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Postharvest UV-C-Irradiated Grapes as a Potential Source for Producing Stilbene-Enriched Red Wines

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The use of postharvest controlled irradiation by UV pulses is proposed as a potential method to produce stilbene-enriched red wine. "Monastrell" grapes were UV-C-irradiated to increase stilbene content. The main inducible stilbenes were resveratrol and piceatannol, which are molecules with reported health-beneficial activities. The evolution of both compounds was followed in the different steps of an "analytical" traditional maceration wine-making process. The final wine made from UV-C-irradiated grapes was enriched about 2- and 1.5-fold in resveratrol and piceatannol, respectively, when compared to the control wine. In addition, no difference was detected regarding the standard enological parameters (color, acidity, etc.). It is strongly suggested that, with the use of more susceptible wine grapes to induce bioactive stilbenes upon UV-C irradiation, the stilbene-enrichment of wine can be much higher.

KEYWORDS: HPLC-DAD-MS-MS; piceatannol; postharvest; resveratrol; stilbene; traditional maceration; UV-C irradiation; viniferin; wine; wine grape

INTRODUCTION

Red wine has been extensively studied because its consumption is inversely related to mortality from coronary heart disease in the so-called "French paradox" (*1*). In addition, moderate wine consumption has been also associated with the delay of tumor onset in both rats and humans (*2*, *3*). These properties have been ascribed to the phenolic compounds which are abundant in red wine (*4*−*6*). In fact, the antioxidant activity of some red wines has been reported to be higher than that of other well-known antioxidant-containing foodstuffs, such as tea or fruit juices (*7*). Among wine phenolics, the stilbenes group is gaining increasing importance (*8*). The main stilbenes found in wine are resveratrol (3,5,4′-trihydroxystilbene) and piceid (resveratrol glucoside) (*9*, *10*). Resveratrol has been reported to have a number of health-beneficial activities, antioxidant (*11*), cardioprotective (*12*), and cancer chemopreventive (*13*, *14*), among others. Stilbene glucosides also show inhibitory activity against tumor and metastasis carcinoma (*15*). Other minor stilbenes found in wine are the antileukemic compound piceatannol (3,5,3′,4′,-tetrahydroxystilbene) (*16*) and the resveratrol polymers viniferins (*17*), the anti-inflamation properties of which have also been described (*18*).

Stilbenes are phytoalexins which are induced in response to a number of biotic and abiotic factors such as injury, pathogen attack, and UV irradiation (*19*, *20*). This naturally occurring induction process has been used to increase the resveratrol content of table grapes by using controlled postharvest UV-C irradiation pulses in order to develop "functional table grapes" with potentially enhanced health-beneficial properties based on their high resveratrol concentration (*21*, *22*). This strategy is the answer to consumers for the increasing demand for "functional foods", i.e., those foods and food derivatives with an "added value" such as increased health-promoting properties resulting from addition of bioactive constituents (ω-3 fatty acids, calcium, fluoride, iron, etc.), removal of undesirable compounds, etc. (*23*). In addition, researchers have also claimed to have developed wines from grapes with high levels of bioactive phenolics (*5*). In this context, the present study will try to throw a light on that demand.

The aim of the present study is to establish the basis for and potential usefulness of UV-C-irradiated grapes to develop a stilbene-enriched red wine. Toward this purpose, the fate of the induced stilbenes from "Monastrell" grapes to the final wine was followed by HPLC-DAD-MS-MS in every step of the "traditional maceration" wine-making process, which is the most typical vinification method followed by wine cellars from the Jumilla region (Spain).

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MATERIALS AND METHODS

Reagents. *trans*-Resveratrol (3,5,4′-trihydroxystilbene), *trans*-piceatannol (3,5,3′,4′-tetrahydroxystilbene), chlorogenic acid (5-*O*-caffeoylquinic acid), and (+)-catechin were purchased from Sigma (Madrid, Spain). Quercetin 3-rutinoside was purchased from Merck (Darmstadt, Germany). Cyanidin 3-rutinoside was purchased from Polyphenols A.S. (Sandnes, Norway). Formic acid and methanol (MeOH) were of analytical grade and also supplied by Merck. Diethyl ether was supplied by Panreac (Barcelona, Spain). Milli-Q system (Millipore Corp., Bedford, MA) ultrapure water was used throughout this research.

Grapes. The red wine grape variety "Monastrell" from the Spanish Appellation of Origin region Jumilla was harvested on September 2001. The Jumilla wine-growing area covers about 40 000 hectares, shared between the provinces of Murcia and Albacete in southeast Spain. It is the one of the world's largest growers for the production of red wine, and the predominant grape is the Monastrell variety (known as "Mourvèdre" in France), which occupies around 85% of the vineyard.

UV Irradiation Treatment. "Monastrell" grape clusters were irradiated with a UV-C treatment previously reported to increase resveratrol content in table grapes (510 W at 40 cm for 60 s) (*21*, *22*). Both irradiated and control (nontreated) grapes were stored for 1 week at room temperature to study the kinetic induction of resveratrol upon storage and to determine the parameter "maximum day" (D_m, defined as the elapsed number of days to achieve the maximum resveratrol concentration; *21*). Once D_m was calculated, the grape clusters to be used for wine-making were irradiated and stored at room temperature until D_m was reached, and then wine-making was carried out.

Traditional Maceration. Analytical (low-scale) "traditional maceration" wine-making was carried out according to the previously reported protocol of Navarro et al. (*24*). Briefly, the analytical wine-making method was carried out as follows. Fifteen kilograms of both control (untreated) and UV-C-irradiated grapes were pressed with a drum press. Stems were removed at this time to avoid giving the must woody flavors. Sulfites (80 mg/kg) were added to the must to prevent the growth of undesired microorganisms. The crushed harvest was allowed to ferment with the skins in 7-L capacity flasks (three replicates per treatment). Fermentation had a regular course (10 days) in all flasks at 28 °C. Flasks were stirred three times per day to stimulate yeast activity and also to favor the release of phenolic compounds from the skin to the must during the 10-day course of maceration. The racking process (to separate wine and lees) was performed 5 days after finishing the maceration. After racking, the wine was clarified (bentonite, 0.4 g/L plus gelatin, 0.08 g/L) and filtered (nylon). Samples, to determine the corresponding amount of stilbenes, were taken in all the steps, i.e., initial (control and UV-C-treated) grapes, must (pre- and postpressed must) and pomace, racked wine and lees, clarified and final wine. This wine-making method is shown in **Figure 1**. However, it should be taken into account that this protocol can be subjected to many possible modifications depending on the wine cellar, and it was our purpose to approach the present protocol as a standard representative example of traditional maceration wine-making.

Enological Parameters. The following parameters were measured according to the applicable methods to the enological sector (Official Diary European Union, 1990): density, pH, volatile and total acidities, color intensity, hue, and alcoholic grade.

Anthocyanins, Flavonols, Hydroxycinnamic Acids, and Flavan-3-ols Extraction. These phenolic compounds were quantified in both grapes and wine. The extraction from grape skins was performed with MeOH/formic acid according to the protocol of Cantos et al. (*21*), with further analysis by HPLC-DAD-MS-MS. Skin represented approximately 25% of the total fresh weight of "Monastrell" grape berries. This was taken into account in order to express the phenolic content as micrograms per gram fresh weight of grape. In the case of wine, the samples were directly filtered through a 0.45-μm membrane filter (Millex-HV$_{13}$, Millipore Corp.) and analyzed by HPLC-DAD-MS-MS.

Stilbenes Extraction. Three-gram portions of solid samples (skin grapes, pomace, and lees) were homogenized in an Ultraturrax T-25 apparatus (Janke and Kunkel, Ika-Labortechnick) at 24 000 rpm for 1 min after addition of 6 mL of diethyl ether. Two milliliters was taken from the supernatant (the total volume was taken into account in every case), concentrated under vacuum, redissolved in 0.5 mL of MeOH, filtered through a 0.45-μm membrane filter (Millex-HV$_{13}$), and further analyzed by HPLC-DAD-MS-MS. Liquid samples (pre- and postpressed must, racked wine, clarified wine and final wine) were also analyzed. Five milliliters of liquid sample was added to five milliliters of diethyl ether and shaken strongly. The diethyl ether phase was recovered and concentrated until dryness, redissolved in 0.5 mL of MeOH, filtered, and further analyzed by HPLC-DAD-MS-MS. In both cases (solid and liquid samples), diethyl ether was used to avoid interference of anthocyanins, which allowed the right identification and quantification of stilbenes.

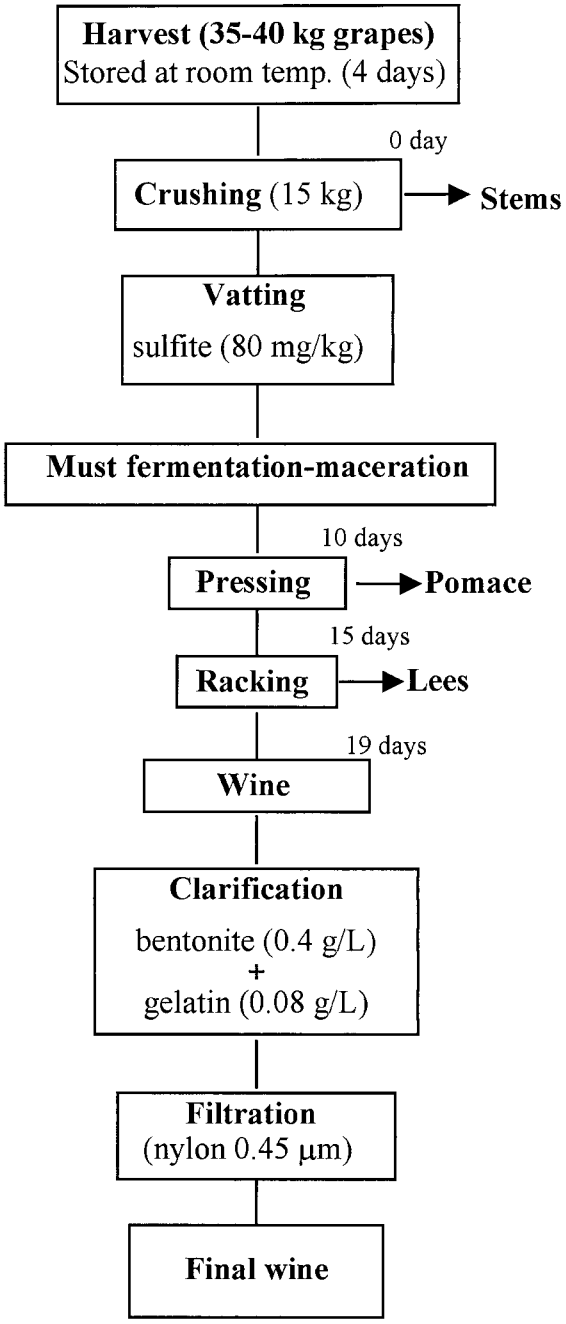

Figure 1. "Analytical" traditional maceration wine-making process used in the present study.

HPLC-DAD-MS-MS. Samples of 20 μL each of the supernatants obtained above were analyzed using an HPLC system equipped with both a DAD and mass detector in series, which consisted of an 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). Chro-

Table 1. Stilbene Content in Traditional Maceration Winemaking:[a] (A) Solid Phases and (B) Liquid Phases

(A) Solid Phases

	piceatannol				*trans*-resveratrol				viniferins			
	CT	UV	ind. (-fold)	LSD	CT	UV	ind. (-fold)	LSD	CT	UV	ind. (-fold)	LSD
grapes	0.78 ± 0.1	1.63 ± 0.2	2.1	(0.03)**	3.18 ± 0.1	8.13 ± 1.7	2.5	(0.17)***	6.37 ± 0.4	11.00 ± 2.4	1.7	(2.7)**
stems	4.68 ± 0.46	5.33 ± 1.8		(2.2)[b]	49.60 ± 4.7	56.40 ± 9.7		(12.2)[b]	11.70 ± 4.5	9.70 ± 4.6		(7.3)[b]
skins												
0d	0.38 ± 0.2	0.62 ± 0.2	1.6	(0.4)*	3.16 ± 1.1	8.90 ± 0.5	2.8	(1.4)***	6.52 ± 2.8	9.60 ± 2.5	1.5	(5.9)[b]
10d	0.15 ± 0.0	0.23 ± 0.05	1.5	(0.06)*	0.26 ± 0.0	0.36 ± 0.01	1.4	(0.04)***	1.63 ± 0.0	3.72 ± 0.4	2.3	(0.4)***
pomace	0.13 ± 0.1	0.16 ± 0.03	1.2	(0.06)[b]	0.18 ± 0.05	0.34 ± 0.01	1.9	(0.09)**	2.20 ± 0.02	2.91 ± 0.4		(1.2)[b]
lees	0.54 ± 0.05	1.14 ± 0.3	2.1	(0.48)*	0.29 ± 0.03	0.59 ± 0.2	2.0	(0.33)**	0.25 ± 0.02	0.28 ± 0.0		(0.08)[b]

(B) Liquid Phases

	piceatannol				*trans*-resveratrol				viniferin-like			
	CT	UV	ind. (-fold)	LSD	CT	UV	ind. (-fold)	LSD	CT	UV	ind. (-fold)	LSD
premust												
0d[c]	36.2 ± 0.3	122.6 ± 25.1	3.4	(28.3)***	79.0 ± 16.2	248.4 ± 85.6	3.1	(98.7)**	0.0 ± 0.0	0.0 ± 0.0		
10d[c]	229.0 ± 15.4	411.0 ± 13.0	1.8	(27.4)***	124.3 ± 23.6	225.0 ± 8.8	1.8	(40.4)**	273.7 ± 47.2	293.2 ± 12.3		(78.4)[b]
must	96.5 ± 44.8	217.4 ± 4.6	2.2	(51.0)**	55.2 ± 15.6	144.6 ± 20.3	2.6	(36.9)**	117.0 ± 47.0	147.9 ± 9.6	1.3	(55.2)[b]
racked wine	205.5 ± 3.4	278.0 ± 9.5	1.3	(15.7)***	62.0 ± 10.4	169.6 ± 19.5	2.7	(32.6)***	159.7 ± 18.3	179.1 ± 20.6		(44.2)[b]
clarified wine	248.3 ± 37.2	305.7 ± 32.1	1.2	(55.5)*	112.5 ± 6.5	171.2 ± 11.5	1.5	(19.9)***	128.0 ± 13.9	140.9 ± 26.8		(45.7)[b]
final wine	208.4 ± 3.6	311.0 ± 44.2	1.5	(63.3)*	90.8 ± 23.0	190.7 ± 34.0	2.1	(60.2)**	160.1 ± 31.2	191.3 ± 25.1	1.2	(53.1)[b]

[a] Values are expressed as μg/g of solid phase and as μg/L of liquid phase. Results are shown as mean ± SD ($n = 3$). "ind.", induction. LSD values are in parentheses. * $P = 0.05$; ** $P = 0.01$; *** $P = 0.001$. [b] Not significant. [c] "Premust 0d" is defined as the must just after crushing. "Premust 10d" is defined as the must just before pressing.

matographic separation was carried out on a reverse-phase C_{18} LiChroCART column (25 × 0.4 cm, particle size 5 μm, Merck). 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. (*21*). UV chromatograms were recorded at 320 (stilbenes and hydroxycinammic acids), 360 (flavonols), 510 (anthocyanins), and 280 nm (flavan-3-ols). The mass detector was an ion-trap mass spectrometer (G2445A, Agilent Technologies) 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 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. Anthocyanins, flavonols, flavan-3-ols, hydroxycinnamic acid derivatives, and stilbenes were identified by their UV spectra, retention times, and MS and MS-MS data after ion isolation using an ion trap (*25*). Anthocyanins were quantified at 510 nm as cyanidin 3-rutinoside, flavonols at 360 nm as quercetin 3-rutinoside, hydroxycinnamic acid derivatives at 320 nm as chlorogenic acid, and flavan-3-ols at 280 nm as catechin (*21*). Pure piceatannol (*m*/*z* 243) and resveratrol (*m*/*z* 227) from Sigma were used as stilbene standards and quantified at 320 nm (*21*). α- and ϵ-viniferins were also quantified at 320 nm according to their UV spectra and MS values (*m*/*z* 679 and *m*/*z* 453, respectively).

Graphs and Statistical Analysis. Graphs of the experimental data were generated by using the Sigma Plot 6.0 program for Windows. Mean values of stilbene contents in both control and UV wines were compared using the least significant difference (LSD), obtained by using the SPSS 11.0 program (SPSS Inc., Chicago, IL). Levels of significance were set as follows: * $P = 0.05$; ** $P = 0.01$; *** $P = 0.001$.

RESULTS AND DISCUSSION

UV-C Irradiation of "Monastrell" Grapes. The first approach in the present study was to obtain resveratrol-enriched wine grapes in order to start the vinification process with the highest resveratrol concentration in grapes. For this purpose, the number of elapsed days to achieve the maximum resveratrol concentration was calculated (D_m; *21*). The maximum resveratrol concentration was detected on the fourth day ($D_m = 4$ d) after UV−C treatment. On this day, the resveratrol and piceatannol concentrations were over 2-fold higher than those of untreated (control) grapes (**Table 1A**). Depending on the grape variety, both the parameter D_m and the induction of stilbenes content can be different. In fact, the resveratrol induction detected in the "Monastrell" wine grape variety (around 2.5-fold) was significantly lower than that previously reported for table grape varieties, in which the induction can be much higher, such as 10-fold (in "Napoleon" table grape; *21*) or even more than 2000-fold in other grape varieties (*26*). This means that the potential induction of much higher resveratrol (and other stilbenes) content after UV-C irradiation cannot be ruled out in other wine grape varieties. However, the grape variety "Monastrell" was used in the present study because it is the most cultivated variety in southeast Spain and it is the basis for the production of the high-quality red wines in that region. Therefore, in the near future, the first step to be investigated in depth should be the different susceptibilities of wine grapes to induce stilbenes as well as possible modification of the UV-C treatment for this purpose.

Monastrell wine grape was mainly characterized by the presence of 10 anthocyanins in a high concentration compared to that of other wine grape varieties (*27*) (**Table 2**). UV-C irradiation did not modify the phenolic pattern of grapes apart from the stilbene content (**Table 2**). However, an increase (independent of UV-C treatment) in anthocyanins was detected after 4 days of storage at room temperature, as previously reported (*28*) (**Table 2**). The identification and quantification

Table 2. Phenolic Composition of "Monastrell" Grapes[a]

	Dp-3-glc	Cy-3-glc	Pt-3-glc	Pn-3-glc	Mv-3-glc	Dp-*p*-coum	Cy-*p*-coum	Pt-*p*-coum	Pn-*p*-coum	Mv-*p*-coum	TA	M-3-glc	Q-3-gluc	Q-3-glc/rut	K-3-glc	TFl	TCatq	Couta
0d	130.2	131.5	138.1	186.8	439.5	19.4	15.3	3.2	27.9	76.5	1168.4	55.3	29.1	53.2	9.0	146.6	154.0	11.3
	(5.9)	(30.3)	(2.4)	(56.5)	(11.7)	(0.9)	(1.4)	(0.9)	(0.7)	(12.1)	(21.4)	(7.9)	(4.0)	(17.2)	(2.9)	(30.3)	(27.3)	(2.9)
CT 4d	174.7	179.5	183.6	190.4	557.0	20.8	20.6	3.5	34.5	75.9	1440.5	63.9	47.7	58.1	11.0	180.7	141.4	13.4
	(18.1)	(0.0)	(27.3)	(0.0)	(50.8)	(1.2)	(0.8)	(0.1)	(1.7)	(10.5)	(288.0)	(10.3)	(5.5)	(13.4)	(1.6)	(12.0)	(7.6)	(3.1)
UV 4d	195.5	177.7	208.1	203.1	617.7	25.0	23.4	3.5	36.7	85.9	1516.7	78.9	48.0	74.7	11.3	212.9	173.8	17.0
	(21.6)	(10.4)	(24.9)	(21.3)	(81.2)	(2.1)	(3.6)	(1.5)	(13.0)	(31.2)	(115.0)	(8.7)	(7.1)	(27.1)	(1.7)	(44.5)	(3.6)	(6.8)

[a] Values are expressed as μg/g fresh weight of total grape berry. Mean values are shown ($n = 3$) with SD in parentheses. Dp-3-glc, delphinidin 3-glucoside; Cy-3-glc, cyanidin 3-glucoside; Pt-3-glc, petunidin 3-glucoside; Pn-3-glc, peonidin 3-glucoside; Mv-3-glc, malvidin 3-glucoside; Dp-*p*-coum, delphinidin 3-*p*-coumaroylglucoside; Cy-*p*-coum, cyanidin 3-*p*-coumaroylglucoside; Pt-*p*-coum, petunidin 3-*p*-coumaroylglucoside; Pn-*p*-coum, peonidin 3-*p*-coumaroylglucoside; Mv-*p*-coum, malvidin 3-*p*-coumaroylglucoside; TA, total anthocyanins; M-3-glc, myricetin 3-glucoside; Q-3-gluc, quercetin 3-glucuronide; Q-3-glc/rut, quercetin 3-glucoside plus quercetin 3-rutinoside; K-3-glc, kaempferol-3-glucoside; TFl, total flavonols; TCatq, total catechins; Couta, *p*-coumaroyltartaric acid.

Table 3. Anthocyanins, Flavanols, Hydroxycinnamic Derivatives, and Flavan-3-ols in Both Control and UV Wines[a]

	Dp 3-glc	Cy-3-glc	Pt 3-glc	Pn 3-glc	Mv-3-glc	Dp-*p*-coum	Cy-3-*p*-coum	Pt-*p*-coum	Pn-*p*-coum	Mv-*p*-coum	TA	M-3-glc	Q-3-gluc	Q-3-glc/rut	Q	TFl	TCatq	Couta
CT	24.03	6.34	41.15	24.57	191.14	8.70	1.91	3.73	4.73	10.68	316.98	35.29	28.02	9.41	22.33	95.05	318.73	8.55
	(1.65)	(0.53)	(2.95)	(1.52)	(9.05)	(0.46)	(0.27)	(0.19)	(0.83)	(0.88)	(17.35)	(1.84)	(1.86)	(1.23)	(1.78)	(5.59)	(38.16)	(0.61)
UV	21.30	6.24	37.00	25.37	178.65	7.29	1.47	3.41	3.67	9.36	293.76	28.88	23.78	8.08	20.65	81.39	320.65	8.57
	(2.18)	(0.70)	(2.43)	(2.25)	(10.53)	(1.03)	(0.23)	(0.54)	(0.54)	(0.40)	(17.84)	(3.62)	(2.71)	(1.87)	(2.32)	(10.51)	(24.08)	(0.48)

[a] Values are expressed as mg/L of wine. Mean values are shown ($n = 3$) with SD in parentheses. Dp-3-glc, delphinidin 3-glucoside; Cy-3-glc, cyanidin 3-glucoside; Pt-3-glc, petunidin 3-glucoside; Pn-3-glc, peonidin 3-glucoside; Mv-3-glc, malvidin 3-glucoside; Dp-*p*-coum, delphinidin 3-*p*-coumaroylglucoside; Cy-*p*-coum, cyanidin 3-*p*-coumaroylglucoside; Pt-*p*-coum, petunidin 3-*p*-coumaroylglucoside; Pn-*p*-coum, peonidin 3-*p*-coumaroylglucoside; Mv-*p*-coum, malvidin 3-*p*-coumaroylglucoside; TA, total anthocyanins; M-3-glc, myricetin 3-glucoside; Q-3-gluc, quercetin 3-glucuronide; Q-3-glc/rut, quercetin 3-glucoside plus quercetin 3-rutinoside; Q, quercetin; TFl, total flavonols; TCatq, total catechins; Couta, *p*-coumaroyltartaric acid.

of phenolics from the "Monastrell" wine grapes assayed in the present study are shown in **Table 2**.

Traditional maceration was used to obtain red wine from both control and UV-C-irradiated "Monastrell" grapes. Traditional maceration involves a close contact between skins and must (**Figure 1**). This wine-making technique was employed in the present study because it is the vinification method most utilized by wine cellars from southeast Spain. Although total phenolics, including flavan-3-ols, flavonols, anthocyanins, and hydroxycinammic acids, were quantified in both control and UV-C-treated grapes (**Table 2**) as well as in the final corresponding wines (**Table 3**), special attention was paid to the stilbene aglycons (resveratrol and piceatannol) (**Table 1**) because these molecules are the main UV-C-inducible phytoalexins (*21*).

Stilbenes Evolution during Traditional Maceration. The main stilbenes found in the solid phase (skins) just after grape crushing were *trans*-resveratrol, *trans*-piceatannol, and viniferins (resveratrol polymers) (**Table 1A**). Astringin (piceatannol glucoside) and piceid (resveratrol glucoside) were detected in trace amount since diethyl ether is not an appropriate solvent to extract glucosides. However, as mentioned above, stilbene glucosides such as piceid are not significantly induced after UV-C irradiation (data not shown), and so their exhaustive characterization was discarded.

After crushing, both skins and liquid (must before pressing) phases were maintained in the flask at 28 °C for 10 days. On the second day after crushing, the fermentation started, as indicated by both bubbling and a decrease in density. When the ethanol content was increased, the extraction of stilbenes from the skins to the liquid phase also increased (*29*). To promote stilbenes extraction, both must and skins were stirred three times every day. The fermentation process finished (a constant density was reached) after 6 days. However, the must was kept in the flasks for four more days in order to complete the usual wine-making method of the local wine cellars. The

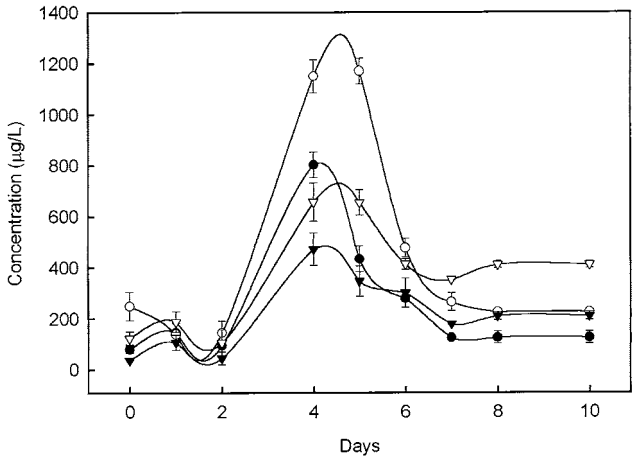

Figure 2. Evolution of resveratrol and piceatannol during the fermentation process in traditional wine-making. (●) Control resveratrol; (○) UV resveratrol; (▼) control piceatannol; (▽) UV piceatannol.

maximum resveratrol and piceatannol contents in must (before pressing) were detected on the fifth day (1170 μg/L resveratrol and 655 μg/L piceatannol in UV must versus 471 μg/L resveratrol and 346 μg/L piceatannol in control must) (**Figure 2**), which was coincident with the end of the fermentation process (*29*, *30*). However, it is remarkable that the resveratrol concentration decreased by about 10-fold on the tenth day with respect to the maximum content detected on the fifth day (**Figure 2**). This decrease could be justified by enzymatic- and nonenzymatic-catalyzed oxidation processes (*31*, *32*), which could also be favored by temperatures above 25 °C, as previously reported (*33*). The oxidation of *trans*-resveratrol in both pre- and postfermentative phases has also been described (*10*, *34*). However, it is somewhat surprising that piceatannol, a molecule very susceptible to oxidation due to its *o*-diphenolic

group, presented an accumulation and decrease in kinetics similar to those of resveratrol (**Figure 2**). It can be assumed that overlapped processes can occur: on one hand, the release of stilbenes from the skin and their increasing solubility in the ethanol-enriched must (net accumulation of stilbenes until the fourth and fifth days), and on the other hand, the decay of stilbenes because of the prevalence of degradation reactions (net decrease from the fifth to tenth days). A likely explanation for the higher depletion of resveratrol from the fifth day could be its lower solubility compared to that of piceatannol. Therefore, this overall process, which can lead to a severe loss of stilbene content during traditional maceration wine-making, is another step which deserves more investigation. Taking into account the stilbenes kinetics in must, the best option could have been to stop the maceration at the fifth day in order to get a wine richer in both piceatannol and resveratrol. The importance of checking the stilbene content every day during the maceration process can be inferred from these results.

After 10 days, the maceration process was stopped, and the must was pressed (**Figure 1**). The solid phase (pomace) contained a low total amount of stilbenes, approximately 1% of the resveratrol initially present in grapes (data not shown), which was in agreement with other reports (*35*). The amount of resveratrol in UV pomace was 2-fold higher than that in control pomace (**Table 1A**). After pressing, a decrease in stilbene content was observed in must (**Figure 1**, **Table 1B**), in accordance with other reports (*36*). The must after pressing was richer in piceatannol than in resveratrol (**Table 1B**). In addition, the UV must contained >2-fold more of both piceatannol and resveratrol than control must (**Table 1B**). After racking, 5 days later (**Figure 1**), the residual solid phase (lees) contained approximately 2% of the total initial resveratrol content of grapes (data not shown). UV lees also presented 2-fold higher resveratrol and piceatannol content than control lees (**Table 1A**). Therefore, about 3% of resveratrol was lost in residual solids (pomace + lees). The next step, clarification (**Figure 1**), was approached by using bentonite plus gelatin, since these fining agents did not shown any effect on resveratrol recovery (**Table 1B**; *37*). At the end of the process, the final wine obtained from irradiated grapes was about 2- and 1.5-fold richer in resveratrol and piceatannol content, respectively, than control wine (**Table 1B**). However, the viniferins (α- and ϵ-viniferin according to *m*/*z* 679 and 453, respectively) induction observed in UV-C-irradiated grapes (1.7-fold, **Table 1A**) were not found in the final wine (**Table 1B**).

Regarding the total resveratrol content in wines, the high variability previously reported should be emphasized (*38*). The resveratrol content in wine reported here in traditional wine-making (**Table 1B**) is equal (*37*, *39*), lower (*36*, *40*, *41*), or higher (*42*, *43*), depending on the previous study considered. The amount of resveratrol can vary considerably, depending on the environmental (agronomic) factors in the vineyard (fungal attack, irrigation, canopy management, soil, etc.), grape variety, maturity stage, and the wine-making techniques, rendering the above-mentioned high variability in resveratrol content (*37*, *38*, *44*). Similar discussion can arise for piceatannol content in wine obtained from UV-C-treated grapes. The amount of 300 μg/L is low in comparison with other reports (*45*). It is also important to emphasize that the total stilbene content in wine could have been higher if the glucoside derivatives had been considered (*45*).

Sensory and Enological Parameters. The general parameters of wine obtained from both control and UV-C-irradiated grapes are shown in **Table 4**. Parameters such as pH, density, acidity, color intensity, hue, and alcoholic grade were not affected by UV treatment (**Table 4**). pH is an important parameter to take into account to determine the wine quality. The low values found for pH imply a high color stability and a good conservation of wine. Both total and volatile acidities showed normal values, which imply a good development of the process as well as a healthy state of the wine grapes (*46*). In addition, no differences were observed in the resulting wines concerning both taste and aroma.

Table 4. Enological Parameters of Both Control and UV Wines

	CT	UV
density (g/L)	990 ± 0.0	990 ± 0.0
pH	3.510 ± 0.020	3.490 ± 0.040
VAc (g/L acetic acid)	0.082 ± 0.007	0.072 ± 0.006
TAc (g/L tartaric acid)	5.820 ± 0.131	6.000 ± 0.110
color intensity	20.380 ± 0.923	22.010 ± 2.447
hue	0.640 ± 0.010	0.670 ± 0.021
alcoholic grade (vol %)	13.70	13.60

VAc, volatile acidity; TAc, total acidity. Color intensity is expressed as absorbance (Abs) at 420 nm + Abs at 520 nm + Abs 620 nm. Hue is expressed as Abs at 420 nm/Abs at 520 nm.

In summary, the present study suggests that postharvest UV-C irradiation of wine grapes could be a valuable tool to obtain stilbene-enriched wines. In the case reported here, UV wine contained an average of 2- and 1.5-fold higher resveratrol and piceatannol content, respectively, than control wine obtained by using traditional maceration. It is reasonable to think that this average 2-fold induction can be significantly increased by using other, more UV-C-inducible wine grapes. In fact, the different susceptibility to induce stilbenes upon UV-C treatment has been recently published for table grapes (*26*). In addition, the overall improvement of the process could be achieved by improving some of the steps involved in the "analytical" wine-making technique assayed here and also by employing other wine-making protocols.

Another proposed approach to increase the stilbene (resveratrol) content in wine is the use of transgenic yeast (*47*). In our opinion, this approach could also be useful in giving a possible positive synergistic effect when combined with the method proposed here.

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LITERATURE CITED

(1) Renaud, S.; De Lorgeril, M. Wine, alcohol, platelets, and French paradox for coronary heart disease. *Lancet* **1992**, *339*, 1523−1526.

(2) Clifford, A. J.; Ebeler, S. E.; Ebeler, J. D.; Bills, N. D.; Hinrichs, S. H.; Teissedre, P.-L.; Waterhouse, A. L. Delayed tumor onset in transgenic mice fed an amino acid-based diet supplemented with red wine solids. *Am. J. Clin. Nutr.* **1996**, *64*, 748−756.

(3) Carbó, N.; Costelli, P.; Baccino, F. M.; López-Soriano, F. J.; Argilés, J. M. Resveratrol, a natural product present in wine, decreases tumour growth in a rat tumour model. *Biochem. Biophys. Res. Commun.* **1999**, *254*, 739−743.

(4) Peña-Neira, A.; Hernández, T.; García-Vallejo, C.; Estrella, I.; Suarez, J. A. A. Survey of phenolic compounds in Spanish wines of different geographical origin. *Eur. Food Res. Technol.* **2000**, *210*, 445−448.

(5) Burns, J.; Gardner, P. T.; O'Neil, J.; Crawford, S.; Morecroft, I.; McPhail, D. B.; Lister, C.; Matthews, D.; MacLean, M. R.; Lean, M. E.; J.; Duthie, G. G.; Crozier, A. Relationship among antioxidant activity, vasodilation capacity, and phenolic content of red wine. *J. Agric. Food Chem.* **2000**, *48*, 220−230.

(6) Soleas, G. J.; Diamandis, E. P.; Goldberg, D. M. Wine as a biological fluid: history, production, and role in disease prevention. *J. Clin. Lab. Anal.* **1997**, *11*, 287−313.

(7) Paganga, G.; Miller, N.; Rice-Evans, C. A. The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitutive? *Free Radical Res.* **1999**, *30*, 153−162.

(8) Bavaresco, L.; Fregoni, C.; Cantù, E.; Trevisan, M. Stilbene compounds: from the grapevine to wine. *Drugs Exp. Clin. Res.* **1999**, *2/3*, 57−63.

(9) Lamuela-Raventós, R. M.; Romero-Pérez, A. I.; Waterhouse, A. L.; de la Torre-Boronat, M. C. Direct HPLC analysis of *cis*- and *trans*-resveratrol and piceid isomers in Spanish red *Vitis vinifera* wines. *J. Agric. Food Chem.* **1995**, *43*, 281−283.

(10) Mattivi, F.; Reniero, F.; Korhammer, S. Isolation, characterization, and evolution in red wine vinification of resveratrol monomers. *J. Agric. Food Chem.* **1995**, *43*, 1820−1823.

(11) Miller, N. M.; Rice-Evans, C. Antioxidant activity of resveratrol in red wine. *Clin. Chem.* **1995**, *41*, 1789 (letter).

(12) Hung, L. M.; Chen, J. K.; Huang, S. S.; Lee, R. S.; Su, M. J. Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. *Cardiovasc. Res.* **2000**, *47*, 549−555.

(13) Jang, M.; Cai, L.; Udeani, G. O.; Slowing, K. V.; Thomas, C. F.; Beecher, C. W. W.; Fong, H. H. S.; Farnsworth, N. R.; Kinghorn, A. D.; Metha, R. G.; Moon, R. C.; Pezzuto, J. M. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* **1997**, *275*, 218−220.

(14) Gusman, J.; Malone, H.; Atassi, G. A reappraisal of the potential chemopreventive and chemotherapeutic properties of resveratrol. *Carcinogenesis* **2001**, *22*, 1111−1117.

(15) Kimura, Y.; Okuda, H. Effects of naturally occurring stilbene glucosides from medicinal plants and wine, on tumour growth and lung metastasis in Lewis lung carcinoma-bearing mice. *J. Pharm. Pharmacol.* **2000**, *52*, 1287−1295.

(16) Wieder, T.; Prokop, A.; Bagci, B.; Essmann, F.; Bernicke, D.; Schulze-Osthoff, K.; Dorken, B.; Schmalz, H. G.; Daniel, P. T.; Henze, G. Piceatannol, a hydroxylated analog of the chemopreventive agent resveratrol, is a potent inducer of apoptosis in the lymphoma cell line BJAB and in primary leukemic lymphoblasts. *Leukemia* **2001**, *15*, 1735−1742.

(17) Baderschneider, B.; Winterhalter, P. Isolation and characterization of novel stilbene derivatives from Riesling wine. *J. Agric. Food Chem.* **2000**, *48*, 2681−2686.

(18) Kitanaka, S.; Ikezawa, T.; Yasukawa, K.; Yamanouchi, S.; Takido, M.; Sung, H. K.; Kim, I. H. (+)-Alpha-viniferin, an antiinflamatory compound from Caragana chamlague root. *Chem. Pharm. Bull.* **1990**, *38*, 432−435.

(19) Soleas, G. J.; Diamandis, E. P.; Goldberg, D. M. Resveratrol: a molecule whose time has come? and gone? *Clin. Biochem.* **1997**, *30*, 91−113.

(20) Douillet-Breuil, A. C.; Jeandet, P.; Adrian, M.; Bessis, R. Changes in phytoalexin content of various *Vitis* spp. In response to ultraviolet C elicitation. *J. Agric. Food Chem.* **1999**, *47*, 4456−4461.

(21) Cantos, E.; Espín, J. C.; Tomás-Barberán, F. A. Postharvest induction modeling method using UV irradiation pulses for obtaining resveratrol-enrichment table grapes: a new "functional" fruit? *J. Agric. Food Chem.* **2001**, *48*, 4606−4612.

(22) Tomás-Barberán, F. A.; Espín, J. C.; Cantos, E. Postharvest treatment of fruits and vegetables by UV irradiation pulses. PCT/ES02/00192, 2001.

(23) Milner, J. A. Functional foods and health promotion. *Am. J. Clin. Nutr.* **1999**, *129*, 1395−1397.

(24) Navarro, S.; Barba, A.; Oliva, J.; Navarro, G.; Pardo, F. Evolution of residual levels of six pesticides during elaboration of red wines. Effect of wine-making procedures in their disappearance. *J. Agric. Food Chem.* **1999**, *47*, 264−270.

(25) Cantos, E.; Espín, J. C.; Tomás-Barberán, F. A. Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC-DAD-MS-MS. *J. Agric. Food Chem.* **2002**, *50*, 5691−5696.

(26) Cantos, E.; Espín, J. C.; Tomás-Barberán, F. A. Postharvest stilbene enrichment of red and white table grape varieties using UV-C irradiation pulses. *J. Agric. Food Chem.* **2002**, *50*, 6322−6329.

(27) Fernández-López, J. A.; Hidalgo, V.; Almela, L.; López-Roca, J. M. Quantitative changes in anthocyanin pigments of *Vitis vinifera* cv Monastrell during maturation. *J. Sci. Food Agric.* **1992**, *58*, 153−155.

(28) Cantos, E.; García-Viguera, C.; de Pascual-Teresa, S.; Tomás-Barberán, F. A. Effect of postharvest ultraviolet irradiation on resveratrol and other phenolics of cv. Napoleón table grapes. *J. Agric. Food Chem.* **2000**, *48*, 4606−4612.

(29) Pezet, R.; Cuenat, P. H. Resveratrol in wine: extraction from skin during fermentation and post-fermentation standing of must from Gamay grapes. *Am. J. Enol. Vitic.* **1996**, *47*, 287−290.

(30) Bavaresco, L.; Fregoni, C.; Trevisan, M.; Fortunati, P. Effect of cluster stems on resveratrol content in wine. *Ital. J. Food Sci.* **2000**, *12*, 103−108.

(31) Traverso-Rueda, S.; Singleton, V. L. Catecholase activity in grape juice and its implication in winemaking. *Am J. Enol. Vitic.* **1973**, *24*, 103−109.

(32) Morales, M.; Alcántara, J.; Ros-Barceló, A. Oxidation of *trans*-resveratrol by a hypodermal preoxidase isoenzyme from Gamay rouge grape (*Vitis vinifera*) Berries. *Am. J. Enol.* **1997**, *48*, 33−38.

(33) Jeandet, P.; Bessis, R.; Maume, B. F.; Meunier, P.; Peyron, D.; Trollat, P. Effect of enological practices on the resveratrol isomer content of wine. *J. Agric. Food Chem.* **1995**, *43*, 316−319.

(34) Castellari, M.; Spinabelli, U.; Riponi, C.; Amati, A. Influence of some technological practices on the quantity of resveratrol wine. *Z. Lebensm. Unters Forsch. A* **1998**, *206*, 151−155.

(35) Bertelli, A. A. E.; Gozzini, A.; Stradi, R.; Stella, S.; Bertelli, A. Stability of resveratrol over time and in the various stages of grape transformation. *Drugs Exp. Clin. Res.* **1998**, *24*, 207−211.

(36) Vrhovsek, U.; Wendelin, S.; Eder, R. Effects of various vinification techniques on the concentration of *cis*- and *trans*-resveratrol and resveratrol glucoside isomers in wine. *Am J. Enol. Vitic.* **1997**, *48*, 214−219.

(37) Threlfall, J. R.; Morris, J. R.; Mauromoustakos, A. Effects of fining agents on *trans*-resveratrol concentration in wine. *Aust. J. Grape Wine R.* **1999**, *5*, 22−26.

(38) Goldberg, D. M.; Yan, J.; Eric, N. G.; Diamandis, E. P.; Karumanchiri, A.; Soleas, G.; Waterhouse, A. L. A global survey of *trans*-resveratrol concentrations in commercial wines. *Am J. Enol. Vitic.* **1995**, *46*, 159−165.

(39) Celotti, E.; Ferrarini, R.; Zironi, R.; Conte, L. S. Resveratrol content of some wines obtained from dried Valpolicella grapes: Recioto and Amarone. *J. Chromatogr. A* **1996**, *730*, 47−52.

(40) Revilla, E.; Ryan, J. M. Analysis of several phenolic compounds with potential antioxidant properties in grape extracts and wines by high-performance liquid chromatography−photodiode array detection without sample preparation. *J. Chromatogr. A* **2000**, *881*, 461−469.

(41) López, M.; Martínez, F.; Del Valle, C.; Orte, C.; Miró, M. Analysis of phenolic constituents of biological interest in red wines by high-performance liquid chromatography. *J. Chromatogr. A* **2001**, *922*, 359−363.

(42) Landrault, N.; Larronde, F.; Delaunay, J. C.; Castagnino, C.; Vercauteren, J.; Merillon, J. M.; Gasc, F.; Cros, G.; Teissedre, P. L. Levels of stilbene oligomers and astilbin in French varietal wines and grapes during noble rot development. *J. Agric. Food Chem.* **2002**, *50*, 2046−2052.

(43) Adrian, M.; Jeandet, P.; Douillet-Breuil, A. C.; Tesson, L.; Bessis, R. Stilbene content of mature *Vitis vinifera* in response to UV-C elicitation. *J. Agric. Food Chem.* **2000**, *48*, 6103−6105.

(44) Jeandet, P.; Bessis, R.; Gautheron, B. The production of resveratrol (3,5,4′-trihydroxystilbene) by grape berries in different developmental stage. *Am. J. Enol. Vitic.* **1991**, *42*, 41−46.

(45) Ribero de Lima, M. T.; Waffo-Téguo, P.; Teissedre, P. L.; Pujolas, A.; Vercauteren, J.; Cabanis, J. C.; Mérillon, J. M. Determination of stilbenes (*trans*-astringin, *cis*- and *trans*-piceid, and *cis*- and *trans*-resveratrol) in Portuguese wines. *J. Agric. Food Chem.* **1999**, *47*, 2666−2670.

(46) Pardo, F. *Jumilla. Viñas, bodegas y vinos*; Consejería de Medio Ambiente, Agricultura y Agua, Com. Aut. Murcia: Murcia, Spain, 1996; 287 pp.

(47) González-Candelas, L.; Gil, J. V.; Lamuela-Raventós, R. M.; Ramón, D. The use of transgenic yeast expressing a gene encoding a glycosyl-hydrolase as a tool to increase resveratrol content in wine. *Int. J. Food Microbiol.* **2000**, *59*, 179−183.

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