

Postharvest Induction Modeling Method Using UV Irradiation Pulses for Obtaining Resveratrol-Enriched Table Grapes: A New “Functional” Fruit?

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A modeling method for the induction of resveratrol synthesis by UV irradiation pulses in Napoleon table grapes is proposed. The method is based on the combination of four main parameters: irradiation power (IW), irradiation time (IT), irradiation distance (ID), and number of elapsed days to achieve the highest resveratrol accumulation (D_m). Maximum resveratrol content (11-fold higher than untreated grapes) was achieved using the combination: IW = 510 W, IT = 30 s, ID = 40 cm, and D_m = 3 days. Sensory characteristics and main features of irradiated grapes (color, weight, firmness, flavor, size, ripening index and vitamin C content) remained unaltered after 1 week of storage. UV induction signal migrated to the hidden side of the grape skin with a delay of 3 days as compared to the directly irradiated side. Phenolic compounds were not detected in Napoleon grape flesh. Resveratrol content per standard serving (200 g) of irradiated grape was about 3 mg, an amount more than 10-fold higher than that of untreated Napoleon grapes. This means that a serving of irradiated grape (unpeeled) could supply the resveratrol content equivalent to 3 glasses of a red wine with high resveratrol content (\approx 1 mg/glass). Therefore, controlled UV irradiation pulses are useful as a simple postharvest treatment (and alternative to genetic engineering) to obtain possible “functional” grapes (with enhanced health-promoting properties) as a dietary source of high resveratrol content.

Keywords: *Functional fruit; postharvest technology; resveratrol; ultraviolet irradiation; table grapes; Vitis vinifera*

INTRODUCTION

trans-Resveratrol (3,5,4'-trihydroxystilbene) is a non-flavonoid phenolic compound not very widely distributed in foodstuffs for human consumption (1). However, this molecule has attracted increasing interest as a health-promoting agent because of its antioxidant (2, 3), antiplatelet (4), anti-inflammatory (5, 6), estrogenic (7), cardioprotective (8), and antitumor (9–12) activities. Even the resveratrol inhibition of herpes simplex virus replication has been recently reported (13). Therefore, the importance of its presence in the diet can be inferred. Red wine is one of the most common products in human diet in which resveratrol is present (1, 14). In fact, phenolic compounds (including resveratrol) present in red wine are held responsible of the so-called “French Paradox”, implicating that moderate drinking of red wine over a long period of time could prevent coronary heart diseases (CHD) (15, 16).

Grape berries are also important as possible dietary source of resveratrol, although the basal concentration of this molecule in table grapes is very low. However, resveratrol is a phytoalexin, which can be induced by a number of biotic and abiotic factors such as injury, fungal infection, and UV irradiation (1, 17). This could open the possibility to modulate the induction process by controlled UV irradiation. Recently, Cantos et al. (18) described the effect of postharvest UV irradiation on

resveratrol and other phenolic compounds of table grapes suggesting that UV irradiation could be used as postharvest treatment to increase resveratrol content in table grapes. The increasing output of studies stressing the health-promoting properties of resveratrol (about 300 scientific publications in the last five years) and the promising results concerning resveratrol bioavailability (19, 20) together with the last approach of Cantos et al. (18) prompted us to develop an induction modeling method to predict and characterize the increase of resveratrol content in table grapes using UV irradiation pulses.

One of the traditional claims in proper dietary habits is the increase in the intake of fruits and vegetables (21–24). 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.

Functional foods try to overcome this problem by approaching different strategies: increasing natural health-promoting compounds of a specific source (higher effect with the same amount), getting rid of some (undesirable) components, adding new ingredients (modifying taste, color; increasing health-promoting properties...), increasing bioavailability of active compounds, etc. (25, 26).

Therefore, the aim of the present study is to develop a possible “functional” table grape with increased health-promoting properties based on their high resveratrol content. For this purpose, an induction model-

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ing method (with industrial applicability) using UV irradiation pulses is developed and characterized.

MATERIALS AND METHODS

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

Plant Material. Red "Napoleon" grapes were harvested in November/December 2000 in Blanca (Murcia, Spain). Grape berries were at mature ripening stage and ready to be commercialized.

UV Irradiation Pulses. EU regulations prohibit the full description (the "know-how") of the methodology, which is currently patent pending, but the essential procedure is described.

Grape berries were placed in plastic wells and irradiated with 17 Sylvania (G30T8; USA) germicidal lamps (30 W each lamp, peak output at 254 nm). The independent switch of the lamps controlled the total irradiation power: irradiation from 30 W (1 lamp) to 510 W (17 lamps). Grapes were irradiated at room temperature in the presence of a fan which prevented the possible increase of temperature. Irradiation was carried out in the range from 20 to 60 cm from the lamps to the sample. Irradiation times ranged from 5 s to 30 min. Both irradiated and control grape berries were stored at 20 °C in perforated plastic bags and at relative humidity of 90–95% to avoid water loss and shriveling. Every day, a set of both irradiated and control grapes was transferred to 2 °C (in the same plastic bags). This was approached to calculate the maximum resveratrol induced since certain resveratrol degradation was observed at 20 °C after reaching the maximum (27). Irradiation experiments were repeated four times.

The parameter "maximum day" (D_m) was defined as the elapsed number of days to achieve the maximum resveratrol concentration. The lower the D_m was, the more quickly resveratrol induction occurred.

Resveratrol induction rate ($V_i = [\text{resveratrol}]/D_m$) was calculated taking into account the maximum amount of resveratrol induced (resveratrol) and the elapsed days to achieve such concentration (D_m). Resveratrol concentration (resveratrol) was expressed as μg of resveratrol/g of grape skin.

Phenolic Compounds Extraction. Resveratrol and the rest of phenolic compounds were extracted by following the procedure of Cantos et al. (18). Briefly, grapes were peeled and the skins were stored at -20 °C until analyzed. Samples were homogenized in an Ultraturrax T-25 device (Janke and Kunkel, Ika-Labortechnik) at 24 000 rpm for 1 min after addition of 4 mL of a solution MeOH/formic acid (97:3) per gram of grape skin. The extracts were centrifuged at 5000g for 5 min, filtered through a 0.45 μm membrane filter Millex-HV₁₃ (Millipore Corporation, USA), and further analyzed by HPLC.

The same methodology, with some modifications, was approached in the extraction of phenolic compounds from the flesh. In this case, 4 g of flesh were homogenized with 2 mL of the MeOH/formic acid solution described above. The extracts were filtered through Sep Pak (a C-18 cartridge; Waters Millipore, USA) which retained phenolic compounds and removed sugars and other highly hydrophilic compounds. Phenolic compounds were further eluted with 1 mL of MeOH. The dead-volume of the cartridge was eluted with air. The cartridge was not washed with water to avoid the loss of some phenolic compounds. The final extract from the flesh was 20-fold more concentrated than that from the skin.

HPLC Analysis. A sample of 20 μL of the extract was analyzed using a Merck-Hitachi HPLC system with a pump model L-7100 and a diode array detector Merck-Hitachi 7455. Separations were achieved on a Liicrochart column (Merck) (RP-18, 12 \times 0.4 cm; 5 μm particle size). The mobile phase was 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. (18). Chromatograms were recorded at 320 and 510 nm. HPLC experiments were repeated three times.

Phenolic Identification and Quantification. The different phenolic compounds were identified and quantified by their UV spectra recorded with a diode array detector and by chromatographic comparisons with standards as previously reported by Cantos et al. (18).

Vitamin C Determination. Both AA and DHA were determined (two replicates) according to the procedure of Zapata and Dufour (28) by HPLC with a Merck-Hitachi (Tokyo, Japan) liquid chromatograph with a L-4000 UV detector and L-7100 pumps. Separations of DHA and AA were achieved on a Grom-Sil 120-Amino-2 PA (25 \times 0.4 cm; 4 μm particle size; Grom, Herrenberg, Germany). The mobile phase was MeOH/H₂O (5:95), 5 mM cetrimide, and 50 mM KH₂PO₄ (pH 4.6). The flow rate was 0.9 mL/min. The detector wavelength was set at 348 and 261 nm.

Graphs and Mathematical Fits. Graphs and fitting of the experimental data were carried out by using a Gauss-Newton algorithm (29) implemented in the Sigma Plot 2.01 program for Windows. Experimental data of resveratrol induction rate (V_i) versus irradiation power were fitted by nonlinear regression to a hyperbolic equation. Experimental data of "maximum day" (D_m) versus irradiation power were fitted by nonlinear regression fitting to a decreasing exponential equation. Experimental data of resveratrol concentration (resveratrol) versus irradiation time using different irradiation powers were fitted by nonlinear regression fitting to both sigmoid (90W and 240W) and hyperbolic (510W) equations.

Color Measurement. A tristimulus color spectrophotometer Minolta CM-508i (Osaka, Japan) was used to obtain the absorption spectra from each grape berry sample. Analyses were performed by reflectance. a^* values were calculated using illuminant D65 and a 10° observer according to the CIELAB 76 convention (30). Data were recorded and processed on a Minolta Software ChromaControl S, PC-based colorimetric data system (18).

Firmness, Size, and Weight. Firmness was measured in the equatorial zone of the grape berries with a device from Universal Instron (Lloyd, Hampshire). Grape berries were placed perpendicularly below the compression axe with an awl (1.13 mm of diameter). The firmness (expressed in Newtons) was measured as the maximum mechanical resistance offered by the fruit to the pressure hold by the press (31). Size of grape berries was determined by using a slide caliper with \pm 0.02 mm of precision (Mitutoyo, Tokyo). Weight of grape berries was determined using a PC-4400 balance with a precision of \pm 0.01 g (Mettler, Madrid, Spain).

Flavor. Ten well-trained people evaluated both visual appearance and flavor of grape berries at harvest and after storage (control and irradiated berries). The evaluation was scored on a ten-point scale (from 0, very bad to 10, excellent).

Ripening Index. Grapes were squeezed with a commercial blender (Moulinex, Barcelona, Spain) and the corresponding juice which was analyzed to determine titratable acidity (TA) and soluble solids content (SSC). TA was measured by titrating 10 mL of juice with 0.1 M NaOH and expressed as percentage of tartaric acid (32). SSC was measured using an Atago N1 refractometer (readings at 20 °C) and expressed in °Brix.

RESULTS AND DISCUSSION

Kinetics of Resveratrol Induction: Modeling Method. Napoleon table grapes were irradiated with a set of different conditions: irradiation power (IW) ranged from 30 to 510 W, irradiation times (IT) ranged from 5 s to 30 min, and irradiation distance (ID) ranged from 20 to 60 cm.

Different combinations IW-IT-ID proved that the best irradiation distance was 40 cm. Resveratrol induction was lower at 20 cm than at 40 cm probably because the stress (UV signal) was too strong and the resveratrol "biosynthetic system" was damaged. The damage pro-

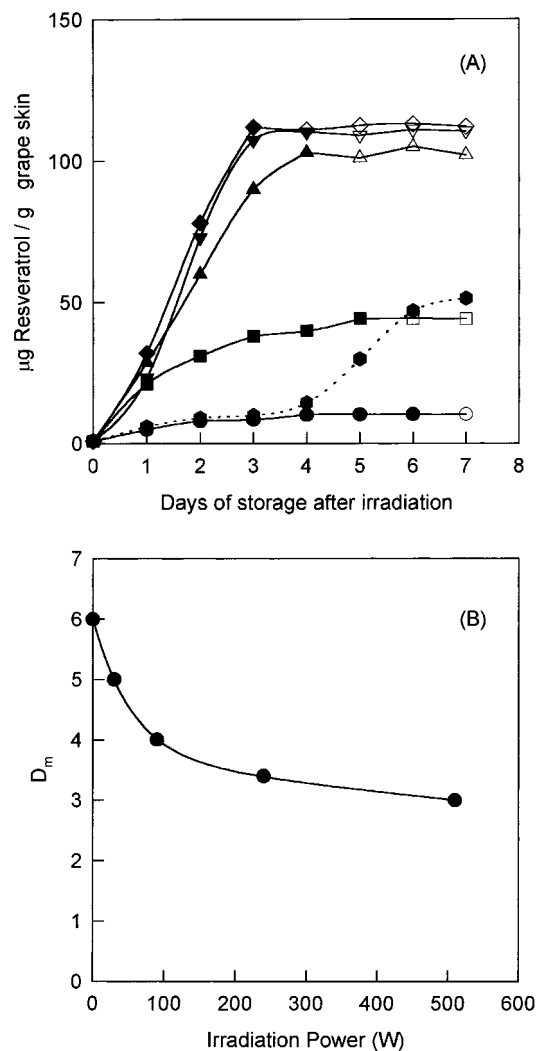


Figure 1. (A) Increasing resveratrol concentration upon storage of irradiated grape berries. Conditions were IT, 60 s; IW: (●) control, 0 W; (■) 30 W; (▲) 90 W; (▼) 240 W; (◆) 510 W. Black symbols, 20 °C. Open symbols, 2 °C. (●) Increasing of resveratrol level in the hidden side of grape skin. Conditions were IT, 60 s; IW, 90 W. (B) Dependence of the number of elapsed days to reach maximum resveratrol concentration (D_m) versus irradiation power (IW). Conditions were the same as in panel A. (●) Experimental data; (—) nonlinear regression fitting of experimental data to a decreasing exponential equation.

voked by an excess of UV irradiation has been previously reported (33, 34). On the other hand, at 60 cm the induction of resveratrol was delayed too much which, in practice, is not feasible. The optimum irradiation distance in the range from 20 to 60 cm was 40 cm. This ID was used for the following experiments.

Different combinations of IW–IT (at 40 cm of ID) were assayed to follow the evolution of resveratrol content upon storage of irradiated grapes (results not shown). Figure 1A shows the evolution of resveratrol content versus days of storage of irradiated grapes for 60 s with different irradiation powers. It is of note that a minimum IW of 90 W is recommended to induce significant resveratrol accumulation. Irradiated grapes were stored at room temperature since refrigerated storage prevented both the induction (18) and the possible degradation of resveratrol (27, 35). Depending on the irradiation power, the number of elapsed days to achieve the maximum resveratrol level (D_m) was different. In

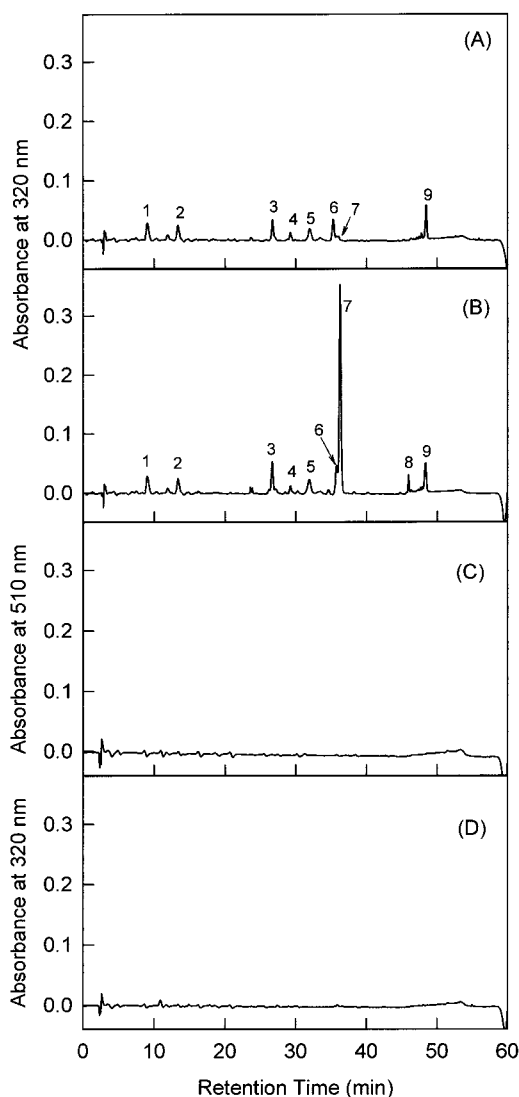


Figure 2. HPLC chromatograms of skin (A, B) and flesh (C, D) extracts of mature Napoleon table grapes. (A) Control grapes. (B) Grape berries irradiated with 510 W for 30 s. (C, D) Control grapes (the same chromatograms were obtained after UV treatment, results not shown). HPLC analysis was also carried out at 510 nm to demonstrate the lack of anthocyanins in the flesh of Napoleon grapes. Peaks (A, B): 1, Caffeic acid derivative. 2, Caffeoyltartaric acid. 3, *trans*-Piceid. 4, Peonidin 3-glucoside. 5, Malvidin 3-glucoside. 6, Quercetin 3-glucuronide + quercetin 3-glucoside. 7, *trans*-Resveratrol. 8, Viniferins (tentatively). 9, Malvidin 3-acetylglucoside + malvidin 3-*p*-coumaroylglucoside. *cis*-Resveratrol was not detected.

fact, D_m exponentially decreased versus irradiation power. This could be useful to predict the required (or desired) time to get the maximum resveratrol concentration depending on the irradiation power (Figure 1B).

When the maximum resveratrol content was determined, the irradiated grapes were transferred to 2 °C to prevent resveratrol degradation (Figure 1A, open symbols) which could be associated with the metabolism turnover and/or oxidative processes (27, 35). The parameter D_m was estimated when the resveratrol content of irradiated grapes started decreasing.

The UV signal to induce resveratrol synthesis migrated to the hidden side of grape berries reaching the maximum of resveratrol concentration 3 days later as compared to the directly irradiated side. Moreover, the resveratrol level reached in the hidden side was around

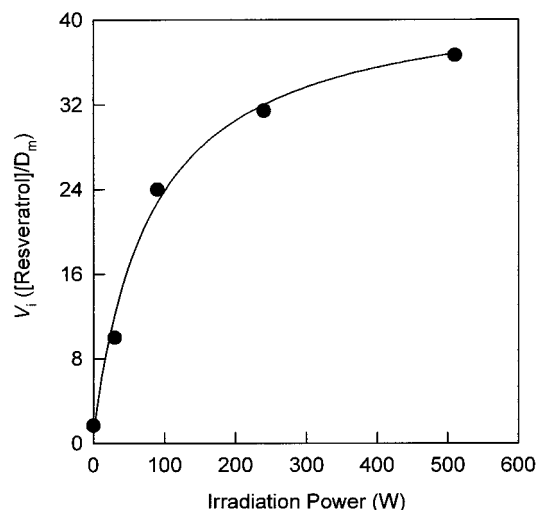


Figure 3. Dependence of resveratrol induction rate (V_i) on irradiation power. Conditions were the same as in Figure 1A. (●) Experimental data. (—) Nonlinear regression fitting of experimental data to a hyperbolic equation.

half of that detected in the irradiated side (Figure 1A). However, resveratrol was not detected in the flesh located under the directly irradiated grape berries. This meant that either there was no a biosynthetic system (probably the lack of stilbene synthase) to catalyze the synthesis of resveratrol in the flesh of Napoleon grapes or there was no migration of the UV signal from the skin to the flesh, or maybe, both circumstances at the same time. The lack of resveratrol in the flesh is in agreement with previous reports (27, 36, 37) which confirmed that independently of the migration of UV signal, the flesh was not able to induce resveratrol synthesis. Furthermore, the migration of the UV signal from the skin to the flesh in other irradiated sources (nectarine) was previously reported to be very low (38).

In addition, the phenolic content of Napoleon grape flesh was negligible as compared to that of skin, although flesh extracts were 20-fold more concentrated than those of skin (Figure 2). Therefore, a strong dietary recommendation is the intake of grape berries with the skin since peeled berries could be devoid of phenolic compounds as in the case of Napoleon table grapes (Figure 2).

Taking into account the resveratrol concentration and the elapsed days to reach it (Figure 1A), the velocity of resveratrol induction ($V_i = [\text{resveratrol}]/D_m$) was estimated. This parameter depended on hyperbolically versus IW (Figure 3). The nonlinear regression fitting of experimental data to the hyperbola gave a maximum induction rate of 42 (μg of resveratrol/g of grape skin/ D_m). From the nonlinear regression fitting, the irradiation to achieve half of maximum resveratrol induction rate was calculated (82 W).

The optimum induction process was finally achieved by combining different IW–IT ratios (Figure 4). Resveratrol induction hyperbolically depended on irradiation time (IT) with 510 W of IW (Figure 4A). The induction presented a sigmoid pattern versus IT with both 90 and 240 W (Figure 4A). Finally, resveratrol induction presented the shape of a progress curve versus IT with 30 W of IW (Figure 4A). Perhaps this progress curve is the first portion of the sigmoid curve detected with both 90 and 240 W. The sigmoid pattern was characterized by the presence of a lag period in the induction of resveratrol. This lag was very long with 30

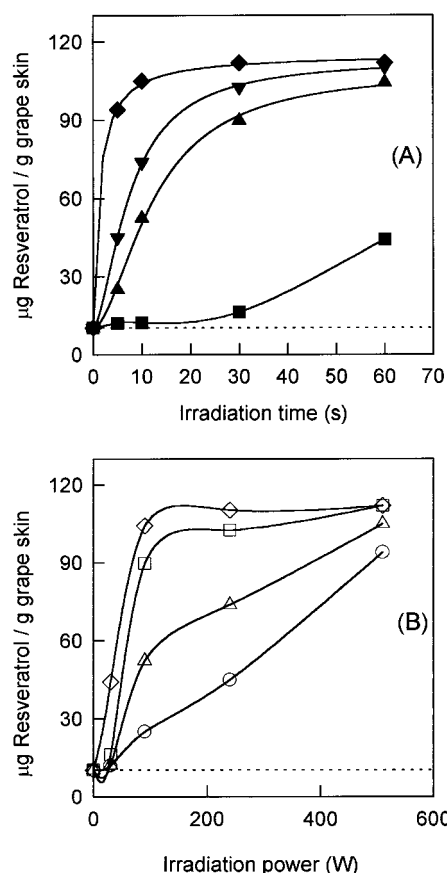


Figure 4. Dependence of [resveratrol] on different IW–IT ratios. Resveratrol concentration was determined at the corresponding D_m for each IW (3 days with 510W; 3.5 days with 240 W; 4 days with 90 W; 5 days with 30 W). (×10³) Basal resveratrol concentration (control). (A) Dependence of [resveratrol] on IT with different IW: (■) 30 W; (▲) 90 W; (▼) 240 W; (◆) 510 W. Black symbols are experimental data. (—) Nonlinear regression fitting of experimental data to both sigmoid (▲, ▼) and hyperbolic (◆) equations. Experimental data using 30 W (■) could not be fitted to any equation. (B) Dependence of [resveratrol] on IW with different IT: (○) 5 s; (△) 10 s; (□) 30 s; (◇) 60 s.

W of IW and progressively was shortened by increasing irradiation power until it virtually disappeared with 510 W of IW. In this last case, the behavior turned to hyperbolic. The maximum [resveratrol] was achieved with IW = 510 W and IT = 30 s (11-fold more resveratrol content than basal level). The nonlinear regression of experimental data of resveratrol concentration on irradiation time (Figure 4) gave the irradiation time needed to achieve half of the maximum resveratrol concentration depending on the irradiation power used: 9 s with 90 W, 4.6 s with 240 W, and 0.5 s with 510 W (Figure 4). This value could not be determined with 30 W since experimental data were not fitted to any equation due to the lack of enough experimental data. The saturation phenomenon (hyperbolic response) observed with 510 W of IW was supported by the fact that irradiation pulses of 5 s were able to multiply the resveratrol content by a factor of 9 with respect to basal levels (only 1.22-fold less than with pulses of 30 s).

Another approach to select the optimum UV treatment is by following the dependence of [resveratrol] versus IW (Figure 4B). At first sight, it is obvious that pulses of 60 s within the IW range from 90 to 510 W are recommended. Different UV pulses can be selected (Table 1). Longer IT resulted either in a decrease of

Table 1. Resveratrol Concentration ([resveratrol])^a with Different IT–IW Ratios^b

irradiation time (ID) (s)	irradiation power (IW)			
	30 W	90 W	240 W	510 W
0 (control)	10.0	10.0	10.0	10.0
5	12.0	25.0	45.0	94.0
10	12.2	52.3	73.8	105.0
30	16.3	89.8	102.5	115.0
60	44.2	104.3	110.3	114.7
120	50.1	104.3	105.3	100.3
300	60.3	104.2	90.3	75.1
1800	65.1	103.2	82.6	60.2

^a Concentration expressed as μg of resveratrol/g of skin. Mean values of three different assays are reported. Variation coefficient of values was always less than 10%. ^b Resveratrol content was analyzed at the corresponding maximum day for each IW (3 days with 510 W; 3.5 days with 240 W; 4 days with 90 W; 5 days with 30 W).

resveratrol induction (probably due to the excessive stress and the corresponding damage) or in the same resveratrol induction (Table 1). The final selection of parameters to induce significant resveratrol level (Figure 2), with short irradiation pulses balancing the irradiation power (Figure 4), will mainly depend on economic criteria.

Sensory Features of Napoleon Table Grapes. Sensory characteristics of irradiated Napoleon table grapes did not significantly differ from those of non-treated grapes. This study was logically approached to propose the present method as a possible tool to obtain functional grapes based on their high resveratrol content. It must be taken into account that most of phenolic compounds ("phytonutrients", including resveratrol) are bitter, acrid, or astringent, and an increase in their content could provoke consumer's reluctance (39). Various determinations were performed to ascertain that no significant differences were found between control and irradiated grapes: color, weight, firmness, flavor, size, and ripening index (Table 2).

Anthocyanin content of Napoleon table grapes was not affected by UV irradiation (results not shown), which could justify that grape color remained unaltered (Table 2), in agreement with previous results (18).

Grape firmness remained unchanged after UV treatment (Table 2), which implied that there was not significant softening. UV irradiation did not affect either grape weight or size (Table 2). However, there was a loss between 5 and 6% of weight after 1 week of storage for both control and irradiated grapes (results not shown).

The only minor difference detected was a slightly higher ripening index ($^{\circ}\text{Brix}/\text{TA}$) for irradiated grapes

as compared to the nontreated samples (Table 2). In fact, UV irradiated grapes were a little bit sweeter than control grapes. This was mainly due to the higher $^{\circ}\text{Brix}$ value in irradiated grapes. At any rate, this little difference was only detected by 3 of the 10 people in the taste panel that positively evaluated both control and irradiated grapes with a final mean score of 8.5 (over 10).

Therefore, UV-irradiated grapes with high resveratrol content did not differ from untreated grapes, which should imply consumers' acceptance of the product.

Vitamin C. There are no previous available reports regarding vitamin C content in Napoleon table grapes. Ascorbic acid was not detected, and only dehydroascorbic acid (DHA) was measured (Table 2). A mean value of 2.3 mg of DHA/100 g of grape berry was calculated. No differences were observed between control and irradiated grape berries.

In summary, a simple and cheap postharvest treatment by UV irradiation pulses can significantly increase resveratrol content of table grapes. This method, patent pending, could be a valuable tool to obtain possible functional grapes with high resveratrol concentration. Dietary habits that are deficient in the intake of bioactive phenolic compounds could be reinforced by "naturally functionalized" fruits. In this way, possible functional grapes obtained by using UV irradiation pulses could be a promising alternative to genetic engineering due to the increasing consumers' resistance to transgenic products.

It is important to stress that a very recent article of Romero-Pérez et al. (40) reports an improved method to extract resveratrol from skin grape berries (extraction in ethanol/water 80:20 maintained at 60 $^{\circ}\text{C}$ for 30 min, without affecting resveratrol stability). This improved extraction protocol (published at the same time of the submission of the present paper) could extract an average of 2.5 more resveratrol than the protocol used in our study. This means that the resveratrol content reported in the present paper, perhaps, is underestimated and could be multiplied by a factor about 2.5 with final concentrations of 290 μg of resveratrol/g of grape skin, which implies an average of 7.5 mg of resveratrol per table grape serving (200 g), taking into account that skin is about 13% of total grape berry.

At present, our research group is applying this induction method to increase resveratrol content in different white and red grape cultivars (cultivar susceptibility to UV induction) as well as to elaborate grape juices and wines from irradiated grapes. In this last approach, many other factors should be taken into

Table 2. Effect of UV Irradiation Pulses on Some Attributes of Napoleon Table Grapes^a

attributes	control	UV-treated
firmness (N)	9.16 \pm 0.99	9.10 \pm 0.97
weight (g of grape berry)	6.5 \pm 0.5	6.4 \pm 0.5
size (length \times diameter) (mm)	(2.57 \times 2.1) \pm (0.04 \times 0.02)	(2.61 \times 2.1) \pm (0.04 \times 0.03)
color (a^* values)	2.5 \pm 0.5	2.5 \pm 0.5
$^{\circ}\text{Brix}$	18.60 \pm 0.4	19.0 \pm 0.13
TA	3.72 \pm 0.15	3.37 \pm 0.09
ripening index	5.00 \pm 0.31	5.63 \pm 0.19
flavor	8.50 \pm 0.35	8.5 \pm 0.30
vitamin C (mg of DHA/100 g)	2.3 \pm 0.15 (whole berry)	2.3 \pm 0.16
	5.24 \pm 0.31 (skin)	5.21 \pm 0.28
	1.88 \pm 0.21 (flesh)	1.91 \pm 0.23

^a Values are the mean of three determinations (two in the case of vitamin C). Conditions were IW = 510 W; IT = 60 s; ID = 40 cm. Irradiated grape berries were stored at room temperature until D_m was reached. Then, berries were transferred to 2 $^{\circ}\text{C}$. Final time of storage was one week.

account (solubility, winemaking procedure such as maceration time, etc.).

In the context of the present paper, we also admit that bioavailability studies should be carried out to determine the real amount of resveratrol absorbed after intake of irradiated grapes with high resveratrol content. Is this therefore a new "functional" fruit?

ABBREVIATIONS USED

D_m , number of elapsed days to reach maximum resveratrol concentration after irradiation; IW, irradiation power; IT, irradiation time; ID, irradiation distance; TA, titratable acidity.

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