JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Postharvest Stilbene-Enrichment of Red and White Table Grape Varieties Using UV-C Irradiation Pulses

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The red table grape varieties Flame, Red Globe, Crimson, and Napoleon, as well as the white varieties Superior, Dominga, and Moscatel Italica, were irradiated with a previously optimized UV-C postharvest irradiation protocol (510 W, 40 cm, 60 s). The induction kinetics of the stilbenes *trans*-resveratrol, *trans*-piceid, *trans*-piceatannol, *trans*-astringin, and viniferins was followed by using HPLC-DAD/MS/MS. The most inducible stilbenes were *trans*-resveratrol, *trans*-piceatannol, and viniferins. Both quantitative and qualitative differences were observed in both the stilbene induction kinetics and stilbene content in the seven table grapes analyzed here. The total resveratrol content ranged from 0.69 mg/100 g fw (Dominga) to 2.3 mg/100 g fw (Red Globe). The net resveratrol induction ranged from 3.4-fold (Flame) to 2315-fold (Red Globe). The highest viniferins content was observed in the variety Flame (0.73 mg/100 g fw), although the variety Red Globe presented the highest viniferins induction (175-fold). The highest content and induction of piceatannol (0.17 mg/100 g fw and 173-fold, respectively) was observed in the variety Flame. It should be stressed that taking into account the health-beneficial effects claimed for stilbenes, the UV-C irradiated table grapes can be considered as new functional fruits that can supply (a serving of unpeeled 200 g table grapes) the resveratrol content (depending on the variety) equivalent to more than seven glasses of red wine (≈1.5 L) with high resveratrol content.

KEYWORDS: Astringin; functional fruit; health-promoting; HPLC-DAD/MS/MS; piceid; piceatannol; resveratrol; stilbene; table grapes; UV-C irradiation; viniferins; *Vitis vinifera*

INTRODUCTION

The inclusion of table grapes in the dietary habits of western countries has increased in the last 20 years. In fact, the consumption of grapes in United States is higher than that of other berries (*1*). Grapes constitute one of the major sources of phenolic compounds among different fruits (*2*). Actually, both table grapes and wine are considered to be important sources of health-promoting properties due to their phenolic composition (*3*−*6*). Some of these phenolic compounds belong to the stilbene group.

Stilbenes are nonflavonoid phenolics that are mainly present in grape berries (*7*) and derived products, such as red wine (*8*). The induction of stilbenes in response to stress, such as pathogenic attack (*9*), as well as preharvest (*10*, *11*) and postharvest UV-C irradiation (*12*−*14*) has been previously described.

One of the most relevant and extensively studied stilbenes is *trans*-resveratrol (3,5,4′-trihydroxystilbene), which has been reported to have a number of health-beneficial properties, such as antioxidant (*15*), antimutagen (*16*), anti-inflammatory (*17*), antiestrogenic (*18*), and antiarrhythmic and cardioprotective (*19*). In addition, resveratrol has been proposed as a cancer chemopreventive drug (*20*−*22*). Moreover, the high bioactivity of resveratrol is also supported by its high bioavailability (*23*) and lack of toxicity after oral administration of high doses to rats (*24*). In addition to resveratrol, other stilbenes have shown health-beneficial effects, such as some stilbene glucosides, which showed inhibitory activity against tumor and metastatic carcinoma (*25*); *trans*-piceatannol (3′,4′,3,5-tetrahydroxystilbene), which is a potent tyrosine-kinase inhibitor (*26*) and antileukemic compound (*27*); pterostilbene (3,5-dimethoxy-4′-hydroxystilbene) and viniferins (resveratrol polymers), the health-beneficial properties of which have also been described (*28*, *29*).

The aim of the present work is to identify and quantify by HPLC-DAD/MS/MS the main inducible stilbenes as well as to study their induction kinetics in four red (Red Globe, Flame, Crimson, and Napoleon) and three white (Superior, Dominga, and Moscatel Italica) table grape varieties after postharvest UV-C irradiation.

MATERIALS AND METHODS

Reagents. Resveratrol (99%) was purchased from Sigma (Madrid, Spain). Formic acid and methanol (MeOH) were of analytical grade and supplied by Merck (Darmstad, Germany). Milli-Q system (Millipore Corp., Bedford, MA) ultrapure water was used throughout this research.

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10.1021/jf020562x CCC: $22.00
Published on Web 09/24/2002

Grapes. The red table grapes Flame seedless, Red Globe, Crimson seedless and Napoleon, as well as the white table grapes Superior seedless, Moscatel Italica, and Dominga were harvested in summer (July−September) 2001 in different locations from Murcia (Spain), transported to the laboratory, and processed the same day that they were at mature ripening stage (soluble solids content ≥ 16 °Brix, according to suppliers' specifications) and ready to be commercialized.

UV Irradiation Treatment. Grape berries were UV-treated as previously described (*13*). Briefly, the standard irradiation parameters used along the present study were irradiation power, 510 W; irradiation distance, 40 cm; and irradiation time, 60 s. Both irradiated and control (nontreated) grape berries were stored at 22 °C in perforated plastic bags and at relative humidity of 90−95% to avoid water loss and shriveling. A set of both irradiated and control grapes was transferred to 2 °C (in the same plastic bags) every day in order to follow induction kinetics of stilbenes at both temperatures. Irradiation experiments were repeated three times.

The parameter "maximum day" (D_m) (*13*) was defined as the elapsed number of days to achieve the maximum resveratrol concentration. The term "D-7" reported in the tables was defined as D_m plus 2 days of conservation at 2 °C, since D_m was the fifth day for all varieties except for Flame and Dominga, which presented this maximum at the third and seventh days, respectively. In the case of Flame, the term D-7 was D_m (3 days at 22 °C) plus 4 days at 2 °C. In the case of Dominga, the term D-9 was used instead of D-7; i.e., D-9 = D_m (7 days at 22 °C) plus 2 days at 2 °C.

Extraction of Phenolic Compounds. Grapes were peeled with the help of a sharp knife, and the skins were stored at −20 °C until analyzed. Skin represented ∼10% of the total fresh weight of grape berry. Samples were homogenized in an Ultraturrax T-25 (Janke and Kunkel, Ika-Labortechnick) at 24 000 rpm for 1 min after the addition of 4 mL of a solution of MeOH/formic acid (97:3) per gram of grape skin. The extracts were centrifuged at 5000*g* for 5 min in a Centromix centrifuge (Selecta, Barcelona), filtered through a 0.45-μm membrane filter Millex-HV_{13} (Millipore Corporation), protected from light to avoid *cis*-isomerization of stilbenes, and analyzed by HPLC-DAD/MS/MS.

HPLC-DAD/MS/MS. A 20-μL sample of the supernatant obtained above was analyzed using an HPLC system equipped with both a DAD and mass detector in series, which consisted of a HPLC binary pump (G1312A), an autosampler (G1313A), a degasser (G1322A), and a photodiode array detector (G1315B) controlled by software (v. A08.03) from Agilent Technologies (Waldbronn, Germany). Chromatographic separation was carried out on a reversed-phase C_{18} LiChroCART column (25 × 0.4 cm; particle size, 5 μm; Merck, Darmstadt, Germany). The mobile phase consisted of water with 5% formic acid (solvent A) and HPLC grade methanol (solvent B) at a flow rate of 1 mL/min. Elution was performed as previously described by Cantos et al. (*12*). UV chromatograms were recorded at 320 nm. The mass detector was an ion-trap mass spectrometer (G2445A, Agilent Technologies, Waldbronn, Germany) equipped with an electrospray ionization (ESI) system and controlled by software (v. 4.0.25). The heated capillary and voltage were maintained at 350 °C and 4 kV, respectively. Mass scan (MS) and MS/MS spectra were measured from *m*/*z* 100 up to *m*/*z* 1500. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, and the collision energy was set at 50%. Mass spectrometry data were acquired in the negative ionization mode. HPLC-DAD/MS/MS experiments were repeated three times.

Phenolic Identification and Quantification. Stilbenes were identified by their UV spectra recorded with a diode array detector and by their ion mass with LC/MS/MS. All stilbenes were quantified at 320 nm as resveratrol. Graphs of the experimental data and their statistic analysis were carried out by using the Sigma Plot 6.0 program for Windows.

RESULTS AND DISCUSSION

Recently, an induction modeling method to increase resveratrol content in Napoleon grapes after postharvest UV-C irradiation was described (*13*). However, the induction kinetics as well as the quantitative concentration of other stilbenes after postharvest UV-C treatment in different white and red table grape varieties has thus far not been described. Seven table grape varieties were irradiated with UV-C, that is, four red (Flame, Red Globe, Crimson, and Napoleon) and three white grape varieties (Superior, Dominga, and Moscatel Italica). All of them induced a number of stilbene derivatives upon irradiation with UV-C. However, in the grape varieties assayed here, there were both qualitative and quantitative differences concerning the induction of these stilbenes.

Trans- and *cis*-resveratrol, *trans*-piceatannol (also named *trans*-astringinin), *trans*-piceid (*trans*-resveratrol 3-*O*-β-glucoside), *trans*-astringin (*trans*-piceatannol-3-*O*-β-glucoside) and α and ϵ-viniferins (trimer and dimer of resveratrol) were identified (**Figure 1**; **Table 1**) and quantified by HPLC-DAD/MS/MS (**Tables 2**, **3**).

***trans*-Resveratrol.** *trans*-Resveratrol content significantly increased in all the table grape varieties after UV-C irradiation and further storage at room temperature. This content reached a maximum at the so-called "maximum day" (D_m, see the Materials and Methods Section) (**Figure 2**, the last solid symbol for each plot) (*13*) which was the fifth day (5 days stored at room temperature after irradiation) for all of the varieties, except for the red variety Flame and the white variety Dominga, which presented their D_m at the third and seventh days, respectively. In addition, the maximum induced resveratrol content was also different, depending on the variety (**Tables 2**, **3**).

To preserve both the maximum induced resveratrol concentration and sensory properties, some of the irradiated and control grapes were transferred to the standard commercial storage temperature (2 °C) after reaching their corresponding maximum day (**Figure 2**, open symbols). Resveratrol content remained approximately constant in the varieties Dominga, Napoleon, and Moscatel Italica. However, the content was reduced by ≈30% in the varieties Crimson and Superior and about 20% in Flame and Red Globe (D-7, **Tables 2**, **3**). The transfer of grapes to 2 °C was necessary in order to maintain the grapes' quality and avoid contamination.

It has been previously published that *trans*-resveratrol is induced in higher amounts by UV-C than are other stilbenes (*10*, *13*), which was in agreement with the results presented here. The *trans*-resveratrol content of red varieties Crimson and Red Globe increased more than 100- and 2000-fold, respectively, when compared to the untreated grapes at the maximum day (D_m) (**Table 2**; **Figure 3**). The huge resveratrol induction in the variety Red Globe was due to the lack of resveratrol in the control (nontreated) Red Globe table grapes. However, the other two red varieties (Flame and Napoleon) induced ∼4-fold more resveratrol than the respective untreated grapes (**Table 2**). The induced resveratrol content in the present study for the red variety Napoleon was 3-fold lower than that previously reported with the same UV-C irradiation protocol (*13*). This difference could be related to agronomic factors, such as soil composition, irrigation, light intensity, etc., since these agronomic factors have been proposed as important determinants of the phenolic composition of fruits and vegetables, including the possible variation of the phenolic profile of grape varieties (*30*). Moreover, it has been described that stilbene biosynthesis greatly depends on grape berry development, since the ability to synthesize *trans*-resveratrol diminishes up to maturity (*11*).

The white varieties Superior, Dominga, and Moscatel Italica increased their *trans*-resveratrol content about 10-, 50-, and 30-fold, respectively, when compared to the untreated grapes (**Table 3** and **Figure 4**). The total induced *trans*-resveratrol content ranged from 0.69 mg/100 g fw for the white variety Dominga

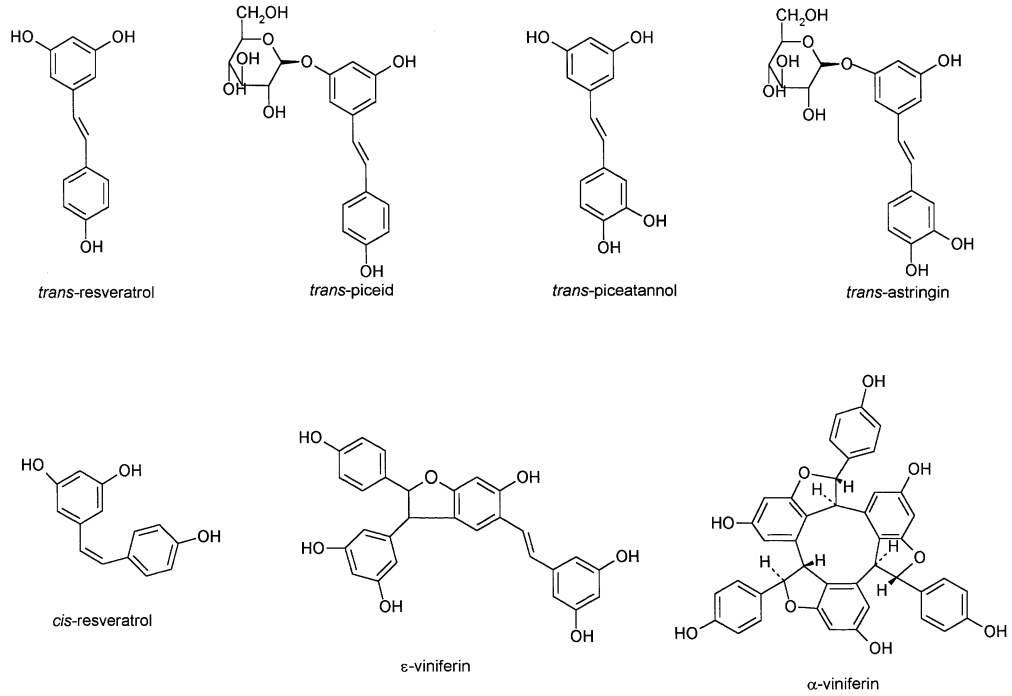

Figure 1. Structures of the UV-C-induced stilbenes in table grapes.

Table 1. HPLC-DAD/MS/MS Analysis of UV-C-Induced Stilbenes of Table Grapes

structure	molecular ion m/z^-	fragment	HPLC R_t (min)
Piceid-*a*	389.4	227.1	23
astringinin	405.5	243.3	26.4
Piceid-*b*	389.4	227.1	28.3
trans-piceid	389.4	227.1	31
piceatannol	243.3	243.3	32.9
trans-resveratrol	227.4	227.4	39.2
α_1-viniferin	679.5	661.0, 585.2, 573.0, 451.3, 345.1, 273.0	46.7
α_2-viniferin	679.5	661.0, 585.2, 573.0, 451.3, 345.1, 273.0	47.1
ϵ_1-viniferin	453.4	435.0, 359.1, 347.05	47.6
α_3-viniferin	679.2	661.2, 585.2, 573.2, 451.1, 345.1, 273.0	48
α_4-viniferin	679.3	661.2, 585.2, 573.2, 451.1, 345.1, 273.0	48.5
ϵ_2-viniferin	453.4	435.0, 359.1, 347.1	49.6

Table 2. Stilbene Content of UV-C-Irradiated Red Table Grapes at the Maximum Day (D_m) and Some More Days Transferred at 2 °C to Complete One Week (D-7)[a]

	Flame		Red Globe		Crimson		Napoleon	
	UV	induction (-fold)	UV	induction (-fold)	UV	induction (-fold)	UV	induction (-fold)
astringin								
D_m	ND[b]	-	ND		ND		47.73 (1.5)	2.3
piceid								
D_m	162.5 (19.0)	3.3	ND		ND		329.9 (38.1)	1.4
piceatannol								
D_m	173.4 (15.6)	173.4	153.6 (14.5)	153.6	54.7 (3.8)	2.2	119.6 (14.3)	2.9
resveratrol								
D_m	2267.2 (220.0)	3.4	2315.9 (190.4)	2315.9	1020.3 (62.4)	100	1149.5 (49.3)	4.4
D-7	1.728.2 (150.3)	2.6[c]	1778.9 (50.0)	1778.9	728.0 (24.9)	62.5	1181.4 (140.2)	4.4
total viniferins								
D_m	732.89 (83.06)	6.3	175.3 (18.6)	175.3	120.0 (9.0)	120	315.3 (9.5)	3.7
D-7	716.4 (78.5)	3.9[c]	124.6 (2.97)	124.6	106.1 (9.5)	106	400.0 (43.6)	6.5

[a] Values are expressed as micrograms per 100 g of fresh weight of total grape berry. Standard deviation is reported between parentheses ($n = 3$). [b] Not detected. [c] Flame variety presented the maximum day at third day. Therefore, in this case, the period of conservation was longer (D-7 = 3 days stored at 22 °C plus 4 days at 2 °C). D_m and D-7 concepts were explained in the Materials and Methods Section.

to 2.3 mg/100 g fw for the red variety Red Globe (**Tables 2**, **3**). On the maximum day, the white varieties reached a *trans*-resveratrol content that was lower than for the red ones, which is not in agreement with other studies in other varieties for which the same resveratrol induction in both red and white grape varieties was reported (*31*). Therefore, resveratrol induction depends on grape variety, veraison and geographical region (*32*), not grape color.

Table 3. Stilbene Content of UV-C Irradiated White Table Grapes at the Maximum Day (D_m) and Some More Days Transferred at 2 °C to Complete One Week (D-7)[a]

	Superior		Dominga		Moscatel Italica	
	UV	induction (-fold)	UV	induction (-fold)	UV	induction (-fold)
astringin						
D_m	ND[b]		22.8 (2.5)	22.8	ND	
piceid						
D_m	116.9 (1.6)	1.2	39.3 (1.9)	1.4	52.2 (5.9)	52.2
piceatannol						
D_m	90.3 (10.4)	41.5	53.1 (6.3)	53	76.8 (8.9)	76.8
resveratrol						
D_m	1626.6 (155.6)	10.3	687.0 (72.0)	50	1030.5 (99.6)	32.5
D-7	1153.9 (30.9)	6.3	690.4 (65.3)[c]	30.5	871.3 (21.1)	25.3
total viniferins						
D_m	567.9 (66.2)	34.6	93.1 (9.2)	6.45	249.6 (23.8)	12.5
D-7	548.8 (35.8)	26.3	157.6 (9.9)[c]	5.0	221.9 (15.8)	20.2

[a] Values are expressed as micrograms per 100 g of fresh weight of total grape berry. Standard deviation is reported between parentheses ($n = 3$). D_m and D-7 concepts were explained in the Materials and Methods Section. [b] Not detected. [c] Dominga variety presented the maximum day at seventh day. Therefore, in this case the term D-9 was used (D-9 = 7 days stored at 22 °C plus 2 days at 2 °C).

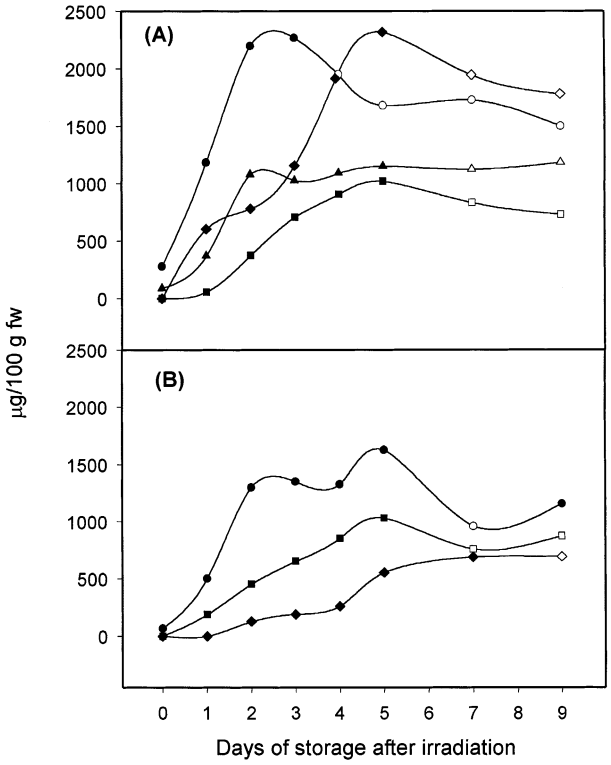

Figure 2. Induction kinetics of resveratrol after UV-C irradiation in table grapes. (A) Red grape varieties: (●) Flame, (◆) Red Globe, (▲) Napoleon, and (■) Crimson. (B) White grape varieties: (●) Superior, (◆) Dominga, and (■) Moscatel Italica. Solid symbols, 22 °C; open symbols, 2 °C. Coefficient of variation ((standard deviation/mean) × 100)) was always <12%.

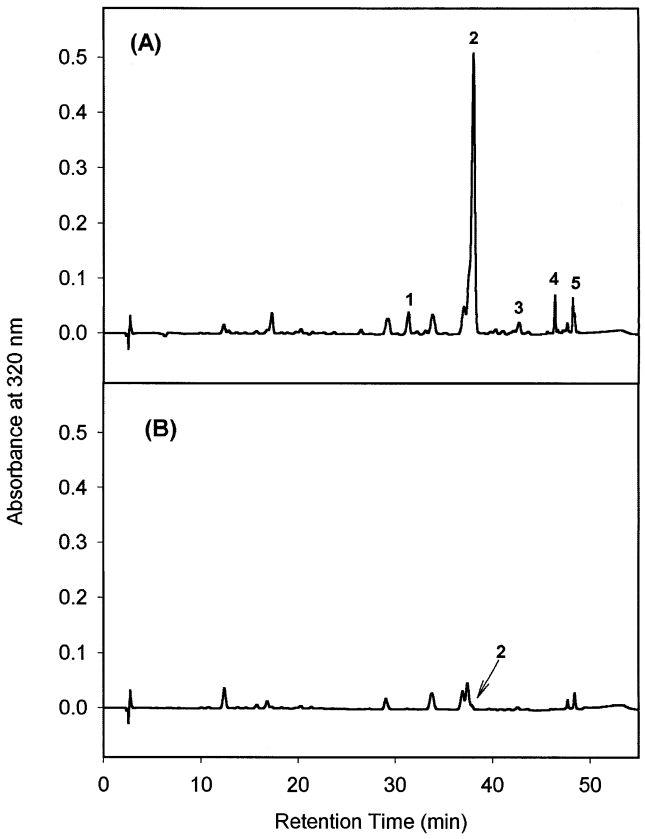

Figure 3. HPLC chromatograms of skin extracts of the red grape variety Red Globe at 320 nm. (A) UV-C irradiated grapes: (1) Piceatannol, (2) *trans*-resveratrol (the shoulder designated by the arrow), (3) *cis*-resveratrol, (4) ϵ_1-viniferin and (5) ϵ_2-viniferin. (B) Control (nontreated) grapes.

According to the results presented here, the red table grape varieties Flame and Red Globe could supply ~5 mg of resveratrol/serving (200 g), which is the same amount supplied by seven glasses of red wine (7 × 200 mL) with high resveratrol content (**Table 2**).

Viniferins. Viniferins are well-known stress-inducible (including UV-C light) resveratrol polymers (**Figure 1**) (*33*, *34*). However, postharvest UV-C irradiation to induce viniferins in table grape varieties has not been reported so far.

Two types of viniferin-like compounds were detected in the seven table grape varieties studied here after UV-C irradiation: dimers of resveratrol (ϵ-viniferin-like viniferins) and trimers of resveratrol (α-viniferin-like viniferins) (**Figure 1**). Both viniferin-like compounds were clearly distinguished by their molecular ion (m/z^-) (**Table 1**) and by their UV spectra. It should be emphasized that viniferins are traditionally classified as α- and ϵ-viniferin. However, up to six resveratrol polymers were quantified in the seven table grapes assayed here: two ϵ-viniferin-like compounds (ion at m/z 453) and four α-viniferin-like compounds (ion m/z at 679) (**Table 1**). These resveratrol polymers could be different viniferin isomers (by m/z relation). The ion at m/z 453 could correspond to ϵ-viniferin, as well as to ampelopsin B and D. The ion m/z at 679 could correspond

Table 4. Single Viniferin Content of Irradiated Table Grapes Varieties at the Maximum day (D_m) of Induction[a]

viniferins	Flame	Red Globe	Crimson	Napoleon	Superior	Dominga	Moscatel Italica
α_1	96.2 (24.7)	ND[b]	ND	46.0 (7.4)	ND	ND	ND
α_2	ND	ND	ND	ND	74.2 (32.3)	ND	ND
ϵ_1	329.5 (32.4)	43.2 (3.6)	42.1 (17.2)	141.1 (2.0)	199.8 (27.7)	47.3 (18.3)	89.5 (9.5)
α_3	44.78 (7.0)	ND	ND	ND	49.9 (16.0)	ND	ND
α_4	67.4 (13.0)	ND	ND	ND	75.1 (30.7)	ND	ND
ϵ_2	195.1 (10.4)	134.9 (22.3)	77.9 (7.9)	128.2 (13.7)	168.9 (21.4)	45.8 (24.3)	160.0 (33.3)

[a] Values are expressed as micrograms per 100 g of fresh weight of total grape berry. Standard deviation is reported between parentheses ($n = 3$). [b] Not detected.

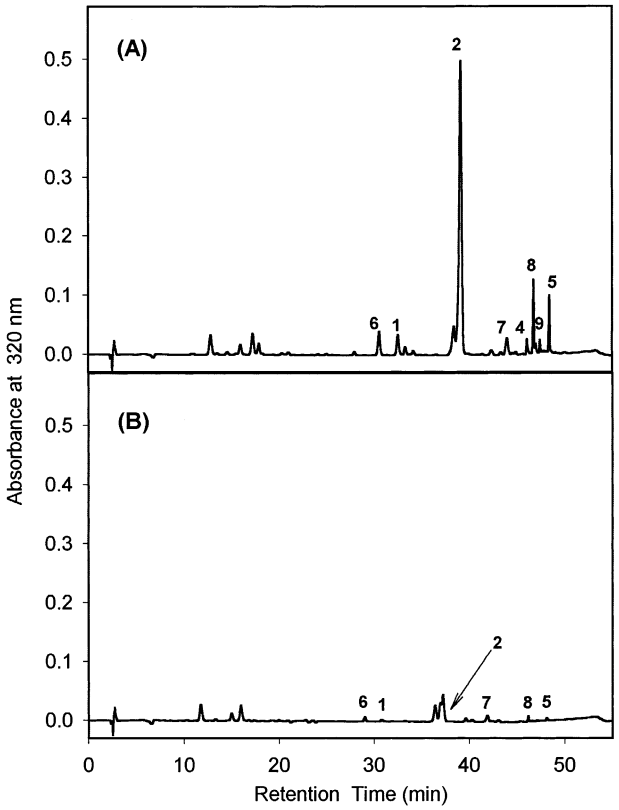

Figure 4. HPLC chromatograms of skin extracts of the white grape variety Superior at 320 nm. (A) UV-C irradiated grape: (6) Piceid, (1) piceatannol (2) *trans*-resveratrol (the shoulder designated by the arrow), (7) α_2-viniferin, (4) ϵ_1-viniferin, (8) α_3-viniferin, (9) α_4-viniferin, and (5) ϵ_2-viniferin. (B) Control (nontreated) grapes.

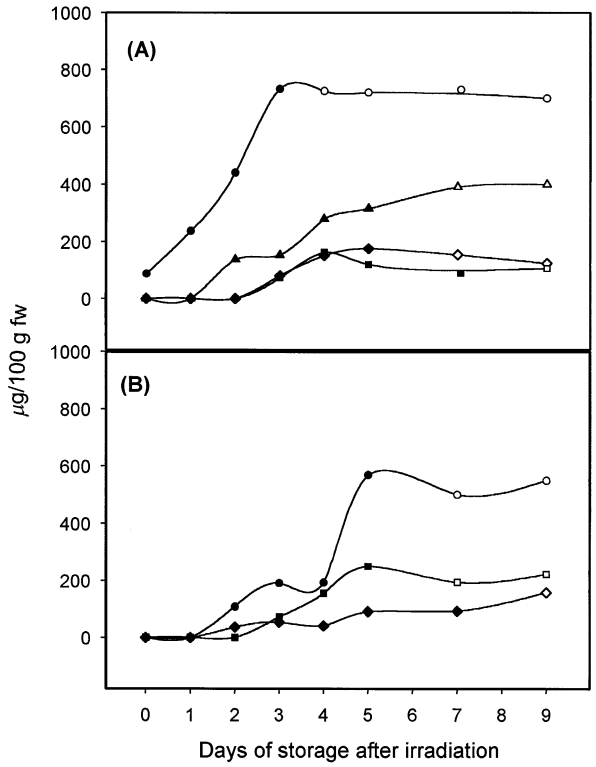

Figure 5. Induction kinetics of viniferins after UV-C irradiation of table grapes. (A) Red grape varieties: (●) Flame; (◆) Red Globe; (▲) Napoleon; and (■) Crimson. (B) White grape varieties: (●) Superior, (◆) Dominga, and (■) Moscatel Italica. Solid symbols, 22 °C; open symbols, 2 °C. Coefficient of variation was always <12%.

to α-viniferin, ampelopsin E, and copalliferol A. All of these compounds have been previously detected in *Vitis sp.* (*35*, *36*). The presence of these compounds (apart from both α- and ϵ-viniferin) has not been previously described in grape berries. However, after UV-C irradiation, some of these resveratrol polymers could have been induced, so their presence in irradiated grapes cannot be ruled out. To follow a coherent nomenclature along the present study, the different dimers and trimers of resveratrol will be termed α_x-viniferin (resveratrol trimers) and ϵ_x-viniferin (resveratrol dimers).

The red variety Flame presented both the highest content in viniferins and the highest number of different viniferins, i.e., three α-like viniferins and two ϵ-like viniferins that are hereafter termed α_1, ϵ_1, α_3, α_4, and ϵ_2 (**Tables 1**, **4**) according to their respective retention times. The second variety with higher content and number of different viniferins was the white variety Superior, which also contained five different viniferins: α_2, ϵ_1, α_3, α_4, and ϵ_2 (**Tables 1**, **4**; **Figure 4**). Therefore, the only difference between Flame and Superior was the occurrence of α_2-viniferin in Superior instead of α_1-viniferin in Flame. The varieties Red Globe, Crimson, Moscatel Italica, and Dominga contained exclusively ϵ_1 and ϵ_2-viniferins, and Napoleon, α_1, ϵ_1, and ϵ_2-viniferins (**Table 4**).

The induction kinetics of viniferins was similar to that above describe for *trans*-resveratrol (**Figure 5**). The induction pattern of total viniferins was parallel to that observed for every single viniferin (results not shown). When grapes were kept at 22 °C (results not shown), total viniferins reached a maximum content 1 or 2 days later than the maximum day for resveratrol. However, when the grapes were transferred to commercial storage temperature (2 °C), viniferins remained approximately constant or slightly increased (D-7 **Table 2**, **3**; **Figure 5**). Therefore, it seems that viniferin induction kinetics is closely related to that of resveratrol, because viniferins content increased in those varieties in which resveratrol content decreased at 22 °C from D_m (results not shown). However, sensory properties of both control and irradiated table grapes were affected at this temperature. When the maximum day (D_m) was reached, the grapes were transferred at commercial storage temperature (2 °C), and then, sensory properties of grapes were not affected, no significant resveratrol depletion was observed, and no significant viniferins increase was detected (**Figures 2**, **5**). This

could be explained by the fact that viniferins are condensation products of resveratrol, and induction kinetics of both resveratrol and viniferins seems to be inversely related from the maximum day. Therefore, viniferins compensate somehow for the resveratrol decrease observed in some varieties after reaching the maximum (**Figures 2**, **5**). This means that taking into account the health-beneficial properties of viniferins (*29*, *37*, *38*), it could be proposed that UV-C irradiated table grapes could keep their health-promoting properties throughout the processing described here.

Total viniferins content was induced from 4-fold (Napoleon) to 175-fold (Red Globe) (**Tables 2**, **3**). As in the case of *trans*-resveratrol, viniferins induction capacity depended on grape variety independently of the color. The highest viniferin content was detected in the red variety Flame, 0.73 mg/100 g fw (about 1.4 mg of total viniferins per serving of irradiated grapes) (**Tables 2**, **3**). It is important to stress that the presence of viniferins in wine has been previously investigated (*8*). However, to our knowledge, the reports concerning viniferin-like compounds in wine are both recent and few (*39*, *40*). The average content of ϵ-viniferin in six red wines was reported to be 0.81 mg/L, ranging from 0.1 to 1.63 mg/L (*40*). Therefore, it is assumed that a serving (200 g) of UV-C irradiated table grapes could supply from 0.19 to 1.4 mg (depending on the variety, **Tables 2**, **3**) of total viniferins. This means that a average serving of irradiated table grapes would supply an amount of viniferins equivalent to that available in almost five glasses of red wine.

Piceids. *trans*-Piceid (**Figure 1**) was found in all the table grape varieties except Red Globe and Crimson. *trans*-Piceid was identified: *m*/*z* 389.4,; MS/MS, 227.1; UV spectra with maximum at 304 and 316 nm; and retention time of 31 min at these chromatographic conditions (**Table 1**), which was in agreement with previous studies (*13*, *41*). The induction of *trans*-piceid was lower than that of its putative precursor *trans*-resveratrol, and it was significant only in Moscatel Italica and Flame (52- and 3-fold, respectively) (**Tables 2**, **3**).

Two more piceid-like compounds were detected. Identification was based on their molecular ion (*m*/*z*, 389.4; MS/MS, 227.1) and UV spectra, which were fully coincident with those above stated (**Table 1**). However, the retention times were different. The first piceid (piceid-*a*) eluted at 23 min (**Table 1**) and was detected in all of the table grape varieties except in Flame, Crimson, and Dominga. The second piceid (piceid-*b*) eluted at 28.3 min (**Table 1**) and was detected in all of the varieties. The full identification of both piceids was not carried out because of both their low content and poor ionization in LC/MS/MS. A likely explanation for the existence of both piceid-like compounds could be the different position of the glucose moiety or also the presence of other type of glycosilation at the same or different position. In fact, the report of resveratrol-4′-O-β-D-glucopyranosides in *Vitis vinifera* cell cultures could support the above hypothesis (*42*).

Other Minor Inducible Stilbenes. *trans*-Piceatannol (**Figure 1**) was significantly present in all the varieties only after UV-C irradiation. Piceatannol was detected in a low amount in untreated grapes, especially in Flame, Red Globe, Moscatel Italica, and Dominga, in which only trace amounts ($\approx$1μg/100 g fw) were found (**Tables 2**, **3**). Although the induced content of *trans*-piceatannol was lower in comparison to that of resveratrol or viniferins, its presence in irradiated grapes should not be dismissed, since *trans*-piceatannol has also been proposed as an anticancer agent, among other activities (*43*). *trans*-Piceatannol was significantly induced from 2.2-fold (Crimson) to 173-fold (Flame) (**Tables 2**, **3**). The highest piceatannol content was detected in the Flame and Red Globe varieties, with 0.17 and 0.15 mg/100 g fw of grape berry, respectively. According to recent publications, *trans*-resveratrol is a prodrug which is converted to piceatannol (the active drug) by an overexpressed enzyme in human tumors (*44*). In fact, low doses of piceatannol (25 μg/kg) have shown antiarrhythmic and cardioprotective properties in rats (*45*).

Astringin (**Figure 1**) was detected in all of the varieties (traces amount), but properly quantified in only Dominga and Napoleon varieties, with an induction of 23- and 2.2-fold, respectively (**Tables 2**, **3**). Astringin has shown a similar antioxidant effect and even higher radical scavenger activity than *trans*-resveratrol, thanks to the *o*-diphenol structure of astringin in positions 3′ and 4′ (*46*).

cis-Resveratrol (**Figure 1**) was detected by LC/MS in Napoleon, Red Globe, Superior and Dominga (traces amount), but it could be properly quantified only in the Red Globe variety (0.018 mg/100 g fw in irradiated grapes, in contrast to control grapes 0.007 mg/100 g fw). This is in agreement with other reports in which it has been either not detected or found in low amount (*7*, *12*).

cis-Piceid in wine grape berries (*7*), in grape juices (*47*), and grapevine leaves (*48*) has been previously described. However, *cis*-piceid was not detected in the seven table grape varieties studied here, which was in agreement with other reports (*10*).

Another inducible stilbene is pterostilbene. However, it was not found in the varieties studied here. It has been described, although in small amount, in grape berries (*9*), grape leaves (*10*), and wine (*49*).

Comparison Among Varieties. Stilbene induction by UV-C was higher in red table grape varieties than in the white varieties (**Tables 2**, **3**). The highest stilbene induction was observed in the variety Flame. This variety induced four different stilbenes: piceid, piceatannol, *trans*-resveratrol, and viniferins (**Figure 1**; **Table 2**). The Napoleon variety induced the above stilbenes and also astringin (**Figure 1**; **Table 2**). The red variety Crimson induced mainly *trans*-resveratrol and a small amount of both piceatannol and viniferins (**Figure 1**; **Table 2**). The red variety Red Globe induced exclusively piceatannol, *trans*-resveratrol, viniferins, and also a small amount of *cis*-resveratrol (**Figure 1**; **Table 2**). It is also of note that both untreated Crimson and Red Globe grapes were almost devoid of stilbenes.

With respect to white table grape varieties, Dominga induced astringin, piceid, piceatannol, *trans*-resveratrol, and viniferins (**Figure 1**; **Table 3**), whereas Moscatel Italica and Superior induced piceid, piceatannol, *trans*-resveratrol and viniferins (**Figure 1**; **Table 3**). Very low stilbene contents were detected in both Dominga and Moscatel Italica untreated grapes.

A serving of unpeeled irradiated table grapes (200 g) could provide up to 6.7 mg of total stilbenes (in the case of the variety Flame, the richest variety) apart from other important nonstilbene phenolic compounds (anthocyanins, flavonols, flavan-3-ols, etc.) which were not significantly affected by the UV-C treatment reported here (results not shown). Moreover, some of the main sensory properties (firmness, color, flavor) were not significantly affected by this protocol (results not shown), so that grapes were perfectly marketable after this UV treatment, in agreement with a previous report (*13*).

Bearing all this in mind, the term "functional fruit" previously suggested for the irradiated table grapes (*13*) should not be dismissed, since consumption of UV-C irradiated table grapes could supply more stilbenes than those provided by normal intake of red wine. In addition, UV-C treated grapes supply

more resveratrol than commercially available resveratrol-containing nutraceuticals.

ACKNOWLEDGMENT

The authors are grateful to Cooperativa Molinense (Molina de Segura, Murcia, Spain) and Coato (Totana, Murcia, Spain) for supplying the table grapes.

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Received for review May 15, 2002. Revised manuscript received August 1, 2002. Accepted August 1, 2002. E.C. is holder of a grant from the "Movilidad de Investigadores y Tecnólogos" program under the action "Mit-Becas; Modalidad F2". This work has been financially supported by the Spanish CICYT: ALI97-0681 and AGL2000-2014.

JF020562X