). Irradiation was carried out under ambient condition for 0 (control), 5 and 10 min. The intensity of radiation was 1.435×10^{-4} W cm^{-2}. Grape clusters were placed at approximately 25 cm from the lamp and rotated to ensure uniform irradiation. After irradiation clusters were stored at the illuminated condition (at ambient temperature) for 72 h.

Extraction of phenolic compounds

Control (no irradiated) and UV-C treated samples (three replicates of four grape clusters) were taken after 72 h storage in illumination. Phenolic compounds extraction was done according by Tsao *et al.* (2003) with some modification. Briefly, grape berries were peeled with a hand peeler (1-2 mm thickness) and were stored at -20 ˚C until analyzed. Samples (approximately 5 g) immediately weighed, and ground in liquid nitrogen in a mortar. The ground sample was then transferred to a beaker with 70% aqueous methanol at a 1:1 (w/v) ratio. The mixture was homogenized using a Polytron blender (Brinkmann Instruments, Westbury, NY) and filtered first through a Whatman no. 1 filter paper under vacuum and then through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, Ann Arbor, MI). The final filtrate was used for HPLC analysis of phenolic compounds.

HPLC analysis of phenolic compounds

An HPLC system (Agilent Technology 1100 series, Palo Alto, CA) equipped with a quaternary pump, an inline degasser and a diode array detector (DAD) was used for identification and quantification of various phenolic compounds in the samples. A Phenomenex Luna C18 analytical column (250×4.6 mm i.d.; particle size, 5 µm) with a C18 guard column (Phenomenex, Torrance, CA) was used for the separation. The binary mobile phase consisted of a 6% acetic acid in 2 mM sodium acetate buffer (solvent A, pH 2.55, v/v) and acetonitrile (solvent B), and the gradient program was as follows: 0% B to 15% B in 45 min, 15% B to 30% B in 15 min, 30% B to 50% B in 5 min, and 50% B to 100% B in 5 min. There was a 10-min postrun going back to the starting conditions for reconditioning. The flow rate was 1.0 mL/min for a total run time of 70 min. The injection volume was 10 µL for all samples. All standards except for anthocyanins were dissolved in methanol. The latter were dissolved in 1% HCl in methanol. The detector was set at 280, 320, 360 and 520 nm for simultaneous monitoring of the different groups of phenolic compounds (Tsao *et al.* 2003). Extracts injected in three replicates.

Sum of phenolic compounds

Sum of phenolic compounds of samples calculated with adding concentration of individual polyphenolic compounds.

Statistical analysis

Mean values of phenolic content in both control and UV-C treated grapes were compared using Duncan multiple range test by using the MSTAT-C statistical software. Levels of significance were set as $P \leq 0.05$.

Results

HPLC-DAD analysis of phenolic compounds of 'Rishbaba' Grape

The methanol extracts of 'Rishbaba' grape were analyzed by HPLC, and 10 polyphenolic compounds belonging to all five major polyphenolic groups were identified from the 'Rishbaba' grapes. They are chlorogenic and *p*-coumaric acid (hydroxycinnamic acids); cyanidin 3-galactoside (anthocyanins); catechin, epicatechin, and procyanidins B1 and B2 (flavan-3-ols/procyanidins); quercetin 3-rhamnoside; quercetin 3-galactoside (flavonols); and phloridzin (dihydrochalcones).

UV-C irradiation and sum of phenolic compounds

Sum of phenolic compounds of 'Rishbaba' grape peel affected by UV-C irradiation ($P \leq 0.01$). There was not significant difference between irradiated berries for 5 min and control berries, but in irradiated berries for 10 min sum of phenolic compounds were 1.9 fold higher than the control and irradiated berries for 5 min and difference between them was significant ($P \leq 0.05$) (Table 1). Correlation between UV-C and sum of phenolic compounds was positive (0.88) (Table 2).

UV-C irradiation and flavan-3-ols/ procyanidins

Procyanidins

Postharvest UV-C irradiation had significant effect on procyanidin content of grape berries peel ($P \leq 0.01$). UV-C had a negative effect on procyanidin B1 and concentration of procyanidin B1 decreased with increasing irradiation time (Table 1). Differences among control berries, irradiated berries for 5 and irradiated berries for 10 min was significant ($P \leq 0.05$) and procyanidin B1 content of them were 4.58, 4.55 and 4.33 mg/100g FW, respectively. However, procyanidin B2 increased with UV-C treatment. Procyanidin B2 content of irradiated berries for 10 min was 1.2 and 2 fold higher than irradiated berries for 5 min and control berries, respectively. Difference between UV-C treatment levels and control fruits was significant ($P \leq 0.05$) (Table 1). Correlation between UV-C treatment and procyanidin B1 and B2 content of berries was -0.91 and 0.99, respectively (Table 2).

Catechin and epicatechin

Treatment of 'Rishbaba' grape berries with UV-C radiation had significant effect on chatechin and epicatechin content of berries peel ($P \leq 0.01$). Catechin content of berries peel increased with increasing UV-C irradiation time and differences were significant ($P \leq 0.05$). Treated berries for 10 and 5 min had 2 and 1.1 fold more catechin than control berries, respectively. Catechin content of irradiated berries peel for 10 min was 1.8 fold higher than irradiated berries for 5 min (Table 1).

Epicatechin was affected by UV-C treatment more than other phenolic compounds, so that,

Table 1. Effect of UV-C irradiation on individual and sum of phenolic compounds (mg/100g FW)

UV-C treatment (min)	Catechin	EPC	ProB1	ProB2	Cyn3gala	Qur3gala	Qur3rha	CGA	*p*-CQA	PLZ	Sum of phenolics
0	9.96 ^{c}	1.23 ^{c}	4.58 ^{a}	2.22 ^{c}	14.00 ^{a}	0.00 ^{b}	0.38 ^{c}	0.95 ^{c}	1.11 ^{c}	0.66 ^{b}	35.09 ^{b}
5	11.18 ^{b}	9.30 ^{b}	4.55 ^{b}	3.59 ^{b}	0.48 ^{b}	0.00 ^{b}	2.39 ^{b}	1.50 ^{b}	1.33 ^{b}	0.66 ^{b}	35.98 ^{b}
10	19.76 ^{a}	26.78 ^{a}	4.33 ^{c}	4.43 ^{a}	0.00 ^{c}	0.89 ^{a}	4.86 ^{a}	2.44 ^{a}	1.39 ^{a}	0.67 ^{a}	65.59 ^{a}

EPC: epicatechin, ProB1: procyanidin B1, ProB2: procyanidin B2, Cyn3gala: cyanidin 3-galactoside, Qur3gala: quercetin 3-galactoside, Qur3rha: quercetin 3-rhamnoside, CGA: chlorogenic acid, *p*-CQA: *p*-coumaric acid, PLZ: phloridzin.

Means with similar letters in each column have no significant difference at $P \leq 0.05$.

Table 2. Correlation among individual polyphenolic compounds and UV-C irradiation

	Catechin	EPC	ProB1	ProB2	Cyn3gala	Qur3gala	Qur3rha	CGA	*p*-CQA	PLZ	Sum of phenolics
UV-C	0.92**	0.98**	-0.91**	0.99**	-0.88*	0.87*	0.99**	0.99*	0.95*	0.43	0.88*
Catechin		0.98**	-0.99**	0.85*	-0.62	0.99**	0.94**	0.97**	0.74*	0.50	0.99**
EPC			-0.98**	0.94**	-0.76*	0.95**	0.99**	0.99**	0.86*	0.48	0.96**
ProB1				-0.85*	0.61	-0.99**	-0.93**	-0.96**	-0.74*	-0.52	-0.99**
ProB2					-0.94**	0.79*	0.98**	0.96**	0.98**	0.40	0.80*
Cyn3glu						-0.53	-0.85*	-0.80*	-0.98**	-0.26	-0.55
Qur3glu							0.89*	0.93**	0.66*	0.49	0.99**
Qur3rha								0.99**	0.93**	0.45	0.90*
CGA									0.89*	0.48	0.94**
p-CQA										0.39	0.68*
PLZ											0.45

EPC: epicatechin, ProB1: procyanidin B1, ProB2: procyanidin B2, Cyn3gala: cyanidin 3-galactoside, Qur3gala: quercetin 3-galactoside, Qur3rha: quercetin 3-rhamnoside, CGA: chlorogenic acid, *p*-CQA: *p*-coumaric acid, PLZ: phloridzin.

* $P \leq 005$, ** $P \leq 001$.

content of this compound in irradiated berries peel for 10 min was 21.7 fold higher than control berries peel. Also, peel of irradiated berries for 5 min had 7.5 fold more epicatechin than control berries (Table 1). Epicatechin content of berries peel increased from 1.23 mg/100g FW in control berries peel to 26.78 mg/100g FW in irradiated berries peel for 10 min. Correlation between UV-C treatment and catechin and epicatechin was 0.92 and 0.98, respectively (Table 2).

UV-C irradiation and hydroxycinnamic acids

Chlorogenic acid and *p*-coumaric acid content of 'Rishbaba' grape berries peel were affected significantly by UV-C radiation ($P \leq 0.01$). There were significant differences between UV-C treatment levels and control berries for chlorogenic acid content ($P \leq 0.05$). Content of chlorogenic acid increased from 0.95 to 2.44 mg/100g FW in control and irradiated berries peel for 10 min, respectively (Table 1).

UV-C treatment had positive effect on *p*-coumaric acid content and peel of irradiated berries for 10 min had higher *p*-coumaric acid content than other treatment and control berries peel (Table 1). *p*-coumaric acid content of peel of control, irradiated berries for 5 and 10 min were 1.11,1.33 and 1.39 mg/100g FW, respectively. The relationship between UV-C irradiation and chlorogenic and *p*-coumaric acids content of berries peel were 0.99 and 0.95, respectively (Table 2).

UV-C irradiation and anthocyanins

Cyanidin 3-galactoside was predominate phenolic compound in untreated 'Rishbaba' grape peel, but UV-C irradiation had negative significant effect on cyanidin 3-galactoside content of berries peel ($P \leq 0.01$) and peel of control berries had higher concentrations of cyanidin 3-galactoside than UV-C treated berries peel. Cyanidin 3-galactoside decreased with increasing UV-C irradiation time and in treated berries, only was detected in peel of irradiated berries for 5 min in very low amounts (Table 1). Correlation between UV-C treatment and cyanidin 3-galactoside was -0.88 (Table 2).

UV-C irradiation and flavonols

Flavonols content of 'Rishbaba' grape cultivar peel was affected significantly by postharvest UV-C irradiation. In untreated berries, only quarcetin 3-rhamnoside detected but after irradiation for 10 min, quarcetin 3-galactoside identified in very low amounts. Concentration of quarcetin 3-galactoside in control berries peel was 0 mg/100g FW but increased to 0.89 mg/100g FW after irradiation for 10 min. For quarcetin 3-rhamnoside content of berries peel, difference between levels of UV-C treatment and control berries was significant ($P \leq 0.05$). After epicatechin, greatest effect of UV-C irradiation observed in quarcetin 3-rhamnoside content of berries peel (Table 1). Quarcetin 3-rhamnosid content of irradiated berries peel for 5 and 10 min was 6.33 and 12.86 fold higher than control berries, respectively. Correlations between UV-C and quarcetin 3-galactoside and quarcetin 3-rhamnoside were 0.87 and 0.99, respectively (Table 2).

UV-C irradiation and dihydrochalcons

Postharvest UV-C treatments had less effect on phloridzin concentration of peel. There was not

significant difference between irradiated berries for 5 min and control berries but irradiation for 10 min had slight increase in phloridzin content of irradiated berries peel (Table 1). Correlation between phloridzin and UV-C was 0.43 (Table 2).

Correlation among phenolic compounds

The scientific interest in the relationship among polyphenolic compounds in fruits is outlined by the fact that phenolic compounds, as the powerful antioxidants, have a pro-oxidant effect, at the same time since they are susceptible to oxidation. In the present study the correlation among the individual polyphenolics of the 'Rishbaba' grape berries peel was investigated and the results are given in Table 2. There was correlation among individual polyphenolic compounds. Catechin and Epicatechin had negative relationship with cyanidin 3-galactoside. Also correlation between cyanidin 3-galactoside and procyanidin B2, quarcetin 3-galactoside, quarcetin 3-rhamnoside, chlorogenic acid, *p*-coumaric acid, phloridzin was -0.94 ($P \leq 0.01$), -0.53, -0.85 ($P \leq 0.05$), -0.80 ($P \leq 0.05$), -0.98 ($P \leq 0.01$) and -0.26, respectively. This shows that there was negative correlation between anthocyanins and flavonols, hydroxycinnamic acids and dihydrochalcons. Individual polyphenolics, also, had strong correlation with sum of phenolic compounds and among these compounds only procyanidin B1 and cyanidin 3-galactoside had negative correlation. Phloridzin did not have significant correlation with other phenolics and sum of henolic compounds.

Discussion

The induction capacity of polyphenolic compounds upon postharvest UV-C irradiation of table grapes (Cantos *et al.* 2003) and apples (Bakhshi and Arakawa 2006) has been previously reported. However, there are no previous studies concerning the induction capacity of phenolics by UV-C irradiation in 'Rishbaba' table grape peel. In general, the induction of polyphenolic compounds in 'Rishbaba' grape showed the same behavior as previously observed in other studies. However, phenolic compound accumulation in the peel of irradiated grape berries influenced by irradiation time. After cyanidin 3-galactoside, catechin was predominant polyphanolic compound in untreated 'Rishbaba' grape berries peel, but after irradiation Cyanidin 3-galactoside decreased and catechin and epicatechin were predominant phenolics. Quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-arabinoside and quercetin 3-xyloside were not detected in control 'Rishbaba' grape peel and only quercetin 3-rhamnoside was identified in peel.

The UV-C treated grapes had more reduced anthocyanin content than the control grapes and these differences were statistically significant. Our result showed that cyanidin 3-galactoside decreased with increasing UV-C dosage. Decrease in anthocyanin content of grapes has been reported by Cantos *et al.* (2000). Longer irradiation time probably cause excessive stress and therefore damage to the anthocyanin biosynthetic system. The damage induced by an excess of UV irradiation has been previously reported by Rodov *et al.* (1992) and D'hallewin *et al.* (2000). Also anthocyanins have

photoprotective function, which reduce the effect of photooxidative damage (Gould *et al.* 2000; Merzlyak and Chivkunova 2000; Pietrini *et al*. 2002; Solovchenko and Schmitz-Eiberger 2003).

In the control berries peel, procyanidin B1 was more abundant than procyanidin B2, but after irradiation, procyanidin B2 was affected more than procyanidin B1. Concentration of procyanidin B1 decreased slightly, but procyanidin B2 content increased. It is known that UV radiation induces free radicals and reactive oxygen species and these very active radicals can cause oxidative degradation of proteins, unsaturated lipids, carbohydrates and DNA (Prasada 1996). The free radical scavenging abilities of proanthocyanidins have been well documented (Bagchi *et al.* 1997; Bagchi *et al.* 1998; Hatano *et al.* 1990). *In vivo* studies have shown grape seed proanthocyanidin extract is a better free radical scavenger and inhibitor of oxidative tissue damage than vitamin C, vitamin E succinate, vitamin C and vitamin E succinate combined, and beta carotene (Bagchi *et al.* 1998). Ricardo da Silva *et al*. (1991) found that procyanidin B2 3-O-gallate was the most effective compound in trapping oxygen free radicals.

'Rishbaba' table grape is used widely as fresh fruit in Iran and increase in polyphenolic composition can improve quality of grapes as the functional food. This study showed that it is possible to increase the level of phenolic compounds and other health related properties of 'Rishbaba' grapes by postharvest UV-C irradiation treatment. The sum of phenolic compounds, catechin, epicatechin, procyanidin B2, chloroghenic acid, *p*-coumaric acid, quercetin 3-galactoside, quercetin 3-rhamnoside and phloridzin were all enhanced with UV-C radiation.

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