Physical Methods of Resveratrol Induction in Grapes and Grape Products – A Review

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Abstract

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Trans-resveratrol ((*E*)-3,4',5-trihydroxystilbene) is a substance that is produced by a large number of plants as a phytoalexin and has a wide range of beneficial biological properties. Resveratrol has been credited as being potentially responsible for the "French paradox" – the observation that the French have a relatively low incidence of coronary heart disease, even though their diet is high in saturated fats. This review deals with the methods serving for the increase of the resveratrol content in wine products – wine and grape juices. The methods reviewed are UV irradiation of grapes and ozonisation of grapes. The discussed methods describe the ways of increasing resveratrol contents in grapes and wine using "natural" methods. Resveratrol is increased endogenously and therefore, it needs not be declared as the added substance on the product labels.

Keywords: trans-resveratrol; methods of enchrichements; UV irradiation; ozonisation

Trans-resveratrol – (*E*)-3,4',5-trihydroxystilbene (Figure 1) is a substance that is produced as a phytoalexin by a large number of plants and has a wide range of beneficial biological properties. Resveratrol has been credited as being potentially responsible for the "French paradox" – the observation that the French have a relatively low incidence of coronary heart disease, even though their diet is high in saturated fats (Renaud & de Lorgeril 1992). Apart from the antioxidant, antimicrobial, and antifungal activities, it also reduces lipid deposition in the liver, inhibits platelet deposition in arteries, inhibits LDL oxidation (Frankel *et al.* 1993), and protects against cardiovascular diseases (Goldberg *et al.* 2001; Stef *et al.* 2006; Fan *et al.* 2008; Berrougui *et al.* 2009).

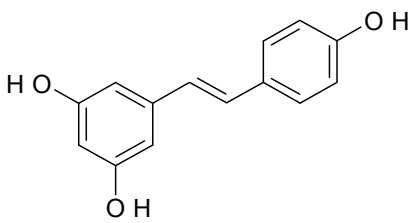

Figure 1. *Trans*-resveratrol – (*E*)-3,4',5-trihydroxystilbene

Mortality rates due to heart disease and stroke increase exponentially with age, which imposes a huge financial burden on the health care systems in the Western world. Therefore, there is an urgent need for effective therapeutic strategies that have the potential to promote cardiovascular health in

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the elderly and prevent or delay the development of atherosclerotic vascular diseases.

During the past decade, dietary supplementation with the plant-derived resveratrol has emerged as a promising approach to counteract aging-induced diseases. Resveratrol also plays an important role in cancer chemoprevention (Ndiaye *et al*. 2011). The literature is full of publications dealing with various aspects of the biological and health effects of resveratrol, and the urgent need to exchange information in this area has led to the organisation of the 1st International Conference of Resveratrol and Health, which was held in Copenhagen in 2010. Additionally, all articles dealing with the health aspects of resveratrol, arranged in twenty one chapters, were published in a special issue of the Annals of the New York Academy of Sciences in 2011 (Vang & Das 2011).

Recently, an interesting effect was discovered by Zga *et al.* (2009), however, it was not included in the above mentioned special issue. The authors found that stilbenoids, *trans*-resveratrol, and (+)-ampelopsin A offered the best anti-amyloidogenic activity in *in vitro* testing. The authors hypothesised that these substances may function as attractive new candidates for the protection against *in vivo* brain cell dysfunction in Alzheimer's disease by inhibiting the aggregation of β-amyloid fibrils.

Montsko *et al.* (2010) demonstrated a considerable variability in the contents of *trans*-resveratrol and *trans*-piceid (major stilbenes) in 42 Hungarian wines of vintages 2003–2007.

In vine plants, stilbenes are produced in response to both biotic stress (mildew and fungal infection caused by *Botrytis cinerea* and *Plasmopara viticola*) and, to a lesser extent, abiotic stress (UV radiation, chemical compounds, climatic conditions, etc.), references see text below.

This variability motivated researchers to study the influence of these factors with the goal of inducing the production of stilbenes in grapes and to modify their levels in grape juice and wine. The goal was not simply to add chemicals in the form of food additives, but to increase the content through natural processes.

Jeandet *et al.* (1995) studied the influence of maceration (physical process) on resveratrol levels in wines. Maceration of grape skins increased the resveratrol concentration ten times compared to wines without maceration (this practice is common for red wines where 'must' (vinum mustum) fermentation is carried out in the presence of grape skins.

Schubert *et al.* (1997) studied the mechanism of stilbenes biosynthesis in small localised regions distributed within the leaf of grapevine plants where the plant reacts, mostly to ozone, by producing stilbenes (stilbene synthase and β-glucuronidase, in these regions, are present in much higher levels than in the surrounding leaf mass).

Adrian *et al.* (1996) studied the induction of phytoalexin resveratrol in grapevine leaves by treating them with aluminium chloride, which acts as an antifungal preparation.

UV-C irradiation of leaves is a practical and reproducible method for inducing grapevine defense responses and can be useful in determining the defense potential of grapevine cultivars (Bonomelli *et al*. 2004).

Another *trans*-resveratrol research objective relates to finding the mechanisms of induction and methods of increasing its production in plants. These studies were logically focused on postharvest induction of *trans*-resveratrol synthesis in grapevine berries using various physical methods, which are the subject of the following review.

UV irradiation of grapes

Initially, most of UV-C experimental studies were carried out with the goal of inhibiting the development of spoilage micro organisms. In 1991, an apparatus was patented which consisted of an array of UV lamps and energy directed reflectors that provide an appropriate level of irradiance; the whole apparatus was able to move through the vineyard. The objective was to eradicate micro organisms and stimulate host plant defence mechanisms (Michaloski 1991). Later, the interest was focused on increasing the production of resveratrol in berries and wine.

The published results on the increased content of *trans*-resveratrol in berries, following UV radiation, showed a sizable variability and ranged from 1.5 to 200 (compared to untreated control samples). The results varied widely depending on: grape variety, degree of maturity, treatment method (e.g. UV wavelength, the distance of grapes from the radiation source, intensity and duration of irradiation), and storage time, as well as the conditions after UV treatment.

Moreover, different analytical methods for the determination of *trans*-resveratrol content in irradiated grapes may also have contributed to

the observed variability in the published results. When grape berries are irradiated with UV-C, the berries react to this stress by mainly inducing the stilbene resveratrol. The data for resveratrol induction (when available) using UV irradiation are summarised in Table 1.

Langcake and Pryce (1976) were the first to study the biosynthesis of *trans*-resveratrol in vine leaves as a non-specific response to fungal infection and exposure to ultraviolet light. Resveratrol was not detectable in healthy leaves, however, after irradiation its content increased to 50–400 μg/g fresh weight (f.w.).

Langcake and Pryce (1977) also studied the influence of UV irradiation on the production of resveratrol and viniferins in grapevines. Maximum production of resveratrol was induced at wavelengths in the region of 260–270 nm. The authors concluded that DNA was the photoreceptor involved in the response. This also explains why the sunlight is not an inducer under the field conditions.

Jeandet *et al.* (1991) described the production of resveratrol in the leaves and grapevine berries of *Vitis vinifera* L. (susceptible) and *Vitis labrusca* (tolerant) in response to UV irradiation; its production decreased during fruit ripening.

Sarig *et al.* (1997) compared the amounts of resveratrol and pterostilbene produced on UV-C irradiation of berries harvested at different stages of development. The cultivars included in this experiment ripen from May (Perlette) to August (Thompson Seedless), thus covering different environmental conditions. After the irradiation of berries with UV-C for 10 min at an intensity of 0.28 mW/cm^2, the highest amount of resveratrol (75 μg/g f.w.) was reached with Thompson Seedless (early stage of development – 4% of SSC – soluble solid content) while the highest amount of resveratrol (41 μg/g f.w.) was elicited in the variety Superior at the latest stage of maturity (16% of SSC). Maximum resveratrol accumulation in grape berries was 4–8.5 times higher than that of pterostilbene, depending on cultivar.

Nigro *et al.* (1998) studied the influence of UV-C irradiation on grape berries inoculated with *Botrytis cinerea.* They found that doses of 0.125–0.5 kJ/m^2 decreased substantially the number of infected berries and lesion diameters.

Douillet-Breuil *et al.* (1999) studied the production of resveratrol and other major phytoalexins in grapevine leaves in response to UV-C irradiation (lamp output 400 μW/cm^2; distance 15 cm; time 15 min). The irradiated and control leaves were stored at room temperature in the dark, petioles were kept in water and the leaves were regularly analysed for stilbene production. It was found that irradiation induced large quantities of resveratrol in leaves. The high resveratrol levels (750 μg/g f.w.) in *V. rupestris* grape leaf extracts persisted for 2 days.

Adrian *et al.* (2000) treated grape berries using UV light. Pinot noir, Gamay, and Chardonnay grape berries were irradiated with UV light (254 nm; 0.36 J/cm^2; 10 min). Both infected and healthy mature grape clusters were treated. Three regions were distinguished on the infected clusters: region I – infected site; region II – surrounding non-infected berries; and region III – berries far from the necrotic area. Berries responded to UV treatment by producing resveratrol (in the skin of the grape) except those seriously infected with *B. cinerea* (zone I). From the resveratrol production point of view, 48-h incubation period, following UV exposure, was better than 24-h period. The berries infected with *B. cinerea* contained lower resveratrol concentrations than the intact berries. The authors hypothesised that either resveratrol was metabolised by the fungus or that UV irradiation was less inductive on fungus stressed berries.

Cantos *et al.* (2000) studied the influence of postharvest treatments on Napoleon grapes using UV-C and UV-B light. Fully pigmented and unripened grape berries were separated from the cluster by cutting the peduncle, which remained attached to the berry, to avoid dehydration and reduce the susceptibility to decay. The grapes were irradiated with UV light for 30 minutes. UV-B irradiation was carried out with a UV-B lamp, VL-340-E (240 W). A similar treatment was used for UV-C irradiation, using three germicidal lamps. The treatments lasted 30 min at room temperature (1780–2300 mW/cm^2). The grapes were then stored at 0°C for 10 days and then transferred to 15°C storage for 5 days to simulate the commercialisation period. The samples were stored in perforated plastic bags at a relative humidity of 90–95% to prevent dehydration and grape decay. This treatment induced a large increase in resveratrol derivatives (3X for UV-B and 2X for UV-C) in mature berries. In immature grapes, no increase in resveratrol content was observed during the storage at 0°C, although after the transfer to 15°C a slight increase was observed in the skin of the control grapes (< 10 mg/kg); UV treatment of immature grapes was able to in-

Table 1. Overview of parameters of treatment of grapes using UV and ozone and results of resveratrol induction

Method	Parameters of treatment	Variety	R_0 (mg/g)	R_{max} (mg/g)	D_{max} (days)	IV (µg/g/day)	Reference
UV-C 254 nm TLC inspection lamp	15 min, 15 cm, 400 µW/cm^2	Pinot Noir (blue) *Vitis labrusca*	nd nd	14 32			Jeandet *et al.* (1991)
UV-C 254 nm	10 min, 0.28 mW/cm^2	Perlette (white)		73	1		Sarig *et al.* (1997)
		Spring Blush (blue)		62	1		
		Early Superior (white)		60	1		
		Superior (white)		54	1		
		Thompson Seedless (blue)		76	1		
UV 254	10 min, 0.36 J/cm^2	Gamay (blue)	5.7	15.9	1	10.2	Adrian *et al.* (2000)
				91.4	2	42.9	
		Pinot Noir (blue)	nd	33.3	1	33.3	
		Chardonnay (white)	6.7	30.2	2	15.1	
				37.2	1	30.5	
				122.9	2	58.1	
UV-B 340 nm UV-C 254 nm	30 min, 240 W 30 min, 1.78–2.3 mW/cm^2	Napoleon (blue)	6.4	11.2 15.0	15 15	0.32 0.57	Cantos *et al.* (2000) (mature grapes, total stilbenes)
UV-C 254 nm	30–510 W 5 s–30 min (30–60 s opt.) 20–60 cm (40 cm opt.) 3 days storage time (opt.)	Napoleon (blue)		114.7–115			Cantos *et al.* (2001)
UV 312 nm	15 min	Corvina (blue)	nd	39.8	1	39.8	Versari *et al.* (2001)
254 nm TLC inspection lamp	10 min, 15 cm	Black Corinth Flame Seedless (blue) (both first shipment)	25.1 13.2	33.2 57.3			Moriartry *et al.* (2001)
UV-C 254 nm	510 W, 40 cm, 60 s	Flame Seedless (blue) Red Globe (blue)	3.0 n. d.	22.67 23.16	3 5	6.6 4.6	Cantos *et al.* (2002)
		Crimson Seedless (blue)	n. d.	10.2	5	2.04	
		Napoleon (blue)	0.5	11.5	5	2.2	
		Superior Seedless (white)	0.3	16.27	5	3.2	
		Moscatel Italica (white)	n. d.	10.31	5	2.06	
		Dominga (white)	n. d.	6.87	7	0.98	

Table 1 to be continued

Method	Parameters of treatment	Variety	R_0 (mg/g)	R_{max} (mg/g)	D_{max} (days)	IV (µg/g/day)	Reference
UV-C 254 nm	30–510 W 5 s–30 min (30–60 s opt.) 20–60 cm (40 cm opt.) 3 days storage time (opt.)	Tempranillo (blue)	2.06	14.06	6	2.0	Cantos *et al.* (2003a)
		Cabernet Sauvignon (blue)	0.47	10.69	6	1.7	
		Merlot (blue)	8.97	25.5	6	2.76	
		Syrah (blue)	1.73	7.9	6	1.03	
		Monastrell (blue)	2.43	1.86	6	–0.095	
		Garnacha (blue)	1.16	7.39	6	1.04	
		Carinena (blue)	1.09	17.16	6	2.68	
UV-C 254 nm	510 W, 40 cm, 60 s	Monastrell (blue)	3.18	8.13	4	1.23	Cantos *et al.* (2003b)
UV-C 254 nm	510 W, 40 cm, 60 s	Superior (white)	1.1	10.8	3	3.2	González-Barrio *et al.* (2005)*
UV-C 254 nm	510 W, 40 cm, 60 s	Superior (white)	nd	13.0	2	6.5	González-Barrio *et al.* (2006)
UV-C 254 nm	0.36 J/cm^2, 10 min 2 days storage	Thompson Seedless (blue) Autumn Black (blue) Emperor (blue)	nd	17.47	2	8.7	Romanazzi *et al.* (2006)
		B36-55 (white)	1.84	18.12	2	8.14	
UV-B 302.1 nm resonant wavelength	0.141 kJ/m^2, 5 ns pulses, 10 Hz frequency, 45 min	Red Globe (blue)	3 µg/ml extract	21.3 µg/ml extract	directly		Jiménez Sánchez (2007)
UV-C 254 nm	510 W, 40 cm, 60 s	Superior (white)	nd	12.0	3	4.0	González-Barrio *et al.* (2009)
UV-C 254 nm	7 W/m^2, 15 cm, 6 min	Pinot Meunier (blue)	nd	15.2	1	15.2	Petit *et al.* (2009)
UV-C 254 nm	510 W, 42 cm, 60 s (14.72 mW/cm^2) Vintage 2007	Graciano (blue)	0.36	1.66	7	0.19	Guerrero *et al.* (2010)
		Merlot (blue)	0.63	2.54	6	0.32	
		Palomino negro (blue)	nd	2.16	7	0.31	
		Regent (blue)	nd	1.61	7	0.23	
		Syrah (blue)	1.26	4.57	7	0.43	
		Tempranillo (blue)	nd	0.99	5	0.20	
		Tintilla de Rota (blue)	nd	2.60	7	0.37	
		V9 (blue)	0.33	2.84	7	0.36	
		V15 (blue)	1.69	10.06	6	1.40	
		V16 (blue)	0.92	4.06	6	0.52	
		Orion (white)	nd	1.23	7	0.17	
		Palomino fino (white)	nd	2.70	7	0.38	

Table 1 to be continued

Method	Parameters of treatment	Variety	R_0 (mg/g)	R_{max} (mg/g)	D_{max} (days)	IV (µg/g/day)	Reference
UV-C 254 nm	510 W, 42 cm, 60 s (14.72 mW/cm^2) Vintage 2008	Graciano (blue)	1.60	6.99	6	0.90	Guerrero *et al.* (2010)
		Palomino negro (blue)	2.82	9.75	5	1.39	
		Regent (blue)	0.57	8.99	6	1.40	
		Syrah (blue)	0.60	10.76	6	1.69	
		Tempranillo (blue)	3.56	19.56	4	4.00	
		Tintilla de Rota (blue)	0.31	3.78	6	0.58	
		V9 (blue)	2.77	5.92	6	0.53	
		V15 (blue)	0.35	10.10	6	1.63	
		V16 (blue)	2.01	12.36	6	1.73	
		Orion (white)	0.71	10.62	5	1.98	
		Palomino fino (white)	n.d	1.68	6	0.28	
			n.d	5.90	6	0.98	
Ozone	8 mg/min, 20 min	Alphonse Lavallée (blue)		20	1		Sarig *et al.* (1996)
		Thompson Seedless (blue)		11	1		
		Zeiny (white)		16	1		
Ozone	8 ppm/30 min every 2.5 h 38 days at 0°C	Napoleon (blue)	1.22	2.81	after ozone		Artés-Hernández *et al.* (2003)
				3.84	6 days at 15°C		
Ozone	3.88 g/h 1.67 g/h	Superior (white)	nd	13.0	2	6.5	González-Barrio *et al.* (2006)
			nd	5.5	2	2.8	
Ozone	2 ppm	Superior Seedless (white)	6.77	5.00/7.93			Cayuela *et al.* (2009)
	2 ppm 12 h/day	Regina Victoria (white)	2.54	1.52/3.95			
	72 days storage at 5°C	Cardinal CL80 (blue)	5.43	5.00/8.10			

R_0 – initial resveratrol content in grape skin before treatment (fresh weight) (µg/g); R_{max} – maximum of resveratrol content in grape skin (fresh weight) (µg/g); D_{max} (maximum day) – number of days of grape storage since treatment (days); IV – induction velocity IV = $(R_{max} – R_0)/D_{max}$ (in µg/g/day)

*development of browning in Superior white grapes after UV-C treatment may be mainly due to the decrease of chlorophylls content

duce higher levels of these compounds. As observed with mature grapes, UV-C irradiation induced higher resveratrol levels (65 mg/kg) than UV-B irradiation (45 mg/kg).

Cantos *et al.* (2001) studied the influence of UV irradiation power, distance from grapes, and irradiation time on resveratrol content in ripe grapes of the red Napoleon table cultivar. They also studied optimum storage times to reach a resveratrol maximum (R_{max}) in grape skins. They found that the distance of 40 cm, irradiation time of 30 s, source power of 500 W, and storage time of 3 days proved to be optimal. It has to be emphasised that this fundamental work found that the grape flesh contained negligible amounts of resveratrol. Therefore, the authors recommended consuming whole, not skinless, berries.

This process was further developed by Cantos *et al.* (2001, 2002) patents WO/2002/085137 and ES 2177465.

Versari *et al.* (2001) treated berries of the Corvina variety. They cut berries from clusters and irradiated them using UV light (312 nm, 15 min). The treated and untreated control berries were incubated in the dark at room temperature (with their petioles in water) for 24 hours. UV treatment applied during the véraison stage of grape maturation caused a substantial increase in *trans*-resveratrol content in the skin of berries (about 40 mg/g f.w.) whereas UV treatment of ripe berries caused hardly any increase in *trans*-resveratrol content during 59 days of storage.

Moriartry *et al.* (2001) tested the influence of UV irradiation on resveratrol levels in the skin of two Californian table grape cultivars. Three shipments of grapes were tested. The best results (highest levels of resveratrol induced) were associated with the first shipment of grapes from the Flame Seedless cultivar (up to 4X compared to untreated controls). The treatment was almost ineffective with the third shipment, which consisted of fully mature grapes.

Cantos *et al.* (2002) studied the influence of UV irradiation on resveratrol production in red table grape cvs Flame, Red Globe, Crimson, and Napoleon, as well as white table grape cvs Superior seedless, Moscatel Italica, and Dominga. Standard irradiation parameters were used throughout the study: irradiation power 510 W, distance 40 cm, and time 60 seconds. Both irradiated and control (not treated) grape berries were stored at 22°C in perforated plastic bags (relative humidity of 90–95%) to avoid water loss and shrivelling. The sets of both irradiated and control grapes were transferred to 2°C (in the same plastic bags) every day in order to follow the induction kinetics of stilbenes at both temperatures. The 'maximum day' was calculated for each variety tested as the number of days in storage (following irradiation) at room temperature after which resveratrol reached its maximum concentration. Total resveratrol content ranged from 0.69 mg/100 g f.w. (cv. Dominga) to 2.3 mg/100 g f.w. (cv. Red Globe). Net resveratrol induction ranged from 3.4X (cv. Flame) to 2315X (cv. Red Globe) as compared to the untreated controls.

Tomás-Barberán *et al.* (2002) patented details of a UV-C irradiation method for fruit and vegetables with the aim of inducing resveratrol production. The patent describes the method of increasing resveratrol content of table grapes by using irradiation pulses in a tunnel of ultraviolet-C lamps. The pulses were applied for less than 1 min and irradiation strength ranged from 30 W to 510 W. 2–4 days after the treatment, resveratrol content of the treated grapes increased at least 10×. In this way, grapes with significantly increased health-benefits can be obtained using a simple, low-cost technique.

Cantos *et al.* (2003a) studied the stilbene induction capacity (*trans*-piceatannol, *trans*-resveratrol, and viniferins) of red wine grape cvs Tempranillo (TEM), Cabernet Sauvignon (CAS), Merlot (MER), Syrah (SYR), Monastrell (MON), Gamacha (GAR), and Cariñena (CAR) exposed to post-harvest UV-C irradiation. The maximum concentration following UV-C irradiation was: 2.5 (mg stilbene/100 g f.w.) for resveratrol in MER, 0.42 for piceatannol in MER, 0.16 for piceid in MON, and 0.39 for ε_1-viniferin in CAR.

Cantos *et al.* (2003b) studied the influence of UV-C irradiation on the Monastrell red variety of grapes relative to inducible stilbene content. Grape clusters were irradiated with UV-C light using the same protocol as previously described for the Napoleon table variety (power 510 W, distance 40 cm, time 60 s). The grapes were then stored for 4 days at room temperature (25°C). This optimum storage time was predicted by preliminary experiments as the storage time at which resveratrol content would reach a maximum for this variety of grapes. Non-irradiated clusters (controls) were stored for the same period of time.

Standard winemaking processes were applied on the stored grapes and the concentrations of the

main stilbenes were measured for each stage of the wine making process (Cantos *et al.* 2003b). The fermentation of crushed grapes (must) without stems was carried out over 10 days. The resveratrol and piceatannol contents in the fermenting must (juice with skins) reached a maximum on the fifth day (1170 mg/l resveratrol and 655 mg/l piceatannol in UV-treated must versus 471 mg/l resveratrol and 346 mg/l piceatannol in control must). During additional fermentation, the levels decreased by about 10× compared to maximum values. The enzymatic- and non-enzymatic-catalysed oxidation processes and increased temperature of must fermenting could have played a role in this decrease. The authors suggest that, by stopping maceration (fermenting with skins) on the 5th day, the opportunity is given to maximise piceatannol and resveratrol contents in the wine. Further losses of stilbenes were observed after pressing, lees separation, and clarification. Similar losses were also observed in UV-C untreated controls. Therefore, the final wine prepared using irradiated grapes had about 2× and 1.5× higher contents of resveratrol and piceatannol, respectively, than the control wine.

Borie *et al.* (2004) tested the influence of three elicitors of the grapevine defence mechanisms: UV irradiation, aluminium chloride, and *Botrytis cinerea*. The leaves from the *Vitis rupestris* and *V. vinifera* cultivars of Pinot noir and Chardonnay plants grown *in vitro* were treated and the corresponding response was analysed with respect to stilbene synthesis products. UV irradiation induced high and relatively stable levels of these products.

Shama and Alderson (2005) reviewed the effect of UV irradiation on the changes in different varieties of fruits generated as a biological response to this abiotic stress. The effect of this type of physical treatment on resveratrol induction in grape berries was also considered. The treatment was found to be an effective method for substantially increasing resveratrol content in table and wine varieties of grapes. The authors pointed out that irradiation involved only individual grape berries and irradiation of whole bunches could create problems with the treatment homogeneity.

González-Barrio *et al.* (2005) observed increased stilbene concentrations after UV-C irradiation, but also some berry surface browning, on the third day of storage at 22°C, with a subsequent deterioration of the sensorial quality of the fruit. After 3 days of storage at 22°C, the level of *trans*-resveratrol in UV-treated grapes reached maximum concentration of 10.8 mg/kg f.w. of berries. Other stilbenes, such as *trans*-piceid, *trans*-piceatannol, and viniferins, not initially detected in control grapes, were also induced in UV-treated grapes with maximum concentrations of 0.35, 1.16, and 8.23 mg/kg f.w., respectively. The results suggest that the development of browning after UV-C irradiation and throughout storage may be mainly due to the loss of chlorophylls and the accumulation of pheophytins as degradation-derived products.

González-Barrio *et al.* (2006) compared the effects of ozone and UV-C treatments on stilbenoid biosynthesis (trans-resveratrol, piceatannol and viniferins (resveratrol dehydrodimers and dehydrotrimers) in white table grapes of the variety Superior. The details for ozone treatment are given in next chapter. UV-C treatment was done on grape berries using standard irradiation parameters: power 510 W, distance 40 cm, and time 60 seconds. Both the irradiated and comparative (not treated) grape berries were stored at 22°C in perforated plastic bags at a relative humidity of 90–95%. UV-C irradiation induced comparable (3.88 g/h ozone treatment lasting 5 h), the maximum resveratrol concentration of 1350 mg/100 g f.w. was achieved after 2 days of storage. The value fluctuated slightly during the following days of storage due to the transformation of *trans*-resveratrol into dehydrodimers and dehydrotrimers (viniferins + trimers) under the influence of stilbene synthesis and in response to abiotic stress. UV-C treatment was more effective than ozone treatment in producing resveratrol.

Romanazzi *et al.* (2006) studied the influence of pre-harvest chitosan spray and postharvest UV irradiation treatment of grape clusters with the goal of decreasing the incidence of gray mold on table grapes. In their study, trans-resveratrol induction in the skin of berries was also monitored.

Mature clusters of table grape cultivars from several grapevines were used. Only clusters with soluble solids content higher than 16% were selected. Berries harvested 2 days after chitosan treatment were irradiated using two germicidal lamps emitting quasi-monochromatic UV radiation at 254 nm. The berries were placed in a single layer on plastic trays, at 10 cm distance from the lamps. After 5 min of treatment, the berries were turned over and the other side of the berries was exposed to the same dose (0.36 J/cm^2) for additional 5 minutes. The irradiated berries and

controls were incubated at 20 ± 1°C and 95–98% relative humidity for 48 h and then inoculated with *B. cinerea.*

Catechin, *cis*- and *trans*-piceid, and *cis* and *trans*-resveratrol contents were quantified in the berry skins of cv. Autumn Black and selection B36-55 (*V. vinifera* L., *V. rupestris* Scheele, and *V. lincecumii* Buchkl.). None of the substances was found in untreated controls or in chitosan treated berries. In chitosan treated and untreated berries, later irradiated with UV-C, catechin was found in the skins of cv. Autumn Black. The berries treated both with chitosan and UV-C irradiation exhibited significantly higher catechin contents than those only irradiated.

Trans-resveratrol was detected in the berries of both cultivars (Autumn Black and selection B36-5) treated with chitosan and UV-C irradiation (Romanazzi *et al.* 2006). UV-C irradiation by itself led to *trans*-resveratrol induction of 17.5 mg/g f.w. in grape berry skins of cv. Autumn Black, and of 18.1 mg/g f.w. in the selection B36-55. A combination of chitosan spray and UV-C irradiation induced significantly higher resveratrol contents than UV-C irradiation alone (23.2 and 22.0 mg/g) in cv. Autumn Black and selection B36-55, respectively. *Cis*-resveratrol, *trans*-piceid, and *cis*-piceid were not detected in any of the samples. In cv. Autumn Black, *trans*-resveratrol and catechin contents were negatively correlated with the decay and disease severity. The authors hypothesised that the presence of these substances could be a significant factor during plant-microbe interactions and could contribute to the suppression of the disease development. We can also hypothesise that UV-C irradiation acts as a direct inactivation factor for micro organisms and, at the same time, as a stress factor for plants that provokes the induction of protective chemical substances with antimicrobial activity (hormesis effect).

González-Barrio *et al.* (2009) irradiated grapes of the white, table, seedless variety Superior using UV-C to induce stilbenes together with the investigation of the maceration time, temperature, macerating enzymes, and the use of β-cyclodextrin. Stilbene content in UV-C non-treated grapes was barely detectable, whereas 12 mg/kg f.w. was detected in UV-C treated grapes. It should be stressed that proper maceration conditions have to be used to extract and solubilise the stilbenes induced in berries. The best results were achieved by maceration of grapes UV-C treated for 2 h at 45°C with 0.2% sodium metabisulfite ($Na_2S_2O_5$) where the phenolics concentration, especially stilbenes, increased up to 35× over the controls.

Petit *et al.* (2009) investigated the plant defence reactions in response to UV-C irradiation in inflorescences and young clusters in great-sized berries stage. They found 15.2 µg/g f.w. of resveratrol after 6 min irradiation and 24 h after treatment (7 W/m^2, 15 cm, 6 min).

Guerrero *et al.* (2010), using UV-C, studied the induction of stilbene biosynthesis in grapes of three varieties of *Vitis vinifera sylvestris*, seven varieties of *Vitis vinifera sativa* and two hybrids. After UV-C treatment (510 W, 42 cm, 60 s), maximum content of total stilbenes (25 mg/kg) was reached in the cv, Syrah, which makes cv. Syrah a suitable candidate for producing "functional wines". Twelve cultivars were tested in this study and grown under the same warm climate conditions found in Spain, grown in the same location and harvested as part of the 2007 and 2008 vintages. Orion (OR) and Regent (RG) varieties are HDPs obtained by crossing interspecies plants with *Vitis vinifera sativa*. Varieties V9, V15, and V16 belong to the *Vitis vinifera sylvestris* species. The main group of *Vitis vinifera sativa* includes the international varieties Merlot (ML) and Syrah (SY), the Spanish national cvs Graciano (GRA) and Tempranillo (TEMP), and the local cvs Palomino fino (PNO), Palomino negro (PN), and Tintilla de Rota (TR). The cvs OR and PNO produce white grapes, while the others (GRA, ML, PN, RG, SY, TEMP, TR, V9, V15, and V16) produce red grapes.

Healthy grape clusters of each variety were manually harvested and UV-C irradiated. Thirty-four UV-C lamps, divided into two panels, positioned above and below the grapes, having the theoretical power of 510 W, were used for irradiation (42 cm, 60 s). The irradiated grape clusters were stored in a stainless steel vessel at 18°C and 75% relative humidity for 7 days. The same conditions were used for the untreated controls.

Trans-resveratrol content was measured in grape skins and recalculated for the fresh weight of grape berries. The number of days of storage needed to reach maximum content (D_m) was determined through day by day analysis. The results were used for the prediction of resveratrol (IV_{resv}) induction velocity and served as one of the parameters for the variety characterisation. This parameter is defined as the concentration of resveratrol on the day of maximum (CD_m) – initial resveratrol content (C_0) divided by the number of days for

maximum content achievement D_m in the skin – $(CD_m - C_0)/D_m$).

The results were obtained from two vintages for each variety. In 2008, the total content of stilbenes in all grapes tested, both initially and after UV-C treatment, was much higher than in 2007. The results provide an additional support regarding the exceptional behaviour of the cv. Syrah. This cultivar reached the highest concentration of resveratrol (19.56 mg/kg f.w.) and also achieved its maximum in the shortest period of time following UV-C treatment (D_m = 4 days). The induction velocity (IV) for resveratrol yields 4 mg/kg f.w./day. This is the fastest rate calculated for any of the varieties tested in the two vintages.

The HDPs (RG and OR) were expected to have higher levels of production because they were designed specifically for increased resistance to pathogens. This resistance has been associated with stilbene production after infection; however, stilbene concentration in the HDPs was only significant in RG for 2008 (10.76 mg/kg f.w.).

UV-C treatment generates abiotic stress in grapes and induces the production of stilbenes. The differences in resveratrol content depend mostly on the variety and vintage. The varieties with a high stilbene concentration before the treatment have better biosynthetic mechanisms to respond to the stress induced by postharvest UV-C treatment. The same variety behaves differently each year. The climate could influence the D_m as well as final resveratrol concentrations.

The cv. Syrah variety exhibited an exceptional behaviour and can be considered the best candidate for the production of stilbene enriched wine, although some variability still occurred between the two years of the study (Guerrero *et al.* 2010).

Regarding the irradiation wavelength used in the previously published works, the reasons for choosing one or another wavelength were not mentioned. The most widely used are 340 nm (UV-B) and 254 nm (UV-C) wavelengths in spite of the fact that UV absorption spectrum of *trans*-resveratrol shows a wide band from 280 nm to 360 nm with the maximum at 306 nm. This is rather an unspecific elicitation method caused by different stresses.

Jiménez Sánchez *et al.* (2007) investigated the effect of the *trans*-resveratrol elicitation using tuneable laser producing UV resonant wavelength (302.1 nm) and a close but non-resonant wavelength (300 nm). After irradiation of the red grapes (cv. Red Globe) for 45 min (optimum), six-fold increase of *trans*-resveratrol content was determined immediately after irradiation at a resonant wavelength while at a non-resonant wavelength, the increase was negligible.

Ozonisation of grapes

The initial work with ozone focused on the prolongation of the shelf life of table grapes. As a side effect, it was found that ozone impulse provokes the synthesis of stilbenes. The data for resveratrol induction (when appropriate) using ozone fumigation are summarised in the second part of Table 1.

Sarig *et al.* (1996) studied the application of ozone for the control of fungi attack during grape storage. They noticed that grapes exhibited increased resistance against fungi development if ozonised both before and after the fungi inoculation. Here, the idea of "vaccination" came into the game. It was found that ozone elicited the induction of phytoalexins, resveratrol, and pterostilbene in berry skins, producing levels comparable with those at UV-C irradiation.

Artés-Hernández *et al.* (2003) studied the composition changes in cv. Napoleon table grapes during post harvest storage. The grapes were kept for 38 days at 0°C, followed by 6 days at 15°C (simulating cold storage and marketing stages). Various storage conditions were applied including modified and controlled atmosphere packaging and ozone. In one treatment, the grapes were thermally sealed in polypropylene perforated bags and placed at 0°C and 90% relative humidity with continuous exposure to 0.1 ppm of O_3. This treatment method was compared to the treatment using O_3 shocks. Hermetically sealed glass jars, each containing one cluster, were conditioned with humidified air at a flow rate of 20 ml/minute. O_3 shocks consisted of O_3 (8 ppm) being flushed into the jars; O_3 shocks lasted 30 min and occurred every 2.5 hours.

O_3-treated clusters, using the shock ozone technique, increased piceid content greatly, compared to piceid content at harvest (from 1.86 ± 0.9 mg/g f.w. to 20.5 mg/g f.w.). However, during storage under standard atmospheric conditions and 15°C, the piceid content decreased to 3 mg/g f.w.

The controlled atmosphere samples (5 kPa O_2 + 15 kPa CO_2 + 80 kPa N_2) and ozonised clusters (continuous and 8 ppm shock treatment) increased their resveratrol content significantly (from 1.22 ±

0.6 mg/g f.w. to 2.38 mg/g f.w. for continuous O_3, 2.71 mg/g f.w. for controlled atmosphere, and 2.81 mg/g f.w. for shock O_3) after cold storage. Standard atmosphere storage at 15°C led to changes in resveratrol levels. Controlled atmosphere and O_3 shock treated samples increased resveratrol levels to 2.87 and 3.84 mg/g, respectively. The samples subjected to continuous O_3 treatment exhibited a slight decrease in resveratrol content to 2.36 mg/g.

Sgarbi *et al.* (2003) studied the production of phytoalexins in *Vitis vinifera* leaves (expressed as resveratrol) in response to ozone fumigation. The concentration of resveratrol reached a maximum 24 h after the ozone treatment.

The conditions for short anoxic treatments are the subject of some patents P200402367 and PCT No.ES2005/000532 (GonzáleZ-Ureña *et al.* (2004) and are described in detail in a publication by Jiménez *et al.* (2007). For the experiments, white table grapes (cv. Aledo) and red grapes (cv. Tempranillo) were selected; from the latter red wine was prepared under standard oenological conditions. The grapes were placed in a vacuum chamber which was slowly evacuated until the vacuum was below 1 mbar. The chamber was then filled with dry nitrogen until the pressure reached 1.1 bars. Anoxic conditions were maintained for 6 h to 48 h, and all the treatments were carried out at room temperature.

A disadvantage of anoxic atmosphere treatment is the reduction of *trans*-resveratrol content in the days following the anoxic treatment, which is caused by the grapes returning to aerobic conditions, oxygen exposure being able to cause serious damage to the plant tissues. This post-anoxic injury is explained by the oxidation of alcohol, which accumulated in the tissues during the anoxic period, to acetaldehyde. The acetaldehyde is most likely responsible for the damage. Also, *trans*-resveratrol itself may be subject of oxidative transformations. To avoid this damage, an optimised method was proposed. After 24 h of anoxic treatment, the berries must be picked, pressed, and transported to a fermentation tank within 35 minutes. With shorter anoxic treatments (6–15 h) no damage takes place, however, a shorter treatment does not achieve any enrichment in *trans*-resveratrol. It is worth mentioning that the analytical methods for the determination of *trans*-resveratrol are a combination of laser desorption, REMPI (Laser Resonance Enhanced Multi-Photon Ionization), and TOFMS (Time-Of-Flight Mass Spectrometry).

Gonzalez-Barrio *et al.* (2006) compared the effects of ozone and UV-C treatments on stilbenoid biosynthesis. Ozone treatment was carried out on 500 g of grape berries placed in a chamber at a constant temperature of 22°C and a relative humidity of 98%. Ozone gas was supplied to the chamber at a flow rate of 0.16 Nm^3/h with ozone production of 3.88 or 1.67 g/h. The treatments lasted 1, 3, and 5 h, and the concentration of ozone in the air was 11 300 and 4 800 ppm (v/v). The ozone treated and untreated control grapes were stored for up to 5 days at 22°C. The 3.88 g/h ozone treatment lasting 5 h induced maximum resveratrol concentration (1250 mg/100 g f.w.) after 2 days of storage, which was almost comparable to UV-C treatment. As concerns total stilbenoids content in the grape skins (resveratrol + piceatannol + viniferins (dimers and trimers)), the ozone treatment induced higher stilbenoid concentrations than UV-C treatment.

Cayuela *et al.* (2009) studied table grapes (cvs Superior Seedless, Cardinal CL 80, and Regina Victoria). The grapes were stored at 5°C for 72 days and treated with ozone either continuously (2 ppm) or intermittently (12 h/day). The intermittent ozone treatment induced a higher *trans*-resveratrol content (17–55%) compared to that in untreated grapes. In fact, the *trans*-resveratrol content in grapes treated continuously (2 ppm of ozone) decreased by 8–67% compared to the untreated grapes. Both ozone treatments considerably reduced the decay of cold stored grapes compared to grapes kept in air, but the former had lower scores in sensory tests and showed higher weight losses than the fruit kept in air.

CONCLUSION

The methods discussed above described the ways of increasing resveratrol content in grapes and wine using "natural" methods. Resveratrol is increased endogenously and, therefore, need not be declared as an added substance on the product labels.

The synthesis of *trans*-resveratrol was mentioned e.g. in a review by Šmidrkal *et al.* (2001). In addition to these synthetic efforts, the production of resveratrol by the roots of *Reynoutria* sp. plants is significant and many products can be found on the market including food supplements in various forms.

Due to a great consumers' interest and many applications including those in the cosmetic industry, there is a considerable interest in finding methods for industrial production using biotechnologies. Donnez *et al.* (2009) reviewed different methods of *trans*-resveratrol production and found plant cell cultures or recombinant microorganisms to be the most promising. Delaunois *et al.* (2009) reviewed the methods of stilbene synthase gene transfer. The gene responsible for resveratrol production in plants is the subject of investigations regarding the possible transfer methods. Transgenic plants containing this gene should have antifungal activity and should also produce fruit with a higher antioxidant activity.

We believe that daily consumption of grapes, grape juice and, to a limited extent, wine also has other positive benefits (synergism with other components) beyond the benefit of consuming the pure chemical substance. A recent review by Mukherjee *et al.* (2010), showed a dose dependent effect of resveratrol: i.e. at lower doses it can be very useful in maintaining human health, while at higher doses, resveratrol has pro-apoptotic effects on healthy cells, but can also kill tumour cells.

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